A NOVEL METHOD TO ASSESS CONSUMER ACCEPTANCE OF BITTERNESS IN CHOCOLATE PRODUCTS

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by
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ABSTRACT

Sensory science provides powerful tools for investigating consumer preferences. Chocolate is a product that comes with a host of special considerations when applying sensory evaluation techniques. Refinement of the methodology, analysis and application of a recently developed method, rejection thresholds, to chocolate products, was the focus of this thesis work. Specifically, rejection thresholds for an added bitter compound (sucrose octaacetate, SOA) were determined in chocolate-flavored fluid milk, milk chocolate-flavored compound coating, and chocolate ice cream. Bitterness was investigated because it is a necessary component of chocolate flavor at low levels but can become objectionable at higher levels. Responses were analyzed based on participant groupings by self-reported preference for milk or dark chocolate. In all three matrices, the group preferring dark chocolate tolerated significantly more SOA than the group preferring milk chocolate. Chocolate milk acted as a model system in which analysis by sigmoid dose-response function was illustrated. In compound chocolate and chocolate ice cream, use of the method in more complex food systems was demonstrated. Finally, rejection thresholds for cocoa powder produced from under-fermented cocoa beans (high cocoa flavanol (CF) natural cocoa powder) was determined in semisweet solid chocolate. This threshold is of interest to the confectionery industry as recent research has focused on potential cardiovascular health benefits from naturally occurring cocoa polyphenols, the content of which is higher in cocoa products subjected to limited fermentation. No significant differences were observed between groups preferring milk chocolate or dark chocolate in this study. The group rejection threshold was 80.7% of the non-fat cocoa solids coming from the high CF natural cocoa powder in a semisweet chocolate.
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“Hem your blessings with thankfulness so that they do not unravel.”

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Chapter 1
Preface

Sensory evaluation provides powerful tools for investigating consumer preferences. When it comes to food choice, taste is the primary driver of selection behaviors, above health, cost and convenience (IFIC, 2011). When considering a distinctive product like chocolate that has a unique balance of flavor, determining tolerance levels for specific attributes and/or compounds becomes paramount, so as not to negatively disconfirm consumer expectations (that is, to taste worse than what was expected). There are many components of chocolate flavor that are necessary in specific proportions. When these components are present at too high of a level, they may become objectionable to the consumer and may ultimately lead to product rejection.

When determining tolerance levels, researchers often look to classic threshold methods, such as detection or recognition thresholds. Detection thresholds are defined as “the lowest physical intensity of a stimulus that is perceived by an assessor a specified percentage of time, usually 50% (ASTM, 2011).” Recognition thresholds are defined as “the lowest physical intensity at which a stimulus is correctly identified by an assessor a specified percentage of the time, usually 50% (ASTM, 2011).” These threshold levels are not the same – at very low concentrations a person may detect a difference between two samples when one contains the stimulus, however they may not necessarily recognize what the difference is. A detection threshold could be of interest because likely an attribute will not be objectionable to the consumer until they can at least tell that something is there. However, detection thresholds do not usually tell whether a product
will be rejected or not. Likewise, recognition thresholds tell us that the difference has come to a level that is recognizable (and therefore generally higher than the detection threshold). While it is possible that an attribute could be rejected immediately upon recognition, and thus tolerance could potentially be inferred from recognition thresholds, this is not always the case. This situation is especially apparent in chocolate. Let us take, for example, the attribute of bitterness. A degree of bitterness is essential for a well-balanced, typical chocolate flavor, the same way it would be expected in a product like coffee or beer. Bitterness needs to be present at a level above both the detection and recognition thresholds. For chocolate flavor, the question then becomes how to determine the intensity at which bitterness is too high and will lead to product rejection.

In the past, researchers have shown that classic thresholds do not associate with predictions of suprathreshold intensity (e.g. Bartoshuk, 1978) or preferences (e.g. Mattes and DiMeglio, 2001; Duffy, Peterson et al., 2004; Lucas, Riddell et al., 2011). More recently, a novel approach was developed to determine ‘rejection thresholds’ (Prescott, Norris et al., 2005). This method is a combination of both psychophysics (examining relationships between measurable physical stimuli and sensory responses) and hedonics. That is, rejection thresholds investigate preferences (hedonic response) in a dose-dependent manner (psychophysics). The strategy is based on paired preference tasks (2-AFC, or 2-Alternative Forced Choice tasks) where participants are presented with successive pairs containing a ‘control’ sample and a ‘spiked’ sample and asked to indicate which they prefer. The spiked sample contains elevated levels of the stimulus of interest, and the pairs are presented in order of ascending spike concentration. The control stimulus contains only the un-spiked vehicle or product. The main goal is to see,
as the attribute of interest gets stronger, at what point do the participants begin to consistently and significantly choose the control sample over the spiked one, therefore reaching a group ‘rejection threshold.’

Prior to the present work, there were only three studies published in the literature where rejection thresholds were determined. Two of these studies determined rejection thresholds for off-flavors in wine: TCA (trichloroanisole, aka cork taint) in white wine (Prescott, Norris et al., 2005) and eucalyptol in red wine (Saliba, Bullock et al., 2009). The other was focused on rejection thresholds for bitterness, sourness, and saltiness in the context of PROP (Propylthiouracil) taster status (Lee, Prescott et al, 2008). While the method was simple and elegant, the results of these studies may have been skewed due to the statistical approach used to analyze the data. In two of these previous studies, the data were plotted as the proportion of participants rejecting the spiked sample versus the concentration of the compound of interest (a preference-indifference function), with the points connected by straight line segments (see figure 1-1). The threshold was estimated as the point where the binomial distribution at \( \alpha = 0.05 \) crossed the fitted line, which is a function of sample size (Prescott, Norris et al., 2005; Saliba, Bullock et al., 2009) (see figure 1-1). In the third study, data was simply interpreted via the binominal distribution, where the first concentration at which the proportion of respondents was significant was considered to be the rejection threshold, even if proportion at the next highest concentration failed to reach significance (Lee, Prescott et al., 2008). However, both of these methods may be biased depending on the concentration range chosen for the spiked samples and the spacing allowed between the concentrations.
Figure 1. Taken from Prescott, Norris et al., 2005. (Left)
Preference/Indifference function for TCA in white wine (n=58) $\alpha=0.05$ at $y=0.66$. (Right)
Preference/Indifference function for TCA in white wine (n=30) $\alpha=0.05$ at $y=0.7$. CRT = Consumer Rejection Threshold.

Theoretically, the underlying preference-indifference function should have an sigmoid or “s” shape (see far right portion of figure 1-2). At low concentrations around or below the detection threshold, we would expect our participants to have no real preference for the control or the spiked sample, so the responses should oscillate around chance performance. When there is no preference, but a choice is forced, the probability of choosing the control sample is equivalent to the probability of choosing the spike (50/50 chance in the case of a 2-AFC task). Once the spike reaches a level at which the participants can detect it and have an opinion about it, we would expect the curve to slope upwards creating a linear portion of the function as more participants prefer the control.
sample as the concentration of the spike increases. Finally, the curve should eventually reach the maximum of 100%, where all of the participants choose the control sample over the spike, which can be referred to as universal rejection. After fitting a sigmoid curve to the data collected experimentally, the rejection threshold can be determined as the point halfway between chance performance (50%) and the maximum (100%), consistent with other threshold determinations. Notably, this does not vary as a function of sample size as the binomial approach does.

If rejection threshold data is analyzed by simply connecting the points from one concentration to the next, the value that is determined will depend greatly on how large the spacing is between the concentrations. See figure 1-2 as an example – while the $R^2$ value is perfectly linear because the line is between just two points, the rejection threshold value could be completely different if the experiment had skipped from the first concentration to the third. Similarly, if a linear fit is forced through all of the data points collected, we can see that the data would be greatly affected by the concentration range selected. In figure 1-2 the concentration range is slightly high, which makes the slope come across as more shallow than it might have, had a broader concentration range been chosen. Additionally, while these other fits can provide an estimate of the rejection threshold, simply applying the binomial distribution as demonstrated by Lee and colleagues (2008) does not provide any means to calculate error. With the binomial approach, there is no way to statically compare rejection thresholds across different groups as there is no variance. When fitting a curve to the data, an extra sum-of-squares F-test can be carried out to determine if differences between calculated group rejection thresholds is significant.
Chocolate is a particularly appealing stimuli to determine rejection thresholds in for many reasons. First, it allows the investigation of consumer response to bitterness and/or astringency, both of which are inherent to chocolate and generally follow the expected dose-response pattern that the rejection threshold method is best suited for. Secondly, the rejection threshold method does not require time and cost intensive trained panels to evaluate. Rather, large-scale tests with numerous untrained participants can be carried out rapidly in a single testing session. In situations of consumer preference, it is not always important to know how the participants describe the attribute of interest; rather, the primary concern is the attribute’s effect on their preference. Chocolate is a well-liked food product that many consumers are familiar with, which again makes it appealing to work with for ease of participant recruitment. Finally, this method is particularly useful in chocolate research because of the potential to group and compare...
respondents based on different demographic information such as gender or other segmentation variables like self-reported preference for milk chocolate or dark chocolate.

The main objective of this thesis work was to refine the methodology and analysis of the rejection threshold method in order to determine rejection thresholds for endogenous compound driving preferences in solid chocolate confections. In order to apply the rejection threshold method to tolerances in chocolate, additional work was required to confirm the utility of the method in complex stimuli. To begin, we performed a series of three experiments measuring rejection thresholds in chocolate flavored products. Initial work was carried out using a model bitter compound (sucrose octaacetate, SOA) and was focused on advancing the methodology and statistical modeling of the data. As previous work with rejection thresholds had only been carried out in liquid systems (Prescott, Norris et al., 2005; Lee, Prescott et al., 2008; Saliba, Bullock et al., 2009), we began initially with liquid (chapter 2), and incrementally increased the level of complexity, moving on to solid (chapter 3) and semisolid (chapter 4) food matrices. These studies utilized the model bitter compound SOA in order to investigate response to increasing bitterness without disturbing other physical or sensory properties of the stimuli. To avoid potentially biased models, we refined the analysis by using a sigmoid function to fit and compare the rejection threshold data that we collected.

In our final study (chapter 5) we focused on the application of the rejection threshold method to actual bitter constituents actually found in chocolate. For this study we used semisweet chocolate as the test matrix and increasing levels of naturally occurring phenolic compounds added to the chocolate as the compound of interest. This last study is particularly relevant to the confectionery industry today as research
continues to unfold the surprisingly positive potential for natural cocoa polyphenols in cardiovascular health (e.g. Corti, Flammer et al., 2009). Cardiovascular health, of course, continues to be of great concern to all, as it remains the leading cause of death worldwide (WHO, 2011). Consumer response to the increase in levels of natural cocoa polyphenols is of interest to those who manufacture and sell chocolate products, as these compounds elicit bitter and/or astringent sensations that are generally aversive to consumers at high intensities (Drewnowski and Gomez-Carneros, 2000).
Chapter 2
Rejection Thresholds in Chocolate Milk: Evidence for Segmentation

Adapted from

Abstract

Bitterness is generally considered a negative attribute in food, yet many individuals enjoy some bitterness in products like coffee or chocolate. In chocolate, bitterness arises from naturally occurring alkaloids and phenolics found in cacao. Fermentation and roasting help develop typical chocolate flavor and reduce the intense bitterness of raw cacao by modifying these bitter compounds. As it becomes increasingly common to fortify chocolate with ‘raw’ cacao to increase the amount of healthful phytonutrients, it is important to identify the point at which the concentration of bitter compounds becomes objectionable, even to those who enjoy some bitterness. Classical threshold methods focus on the presence or absence of a sensation rather than acceptability or hedonics. A new alternative, the rejection threshold, was recently described in the literature. Here, we sought to quantify and compare differences in Rejection Thresholds (RjT) and Detection Thresholds (DT) in chocolate milk spiked with a food safe bitterant (sucrose octaacetate). In experiment 1, a series of paired preference tests was used to estimate the RjT for bitterness in chocolate milk. In a new group of participants (experiment 2), we determined the RjT and DT using the forced choice ascending method of limits. In both
studies, participants were segmented on the basis of self-declared preference for milk or dark solid chocolate. Based on sigmoid fits of the indifference-preference function, the RjT was ~2.3 times higher for those preferring dark chocolate than the RjT for those preferring milk chocolate in both experiments. In contrast, the DT for both groups was functionally identical, suggesting that differential effects of bitterness on liking of chocolate products are not based on the ability to detect bitterness in these products.

Introduction

Bitterness is generally considered a negative attribute in food, yet many individuals enjoy some amount of bitterness in products such as coffee, beer, or dark chocolate. In chocolate, bitterness arises from naturally occurring alkaloids and phenolic compounds. Cacao undergoes many processing steps to develop the typical flavor and aroma of chocolate (Hoskin, 1994; Adriaenssens, 2010). Many of the crucial steps in processing, such as fermentation and heat treatments (e.g. roasting, conching) significantly reduce the intense bitterness of ‘raw’ cacao by transforming a large portion of the bitter compounds (via oxidation and condensation reactions). The standard processing that most chocolate undergoes today is a result of consumers’ sensitivity to and rejection of highly bitter products. Multiple studies suggest taste is the primary driver of consumers’ food selection behaviors, eclipsing cost, convenience and even potential health value (e.g. Glanz, Basil et al., 1998; IFIC, 2011).

Notably, many of the aversive bitter compounds found in chocolate have also been studied for their apparent cardiovascular health benefits (e.g. Corti, Flammer et al., 2009; Visioli, Bernaert et al., 2009; Djousse, Hopkins et al., 2011) – cacao is abundant in
potentially healthy compounds (e.g. the monomer epicatechin (flavan-3-ols) and its polymer, procyanidins) that elicit very bitter and also often astringent sensations (Drewnowski and Gomez-Carneros, 2000; Stark, Bureuther et al., 2006). However, the same processing that cacao undergoes to develop its well-loved flavor and aroma destroys up to 85% of these compounds (Visioli, Bernaert et al., 2009). For this reason, some manufacturers are working to develop processes to make chocolate that preserve more of these compounds, or alter formulations in an effort to boost the amount of these compounds in the finished product (Visioli, Bernaert et al., 2009). For example, Kealey and colleagues (2011) at Mars recently patented processes and formulations that preserve polyphenol content in chocolate. This provides a challenge in formulation, however, as these changes may significantly alter the sensory profile of the product in a way that is aversive to the consumer. To meet the demands of consumers for health enhancing products, it will be important to identify the point at which the concentration of bitter compounds in chocolate becomes objectionable, even to those who enjoy some bitterness.

The measurement of detection thresholds dates back to Fechner and the dawn of psychophysics 150 years ago (Engen, 1972; Duffy, Hayes et al., 2009a). Unfortunately, detection thresholds often fail to predict suprathreshold intensity (e.g. Bartoshuk, 1978; Frijters, 1978) or food liking (e.g. Duffy, Peterson et al., 2004; Lucas, Riddell et al., 2011). In the case of off flavors (taints) or for attributes where only low levels are desired, it is more critical to determine the point at which liking is adversely affected. The question becomes not can the consumer sense the attribute, but do they care? Recently, Prescott and colleagues elegantly adapted classical threshold methods to
directly address the question of acceptability of cork taint (TCA) in white wine (Prescott, Norris et al., 2005). Subsequently, this approach has been applied to eucalyptol in red wine (Saliba, Bullock et al., 2009), caffeine in coffee, citric acid in orange juice, and salt in beef broth (Lee, Prescott et al., 2008). Notably, rejection never reached 100% in any of these studies. Also, the statistical analysis strategies in these prior studies have been based on linear or segmental fits and binomial distributions tables for preference tests (Prescott, Norris et al., 2005; Lee, Prescott et al., 2008; Saliba, Bullock et al., 2009).

Here, we sought to quantify and compare differences in Rejection Thresholds (RjT) and Detection Thresholds (DT) across individuals who differed in their preference for solid chocolate (dark chocolate versus milk chocolate). The present study aimed to answer three questions. First, was it possible to reach universal rejection? Second, would solid chocolate preferences generalize to flavored fluid milk? Third, if segmentation was present in fluid milk, could it be explained by differences in bitterness detection thresholds? Finally, we sought to use a sigmoid fit in place of a linear fit to be consistent with the expected shape of the underlying psychometric function.

**Methods**

**Overview of Methods**

Chocolate milk was chosen as the model system, and sucrose octaacetate (SOA) was selected to be the model bitter compound. SOA is a highly bitter acetylated sugar that is generally recognized as safe (GRAS) by the FDA as a direct and indirect food additive (21 CFR 172.515 and 21 CFR 175.105, respectively), and it shows little or no evidence of genetic variation in humans (Boughter and Whitney, 1993; Hansen, Reed et
al., 2006). Compusense five software v5.2 (Guelph ONT) was used for sample randomization and data collection. All tests occurred in individualized test booths in the Sensory Evaluation Center in the Department of Food Science at Penn State. Procedures were exempted from Institutional Review Board review by the Penn State Office of Research Protections under the wholesome foods/approved food additives exemption in 45 CFR 46.101(b)(6). Participants provided informed consent and were paid for their time. In experiment one, we determined group rejection thresholds in 55 participants in a single session. In experiment two, we recruited a new group of participants and determined detection thresholds and rejection thresholds on separate days (80 recruits; 70 completers).

**Experiment One**

**Participants**

Fifty-five reportedly healthy, non-smoking individuals were recruited from the campus of and community around the Pennsylvania State University (State College, PA) via email for their willingness to consume chocolate milk. Forty-seven women and eight men, aged between 18 – 45 years participated in the study. Of these participants, 24 endorsed preferring dark chocolate and 31 endorsed preferring milk chocolate when asked about their preference when eating solid chocolate at the conclusion of the test session.
Stimuli

The chocolate milk samples were spiked with varying concentrations of SOA: 0 (blank), 3.2, 5.6, 10, 18, 32, and 56 μM. These concentrations were selected based on published threshold values for SOA in water (Boughter and Whitney, 1993), the median of which was 4μM (ranging from 0.25 – 16 μM across a unimodal distribution), and adjusted for milk via informal piloting at the lab bench. Each spiked sample was paired with a blank containing only chocolate milk. Samples (10mL) were pre-poured in small clear plastic soufflé cups, kept refrigerated until testing (4C), and served cold.

Procedure

This first test was a two-alternative forced choice (2-AFC) preference test carried out according to the American Society for Testing and Materials (ASTM) method E-2263 (ASTM, 2004). In this test, six pairs of samples were presented to each participant, with each pair containing a spiked and blank sample. The pairs were presented in order of ascending concentration, with three pairs on the first tray, and 3 pairs on the second tray. Within a pair, the order of the samples was randomized. The participants were asked to move through the sample pairs one at a time by tasting both of the samples in the pair and then indicating which of the samples in that pair that they preferred by clicking the appropriate button on the computer screen before moving on to the next pair of stimuli.

A ‘no preference’ option was not provided for two reasons: recent work suggests a ‘no preference’ option does not clarify an ambiguous 50/50 result as was previously argued (see Chapman & Lawless, 2005), and it would have complicated the present analysis unnecessarily. In contrast to earlier ‘identical sample’ studies on the incidence of
no preference, the rejection threshold model covers the entire range of the response function, beginning with samples that are essentially identical (e.g. below detection threshold) before progressing to samples that are obviously different. For more information on the recent debate over use of a ‘no preference’ option, the interested reader is referred to (Marchisano, Lim et al., 2003; Chapman and Lawless, 2005; Chapman, Lovelace et al., 2010; Ennis and Ennis, 2012).

**Experiment Two**

**Participants**

Eighty reportedly healthy, non-smoking chocolate consumers aged 18-45 were recruited as above (of the eighty, twenty five had participated in Experiment 1). Fifty-six women and twenty-four men participated in the detection threshold portion of the experiment (2A). Of these participants, 39 endorsed preferring dark chocolate and 41 endorsed preferring milk chocolate when asked about their preference when eating solid chocolate. The participants in this experiment were randomly assigned to two different groups, stratifying for chocolate preference (Group A, total n = 40, milk n = 20, dark n = 20; and Group B, total n = 40, milk n = 21, dark n =19).

Seventy of these individuals (52 women and 18 men) returned on another day for determination of SOA rejection thresholds in chocolate milk (Experiment 2B). Of these, 38 endorsed preferring dark chocolate and 32 endorsed preferring milk chocolate.
Stimuli

For experiment 2A (detection thresholds), the chocolate milk samples were spiked with increasing concentrations of SOA: 0.056, 0.18, 0.56, 1.8, 5.6, and 18 μM. For experiment 2B (rejection thresholds), the concentrations were revised based on Experiment 1, resulting in 1, 2.1, 4.6, 10, 21, and 46 μM samples. Each spiked sample was paired with a blank sample containing only chocolate milk. Ten mL samples were prepared and presented as above.

Procedure

In Experiment 2A, detection thresholds were determined in accordance with ASTM method E-679 Forced Choice Ascending Method of Limits (ASTM, 2011). Briefly, participants execute a series of triangle tests, where every triad of samples contains one spiked sample and two blank samples. The triads were presented in order of ascending concentration, and the order of samples within a triad was randomized. To limit fatigue, the participants were randomized into two groups, where each group tasted three of the six concentrations, as described in E-679. Therefore, each participant tasted three triads of samples total. Group A tasted the concentrations 0.056, 0.56 and 5.6 μM, while Group B tasted the concentrations 0.18, 1.8 and 18 μM. The participants were asked to taste each of the samples in the triad and indicate of the three samples tasted, which of the samples was different before moving on to the next triad. If the participants were unsure of their answer, they were forced to choose a sample; degree of certainty was not assessed. For Experiment 2B (rejection thresholds), the procedure was identical to that used in Experiment 1.
We considered using a 2-AFC task instead of a triangle test, as it is a more powerful method able to find smaller differences at the same number of participants (Ennis, 1993). However, after much discussion, we chose to use a triangle here instead of a 2-AFC to avoid having to specify a particular attribute, given the complex matrix we were working with. Specifically, adding SOA might alter the chocolate milk samples in attributes beyond bitterness. For example, perithreshold levels of the bitterant might reduce sweetness via mixture suppression (Lawless, 1979; Hayes, Wallace et al., 2011b), or increase perceived cocoa flavor via learned associations (Keast, 2008), without increasing perceived bitterness. Thus, we felt a 2-AFC was too narrow a task for this exploratory work, in spite of the additional power and sensitivity it provides over a triangle test.

**Statistical Analysis**

To determine the group rejection thresholds (RjT) in experiment 1 and 2B, we adapted the method described by Lawless (2010). That is, we plotted the proportion of responses (i.e., preference for the blank) against concentration and fit a curve through the points. The point at which performance exceeds chance by 50% can then be determined via Abbott’s formula (Lawless, 2010), resulting in chance corrected probabilities of 75% for 2AFC tasks. Instead of using OLS regression or a logit model as recommended by Lawless (2010), we chose to use a sigmoid model, as we expected preference to oscillate around chance until the attribute was not only detectable but objectionable, leading to a steep climb to an asymptote near universal preference. Specifically, we fit a sigmoid
variable slope dose-response function using the following four parameter logistic equation:

\[ Y = Min + \left( \frac{(Max - Min)}{1 + 10^{(\log RjT_{50} - X) \times HillSlope}} \right) \]

Adapted from the Hill equation commonly used in pharmacology, this model describes the top of the curve (max), the bottom of the curve (min), the spot halfway between min and max (classically known as the “effective concentration for 50% of the population tested” or EC\(_{50}\); called RjT\(_{50}\) here), and the slope of the curve (the Hill coefficient). Notably, the EC\(_{50}\) in the traditional Hill equation corresponds to the traditional definition of a sensory threshold used in psychophysics (Engen, 1972).

Here, we fit the rejection functions using GraphPad Prism 5.0C for OSX (GraphPad Software, San Diego CA) and RjT\(_{50}\) values were calculated directly in software. In experiment 1 and 2B, we compared the rejection thresholds between those preferring milk chocolate and those preferring dark chocolate via the ‘compare two data sets’ option in Prism, which generates an F-statistic and p-value for the difference between the EC\(_{50}\) values. Prism generates this statistic by fitting the data twice: once for each group separately (two curves), and once for a global fit (i.e. a common EC\(_{50}\)) across all of the data. Because the global model is nested within the two group (separate curve) model, the two models can be tested with a standard extra sum-of-squares F test (Motulsky, 2004). The equation for this F value is:
In experiment 1, the dose range did not include chance performance (near 50%) for one group, so we constrained the fit in Prism and refit the curve (described below). Finally, we used the ECx feature in Prism to determine the RjT value for both preference segments (i.e., the threshold where performance was 20% above chance.)

To determine the group detection thresholds in Experiment 2A, we again adapted the method of Lawless (2010). The proportion of individuals correctly identifying the odd sample in each triangle test was plotted against concentration. For this, we used linear regression to fit the data, after excluding the lower concentrations that oscillated around chance. The group detection threshold was defined as the concentration at which the chance corrected proportion was 66% (i.e., halfway between chance (0.33) and perfect performance (1.0)).

**Results**

**Experiment One**

The rejection threshold (RjT) reflects the concentration at which the participants preferred the un-spiked sample. As shown in Figure 2-1, as concentration increased, more participants chose the blank over the spiked sample, since bitterness is generally considered a negative attribute in food products. This was true here, as the participants unanimously rejected the spiked sample at the top two concentrations tested. Figure 2-1 also shows that the rejection thresholds for individuals who report preferring solid milk...
chocolate was noticeably lower than those who report preferring solid dark chocolate. Due to the lack of points near chance for the milk chocolate group, the original sigmoid fit had an ambiguous $R_{JT_{50}}$ value (see Supplemental Figure 2-1). Therefore, we constrained two of the four parameters in the Hill equation, minimum and maximum, to their theoretically indicated values of 0.5 and 1.0, and refit the curve. When we did so, the rejection threshold for the dark chocolate group was 2.37 times higher [$F(1.8)=25.1; p=0.001$] than the milk chocolate group (9.35 µM versus 3.95 µM, respectively). As a cross check, we also analyzed these data using OLS regression and determined the group thresholds at 75% of chance via the Lawless (2010) method; the values (not shown) were not substantially different from the results above.
Figure 2-1. Proportion of participants preferring the unspiked chocolate milk samples plotted against concentration of SOA in the spiked sample in Experiment 1.

At the highest concentrations (right side), the spiked samples were universally rejected by all participants. The squares (light brown) represent individuals who prefer milk chocolate when eating solid chocolate; the circles (dark brown) represent those who prefer dark chocolate. The concentration at which 50% of participants preferred the unspiked control (after correcting for chance) was substantially higher in those who prefer eating solid dark chocolate (p<.0001).
Supplemental Figure 2-1. Proportion of participants preferring the unspiked chocolate milk samples plotted against concentration of SOA in the spiked sample in Experiment 1 with an unconstrained fit.

The squares (light brown) represent individuals who prefer milk chocolate when eating solid chocolate; the circles (dark brown) represent those who prefer dark chocolate.
Experiment Two

As shown in Figure 2-2, the group detection thresholds (DTs) were functionally identical across both groups (5.86 uM versus 5.38 uM for dark and milk chocolate groups, respectively). Regarding rejection thresholds, Figure 2-3 shows that the participants almost unanimously rejected the spiked sample at the highest concentration tested. Extension of the concentration range downward relative to experiment 1 resulted in proportions near the theoretical minimum and maximum (0.5 and 1.0, respectively) at either end of the curve, allowing for an unconstrained fit. Similar to Experiment 1, the rejection thresholds for individuals who report preferring dark chocolate were 2.3 times higher \([F(1,4)=11.90; \ p=0.026]\) than those preferring milk chocolate. For completeness, the constrained and unconstrained RjTs from experiments 1 and 2 are summarized in Table 2-1.
Figure 2-2. Proportion of participants correctly identifying the spiked sample in a triangle test with two blanks in Experiment 2A.

Squares (light brown) indicate those who prefer solid milk chocolate while circles (dark brown) indicate those who solid dark chocolate. The detection thresholds for SOA in chocolate milk did not differ by group.
Figure 2-3. Proportion of participants preferring the unspiked chocolate milk samples plotted against concentration of SOA in the spiked sample in Experiment 2B.

Again, the concentration at which 50% of participants preferred the control was substantially higher (p<.05) in those who prefer eating solid dark chocolate.
**Table 2-1. Rejection Threshold Values for Each Group- from experiments 1 & 2B**

<table>
<thead>
<tr>
<th></th>
<th>RjT50</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk Chocolate Group</td>
<td>Dark Chocolate Group</td>
<td>Fold Difference</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td>3.95 (±1.11 SE)</td>
<td>9.35 (±1.14 SE)</td>
<td>2.37</td>
<td>0.001</td>
</tr>
<tr>
<td>(Constrained Fit)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2B</strong></td>
<td>3.90 (±1.22 SE)</td>
<td>9.00 (±1.18 SE)</td>
<td>2.30</td>
<td>0.026</td>
</tr>
<tr>
<td>(Unconstrained Fit)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The present work extends prior findings in several ways. First, we were able to demonstrate that it is possible to reach universal rejection (right side of Figures 2-1 and 2-3). We were also able to see clear segmentation based on preference for solid chocolate despite the fact that we were working with a model system of chocolate milk. This indicates that solid chocolate preference may generalize to preferences for other chocolate products. For those preferring dark chocolate, the rejection threshold was approximately 2.3 times higher than the rejection threshold for those preferring milk chocolate. In contrast, the detection threshold (DT) for both groups was functionally identical. This suggests that tolerance for bitterness in chocolate products is not based on differential ability to detect bitterness in those products. Finally, we were able to describe the dose-response function for bitterness in chocolate milk using a sigmoid model as opposed to the linear fits that had been used previously.

Although experiments 1 and 2 contained a few of the same participants, the consistency of the data across both experiments suggests these effects are robust and may generalize to other populations. In experiment 2B, we were able to clearly see the sigmoid shape of the entire function with the proportion preferring the control starting
near chance, and plateauing once the concentration reached a level that was objectionable to all (unlike prior work on rejection thresholds). Notably, while the detection thresholds from experiment 2 were slightly higher than the RjT for the milk chocolate groups in experiments 1 and 2, they were not significantly different as the DTs fell within the 95% confidence intervals for the milk chocolate group RjTs. In contrast, within each experiment, the dark chocolate group RjTs were significantly higher that those for the milk chocolate group. This result suggests that market segmentation in solid chocolate preference is not driven by differences in perception.

Use of a four parameter logistic equation (the Hill equation) to describe the group rejection function has multiple advantages over prior attempts to model rejection thresholds. First, unlike earlier examples that use binomial tables to determine where to put the rejection threshold (Prescott, Norris et al., 2005; Lee, Prescott et al., 2008; Saliba, Bullock et al., 2009), the cutoff of interest here does not change with the number of participants, facilitating comparisons across groups of different size. Also, this neatly avoids the issue that binomial models may not accurately reflect human choice behavior (see Chapman & Lawless, 2005). Second, it is theoretically attractive, as the expected psychometric function is a sigmoid, not linear. The use of a sigmoid captures the entirety of the relationship, where at levels around the detection threshold, we expect to see preference oscillate around chance (due to momentary differences in perception (see Chapman et al., 2010)), then climb steadily upwards as more people begin to reject the spiked sample at higher concentrations and eventually plateau at universal rejection. Third, using the Hill equation precludes the need to adjust for chance via Abbott’s formula, as the definition of EC_{50} is the halfway point between chance and perfect
performance (e.g. the classical definition of a threshold (Engen, 1972)). Fourth, it appears to be robust to small errors in dose-finding (e.g. Experiment 1) as the minimum and maximum values are easily constrained to their theoretical values. Fifth, this type of model allows for the calculation of an ‘acceptable concentration limit’ (ACL) for any desired percentage of the respondents (i.e., $R_{jT_x} / \text{EC}_x$). For example, based on the results of experiment 2B, the $R_{jT_{20}}$ for the milk chocolate preferring group is 2.175 $\mu$M and for the dark chocolate preferring group is 4.325 $\mu$M. This may be an advantage in quality assurance or brand management situations where one might want to be more conservative than a binomial distribution table, by setting the proportion of participants rejecting a product at a lower value.

This technique is most applicable for attributes that show a monotonic liking function. Here, we show a typical example of this by using bitterness, which shows a sigmoid relationship as a function of concentration. In contrast, this method may not work for stimuli that have a clear optimum. When considering an attribute with an optimum, one could plot the proportion preferring the spiked sample, which would result (initially) in a curve similar to those seen here. However, once the optimum was exceeded, the proportion preferring the spike would drop back toward chance as the respondents then switch their preference back to the blank sample. While it would be possible to analyze the data up to the point of optimum by treating the optimum as a local maximum, difficulty in fitting the complex curve shape would limit the utility of this approach, especially if the drop was rapid, resulting in an asymmetric curve. Thus, this approach is best suited to negative attributes whose physicohedonic (concentration-loving (Hayes and Duffy, 2008)) function is monotonic. Notably, this method could also be used
for variables other than physical concentration, where one might also expect to see a monotonic relationship. For example, it might be possible to test for shelf life, with time on the x-axis.

The concept of a preference threshold has been used in animal psychophysics since the 1930’s (e.g. Richter, 1936). However, the rejection threshold method here is quite different in spite of the similar name and goals. In a preference threshold task, an animal is allowed to feed from two bottles. When faced with two bottles that are indistinguishable, the animals will drink from both bottles equally. This is similar to what we see as respondents choose a sample randomly when the levels of the spike are below the detection threshold. In a preference task, the animal is then presented an ascending series of concentrations paired against a blank. At the end of each session, the amount consumed from each bottle is measured, and relative preference is inferred from the amount consumed. Thus, the preference threshold is defined as the concentration at which the animal consumes significantly more of one solution than the other (Richter, 1936). However, this method implicitly conflates the ability to tell a difference with a preference (e.g. Fregly, 1973). That is, an animal (or nonverbal humans) could conceivably be able to distinguish between stimuli while remaining indifferent. In adult humans, we have the luxury of defining the task more narrowly, as was done here. Thus, while the two approaches have similar goals, they have different underlying assumptions. Finally, while prior reports have referred to the present technique as a ‘consumer rejection threshold’, we suggest dropping the prefix consumer in the future, as the technique need not be limited to consumer-oriented research. For example, it could be
used to characterize different hedonic phenotypes in genetic research (e.g. Duffy, Hayes et al., 2009a; Duffy, Hayes et al., 2009b).

**Limitations and Conclusions**

Here, we used a triangle test method to determine the group detection threshold using ASTM method E-679, in spite of the fact that n-AFC tasks are more powerful than triangle tests (Ennis, 1993). The triangle method and the n-AFC methods require different cognitive strategies on the part of the participant: in a triangle test, the participant must pick out the odd sample, whereas in the n-AFC approach, participants can skim across the samples, seeking to identify the weakest or strongest sample. Thus, the n-AFC approach is an easier cognitive task, which makes it more sensitive with the same number of participants and samples. (Astute readers may note ASTM E-679 refers to 3-AFC in places; however, this refers solely to the sample presentation – one test and two blanks – and not the cognitive strategy to be used. The E-679 method explicitly indicates panelists should identify the different sample.) As noted by an anonymous reviewer, the rejection threshold task used here is much more analogous to a directed n-AFC task than a triangle test, so it may have been more appropriate to determine our detection thresholds using a 2-AFC or 3-AFC task for a more direct comparison. When designing our study, we decided against this experimental approach for the reasons outlined in section 2.2.3, but we agree that additional work is needed to explore how the results might differ with an alternative detection threshold method. Because the group detection threshold may be lower with a more sensitive task, present results may be overly conservative, underestimating the gap between detection and rejection thresholds. In addition, more
work is needed to determine whether inclusion of a ‘no preference’ option alters rejection threshold estimates.

Another potential limitation to the study is the study population. The sample was limited to consumers from central Pennsylvania, the majority of whom were women. More work is needed to explore any potential sex differences. Also, SOA is not an endogenous compound found within chocolate. With regard to validation of the method itself, this is not an issue, but inferences about the absence of perceptual differences in chocolate bitterness should not be made from present data; it remains possible that endogenous bitter compounds found in chocolate could exhibit genetic variation in perception (e.g. Hayes, Wallace et al., 2011a). Here, SOA was chosen for its ease of acquisition and use, potency, and GRAS status. We also note that “which do you prefer” and “which do you reject” are two different cognitive tasks. Here, we only asked our participants which sample they preferred, to be consistent with prior studies. Additional work is needed to verify that the curves obtained under both tasks would be symmetric. Finally, further work is also needed to see if this method will be effective for quantifying segmentation in solid products, as all work to date on this method have occurred in liquid food systems. In conclusion, we demonstrated that rejection thresholds can be used to study market segmentation and that the differences that we observed in rejection thresholds were not due to differences in the ability of our participants to detect the bitter compound.
Acknowledgements

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Chapter 3

Rejection Thresholds in Solid Chocolate-Flavored Compound Coating

Adapted from


Abstract

Classical detection thresholds do not predict liking, as they focus on the presence or absence of a sensation. Recently however, Prescott and colleagues described a new method, the rejection threshold, where a series of forced choice preference tasks are used to generate a dose-response function to determine hedonically acceptable concentrations. That is, how much is too much? To date, this approach has been used exclusively in liquid foods. Here, we determined group rejection thresholds in solid chocolate-flavored compound coating for bitterness. The influences of self-identified preferences for milk or dark chocolate, as well as eating style (chewers versus melters) on rejection thresholds were investigated. Stimuli included milk chocolate-flavored compound coating spiked with increasing amounts of sucrose octaacetate (SOA), a bitter GRAS additive. Paired preference tests (blank vs. spike) were used to determine the proportion of the group that preferred the blank. Across pairs, spiked samples were presented in ascending concentration. We were able to quantify and compare differences between two self-identified market segments. The rejection threshold for the dark chocolate preferring group was significantly higher than the milk chocolate preferring group (p = 0.01). Conversely, eating style did not affect group rejection thresholds (p = 0.14), although this
may reflect the amount of chocolate given to participants. Additionally, there was no
association between chocolate preference and eating style (p = 0.36). Present work
supports the contention that this method can be used to examine preferences within
specific market segments and potentially individual differences as they relate to ingestive
behavior.

**Practical Application**

This work makes use of the rejection threshold method to study market
segmentation, extending its use to solid foods. We believe this method has broad
applicability to the sensory specialist and product developer by providing a process to
identify how much is too much when formulating products, even in the context of
specific market segments. We illustrate this in solid chocolate-flavored compound
coating, identifying substantial differences in the amount of acceptable bitterness in those
who prefer milk chocolate versus dark chocolate. This method provides a direct means to
answer the question of how much is too much.

**Introduction**

Recently, Prescott and colleagues conceptualized a new psychophysical method,
the rejection threshold (RjT), that combines both dose-response and preference measures
(Prescott, Norris et al., 2005). In this method, a forced choice preference task is used to
generate a dose-response function to determine acceptable concentrations. This method
avoids the disconnect between detection thresholds and hedonics. Detection thresholds
frequently fail to predict suprathreshold intensity (Bartoshuk 1978; Frijters 1978) or food
liking (Duffy, Peterson et al., 2004; Lucas, Riddell et al., 2011). This suggests trying to predict product acceptability via detection thresholds is likely to fail. For example, even if I can detect a hint of cork taint in a wine, the amount of cork taint may not be sufficient to make the wine objectionable (see Prescott and others 2005). Using a detection threshold to try to determine acceptability ignores this subtle but important distinction. Thus, instead of making this flawed assumption, the rejection threshold method directly assesses ‘how much is too much?’ by asking the participant to make a hedonic judgment.

To date, all applications of rejection thresholds have been in liquid food systems (Prescott, Norris et al., 2005; Lee, Prescott et al., 2008; Saliba, Bullock et al., 2009; Harwood, Ziegler et al., 2012a). Here, we apply this method in a solid food system.

Rejection thresholds have been successfully used to investigate group rejection thresholds based on different market segments (Harwood, Ziegler et al., 2012a) as well as differences in PROP taster status (Lee, Prescott et al., 2008). These studies suggest that the rejection threshold method may be a powerful tool for the investigation of other individual differences that influence preference and potentially ingestive behavior.

For chocolate products, one behavior of particular interest is eating style. Rates of mastication and differences in melting have the ability to influence flavor release and overall perception of a product (Wilson and Brown, 1997; Afoakwa, Paterson et al., 2007). A recent study characterized chocolate eating patterns, using cluster analysis to identify three main behaviors relating to mastication and swallowing that occur when people eat chocolate (Carvalho-da-Silva, Van Damme et al., 2011). Variation in eating style could potentially alter the perception of and therefore rejection or acceptance of
chocolate products. Here, we explore this possibility without advancing any specific a priori hypotheses.

The objective of this study was to investigate the effects of self-identified preference for milk or dark chocolate as well as eating style on group rejection thresholds for bitterness in solid chocolate-flavored compound coatings. Our aim was to quantify and compare differences between these self-declared groups. A secondary goal of this study was to confirm the utility of this method for use with solid samples.

Materials and Methods

Overview of Methods

Commercially available solid milk chocolate compound was chosen as the sample matrix for ease of sample preparation as chocolate-flavored compound coating does not require tempering before molding. (Chocolate differs from compound coating in regard to the fat source; where cocoa butter must be used in chocolate, compound coating can be made from other fats with similar melting characteristics such as palm kernel oil. We will refer to this material as solid milk chocolate compound for the rest of the article.) Sucrose octaacetate (SOA) was used as a model bitter compound as it is highly bitter and generally recognized as safe (GRAS) as both a direct and indirect food additive (21 CFR 172.515 and 21 CFR 175.105). The tests were carried out in individual booths in the Sensory Evaluation Center in the Department of Food Science at Penn State. Procedures were exempted from Institutional Review Board review by the Penn State Office of Research Protections under the wholesome foods/approved food additives exemption in 45 CFR 46.101(b)(6).
Participants

Eighty-five individuals (22 men) aged between 18 – 45 years old who were reportedly healthy, non-smoking, chocolate consumers participated in the study. The participants were recruited from the campus and community surrounding the Pennsylvania State University via email. All participants provided informed consent and were paid for their time.

Stimuli

Solid milk chocolate compound was obtained from a commercial source (Chocoley.com), melted down, spiked with varying concentrations of SOA, molded, and stored for 2 weeks at ambient temperature (23°C) before the test. The concentrations of SOA used were 0 μM (blank), 7.5 μM, 15 μM, 30 μM, 60 μM, and 120 μM. These concentrations were chosen based on rejection thresholds for SOA reported in chocolate milk (Harwood, Ziegler et al., 2012a) and adjusted for solid chocolate via informal bench testing. Samples were 10mm x 5mm x 13mm, and ~0.63g each (Figure 3-1). Each individual sample was presented as one piece to prevent re-tasting, in a small, clear plastic cup with a lid that was labeled with a random three-digit blinding code. All samples were served at ambient temperature under white light.
Figure 3-1. Sample Dimensions

**Shape and Measurements:** One sample piece is shown from above and another is shown from a side-view with references for the dimensions of the samples, which are 10 x 13 x 5 mm. Each sample piece was approximately 0.63g.

**Procedures**

A Two-Alternative Forced Choice (2-AFC) test was performed to measure preference of the spiked samples compared to the blank samples (rejection thresholds). This test was carried out in accordance with American Society for Testing and Materials (ASTM) standard method E-2263 (ASTM, 2004). Each participant was served five pairs of samples total, each pair containing a blank and a spike. Pairs were presented in order of ascending concentration. Sample order within a pair was randomized to avoid order effects. All of the samples were presented on a single tray, and the participants were asked to taste from left to right and front to back, tasting one pair at a time and indicating on the computer which of the two samples they preferred before moving on to the next.
pair. Compusense *five* software v5.2 (Guelph, ONT) was used for sample randomization and data collection. As discussed previously, a ‘no preference’ option was not provided (Harwood, Ziegler et al., 2012a).

At the conclusion of the test session, the participants were then asked to indicate their preference for milk or dark chocolate. They were also asked to identify the best descriptor of their chocolate eating style from a list of three descriptions. The eating style descriptions were based on the results of a recent study on chocolate eating styles by Carvalho-da-Silva and colleagues (2011). Participants were asked to select one of the following descriptors: ‘I chew the chocolate until smooth before swallowing,’ ‘I chew the chocolate quickly,’ or ‘I suck on/melt the chocolate in my mouth before chewing/swallowing’.

**Statistical Analysis**

To determine the group rejection thresholds, we used the sigmoid curve fitting approach described previously (Harwood, Ziegler et al., 2012a), with the minimum and maximum values constrained to their theoretical values (0.5 and 1.0, respectively). To investigate the breakdown of participants into eating style groups, Fisher’s exact test (2-tailed) was performed based on eating style and preference for milk or dark chocolate.

**Results**

The rejection thresholds for the group as a whole, as well as for preference groups and each eating style group are summarized in *Table 3-1*. As expected, as the concentration of SOA increased, the rejection of the spiked samples also increased (see
Figures 3-2 and 3-3). Across all 85 participants, 43 reported preferring milk chocolate and 42 reported preferring dark chocolate. There was a significant difference between the rejection thresholds based on preference, where the dark chocolate preferring group had a RjT significantly higher than the milk chocolate preferring group \[F(1,6)=13.37; \ p=0.0106\] (see Figure 3-2).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Rejection Threshold</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Participants</td>
<td>85</td>
<td>81.5 μM (±1.12 SE)</td>
<td>n/a</td>
</tr>
<tr>
<td>Milk Chocolate</td>
<td>43</td>
<td>43.9 μM (±1.21 SE)</td>
<td></td>
</tr>
<tr>
<td>Dark Chocolate</td>
<td>42</td>
<td>113.5 μM (±1.07 SE)</td>
<td>0.011a</td>
</tr>
<tr>
<td>Thorough Chewers</td>
<td>45</td>
<td>70.0 μM (±1.13 SE)</td>
<td></td>
</tr>
<tr>
<td>Quick Chewers</td>
<td>8</td>
<td>–</td>
<td>0.144</td>
</tr>
<tr>
<td>Melters</td>
<td>32</td>
<td>93.3 μM (±1.10 SE)</td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant across the respective groups.

Among those preferring milk chocolate, 26 were thorough chewers, 2 were quick chewers, and 15 were melters; for those preferring dark chocolate, the respective numbers were 19, 6, and 17. A Fisher’s exact test suggested there was no association between milk/dark preference and self identified eating style \(p=0.36\). When comparing the thorough chewers and the melters, there were no significant differences in the rejection thresholds based on the identified eating style \[F(1,6)=2.819; \ p=0.1441\]. A rejection threshold was not determined for the quick chewers due to the low number of participants in this group \(n=8\).
Figure 3-2. Group Rejection Thresholds Based on Preference for SOA in Solid Chocolate

Proportion of participants preferring the un-spiked samples is plotted against concentration of SOA in the spiked sample. The circles (light brown) represent individuals who prefer milk chocolate when eating solid chocolate; the squares (dark brown) represent those who prefer dark chocolate. The chance-corrected concentration at which 50% of participants preferred the control, the rejection threshold, was significantly higher for those who prefer dark chocolate (p = .01).
Figure 3-3. Group Rejection Threshold of all Participants for SOA in Solid Chocolate

Proportion of participants preferring the un-spiked samples is plotted against concentration of SOA in the spiked sample. The concentration at which 50% of participants preferred the control after correcting for chance represents the rejection threshold. The rejection threshold for the entire sample combined (n = 85) was 81.4 μM.

Discussion

The present study successfully utilized the rejection threshold to investigate the effects of self-identified preference for milk/dark chocolate as well as eating style on
group rejection thresholds for bitterness in solid chocolate compound. Our aim was to quantify and compare differences between these self-identified groups.

We were able to successfully employ the rejection threshold method in solid chocolate compound, satisfying the secondary aim of this work. Consistent with the reported rejection thresholds for SOA in chocolate milk (Harwood, Ziegler et al., 2012a), we saw that the RjT for the dark chocolate preferring group was approximately 2.58 times higher than that of the milk chocolate preferring group, a significant difference indicating that those who prefer dark chocolate are much more tolerant of bitterness in chocolate products. Although the current study did not specifically test for differential ability to detect SOA, it seems unlikely that this would explain the difference in rejection thresholds across the two groups. In fluid milk, we observed a similarly sized difference (~2.3 fold) in rejection threshold between the two groups, in spite of the groups having detection thresholds that were functionally identical (Harwood, Ziegler et al., 2012a). This indicates the increased tolerance for bitterness is not dependent on differences in sensory ability. Additionally, the group RjTs were much higher in the solid chocolate product (here, 43.9 and 113.5 μM) compared to fluid milk (3.95 and 9.00 μM) (Harwood, Ziegler et al., 2012a), as might be expected as solid chocolate is a more complex stimuli. These data suggest the slope of the indifference-rejection function for bitterness is much shallower in solid chocolate than in chocolate milk.

We failed to find any evidence that rejection threshold varies across eating style, although this may be result of the size of our samples. Because our samples were quite small (~0.63g), they effectively melted as soon as they were placed in the mouth. Thus, while useful in removing any potential confound from eating style in the present data,
caution should be taken in generalizing to larger samples. It remains possible that given larger pieces, there may be more differences in perception and therefore rejection thresholds may vary with eating style. Here, we deliberately used small samples to prevent re-tasting.

Present work partially confirms the findings of Carvalho-da-Silva and colleagues (2011) on chocolate eating styles. They found approximately half of their participants were quick chewers, with smaller numbers of thorough chewers (~36%) and melters (~15%). In our participants, thorough chewers were the largest group (53%), followed by melters (38%) and quick chewers (9%). Whether this discrepancy results from classification method (EMG/EEG versus self report), chocolate size and/or type, or culture (UK versus US) is unclear. In particular, the higher number of melters in our study may be an artifact of the small amount of chocolate we provided. One potential limitation of this study is the fact that eating style was determined via self-report. Differing amounts of self-awareness while eating, as well as difficulty of characterizing one’s own behavior, may have been limiting factors. Here, only three options were provided; hopefully, these choices adequately described all potential eating styles. Also, asking participants to describe their eating style after consuming 10 small samples of chocolate may have altered their response pattern; whether these self-identified styles are stable over time is unknown. Another potential limitation relates to the size of our stimuli; as noted above, we cannot speculate on whether eating style might influences rejection thresholds when larger samples are provided.
Conclusion

This work further validates the use of rejection thresholds to study market segmentation, and extends it to solid foods. We believe this method has broad applicability to the sensory specialist and product developer by providing a means to identifying how much is too much when formulating products. This method could also be used in more basic research to study individual differences as they relate to ingestive behavior.

Acknowledgments

This manuscript was completed in partial fulfillment of the requirements for a Master of Science degree at the Pennsylvania State University by MLH. The authors would like to thank Samantha Bennett, Nadia Byrnes, Alissa Allen, and Elise Boretz for their assistance in sample preparation and data collection. We also thank our study participants for their time and participation.

MLH is supported by funds from the Pennsylvania Manufacturing Confectioners’ Association (PMCA) and JEH and GRZ receive support from the Pennsylvania State University. JEH received additional support from National Institutes of Health grant number DC010904.
Chapter 4
Explaining Tolerance for Bitterness in Chocolate Ice Cream Using Solid Chocolate Preferences

Adapted from

Abstract
Chocolate ice cream is commonly formulated with higher sugar levels than non-chocolate flavors to compensate for the inherent bitterness of cocoa. Bitterness, however, is an integral part of the complex flavor of chocolate. In light of the global obesity epidemic, many consumers and health professionals are concerned about the levels of added sugars in foods. Once a strategy for balancing undesirable bitterness and health concerns has been developed, the task becomes determining whether that product will be acceptable to the consumer. Thus, the purpose of this research was to manipulate the bitterness of chocolate ice cream in order to examine how this influences consumer preferences. The main goal of this study was to estimate group rejection thresholds for bitterness in chocolate ice cream, and to see if solid chocolate preferences (dark versus milk) generalized to ice cream. As anticipated, the group containing individuals who prefer milk chocolate had a much lower tolerance for bitterness in their chocolate ice cream compared to the group of individuals who prefer dark chocolate; indeed, the dark chocolate group tolerated almost twice as much added bitterant to the ice cream before indicating a significant preference for the unspiked (control) ice cream. This work
demonstrates the successful application of the rejection threshold method to a complex
dairy food. Estimating rejection thresholds could prove to be an effective tool for
determining acceptable formulations or quality limits when considering attributes that
become objectionable at high intensities.

**Introduction**

Chocolate is an extremely complex flavor, to which a degree of bitterness is
essential for an acceptable overall flavor profile (Harwood, Ziegler et al., 2013). Making
a chocolate ice cream, therefore, can be complicated, as ice cream contains high levels of
fat and sugar which have the ability to significantly decrease perceived bitterness. Fat can
influence perceived bitterness as bitter compounds may partition away from the aqueous
phase of an emulsion and therefore become less available to act on taste receptors (e.g.
Metcalf and Vickers, 2002). However, this effect may vary from compound to compound
(e.g. Keast, 2008; Bennett, Zhou et al., 2012), as great structural diversity exists among
compounds that elicit bitterness via human bitter taste receptors (Meyerhof, Batram et al.,
2010). Additionally, crystallinity of the fat can also influence the partitioning of small
particles into or out of the aqueous phase (Ghosh, Peterson et al., 2007).

Sweetness perceptually masks bitterness in the central nervous system (Lawless,
1979). It has been shown that there is an optimum level for sugar in vanilla ice cream at
about 13.5% (Guinard, Zoumas-Morse et al., 1996). In chocolate ice cream however,
sugar levels are generally increased compared to other ice cream flavors in order to
compensate for the bitter compounds inherently present in chocolate (Prindiville,
Marshall et al., 1999; Marshall, Goff et al., 2003). It has been recommended to increase
sweetener by using equal weights of sucrose (or equivalent sweetener) and cocoa in addition to the initial amount of sweetener to achieve an optimal balance of sweetness and bitterness (Marshall, Goff et al., 2003). While this may seem like an effective strategy for maintaining flavor balance, levels of added sugar in foods are of great concern in the United States today as the obesity epidemic is on the rise (Ogden, Carroll et al., 2012). Moreover, consumers are becoming increasingly aware of and concerned about the amount of sugar in the foods that they choose to eat, and approximately 56% of Americans have reported trying to limit sugars in their diet (IFIC, 2011). For the production of ice cream in particular, strategies to limit sugar may be especially challenging for producers to meet consumer demand since sugar is integral for the perceived creaminess (Stampanoni, 1993; Guinard, Zoumas-Morse et al., 1997), quality, and acceptance of ice cream products (Guinard, Zoumas-Morse et al., 1996). Additionally, several processing parameters (i.e. freezing point) are highly dependent on the sugar content of the final product and thus the reduction of sugar can lead to manufacturing complications.

A further potential influencing factor on the balance of bitterness in chocolate ice cream is the temperature at which ice cream is served. Both hot and cold serving temperatures, for example, have been demonstrated to have the ability to significantly enhance or decrease the perceived intensity of some sweeteners, though the effect sizes are small (Schiffman, Sattely-Miller et al., 2000). Paulus and Reisch (1980) investigated detection, recognition, and terminal thresholds for the five prototypical tastes, finding that for bitter tastants (specifically quinine and caffeine) both cold and hot temperatures elevated the amount of stimulus required to be present for detection and recognition
thresholds, with the effect much greater at higher temperatures. While these effects may not generalize to supra-threshold concentrations or different bitter compounds, it remains possible that serving temperature may affect the perceived bitterness of ice cream.

It is important to consider that the degree of bitterness that is desirable in chocolate products may differ largely from consumer to consumer as dictated by preferences for milk or dark chocolate (e.g. Harwood, Ziegler et al., 2012a, b). Additionally, it is unknown whether or not preference for vanilla or chocolate ice cream is influenced by preference for milk or dark chocolate when considering an individual’s solid chocolate preferences. Recently, we demonstrated that bitterness tolerance in chocolate flavored fluid milk can be explained, in part, by solid chocolate preferences, as dark chocolate preferring individuals much more tolerant of bitterness than those who prefer milk chocolate (Harwood, Ziegler et al., 2012a). It seems reasonable to ask whether this pattern also generalizes to ice cream. Here, our main objective was to investigate if rejection thresholds for added bitterness in chocolate ice cream are influenced by individual preference for milk or dark solid chocolate.

**Materials and Methods**

**Treatments**

In ice cream, as sugar content is decreased, significant textural and flavor changes occur. After pilot testing, it became clear that while some of these textural aspects may be overcome by the addition of bulking agents, the difference was still large enough that preferences could be affected regardless of differences in flavor. Therefore, in order to study how the balance of bitter flavor affects preferences in chocolate ice cream, it was
decided that the most effective way to manipulate the bitterness while holding all other sensory attributes constant would be to add small amounts of a highly potent bitter compound. Therefore, application of the findings from this study should be cautiously interpreted when examining bitterness in chocolate ice cream caused by other bitter agents. Sucrose octaacetate (SOA) (SAFC, Lenexa, KS) was chosen as the added bitter ingredient because it is strongly bitter at micro-molar (µM) concentrations, and generally recognized as safe as a direct and indirect food additive (21 CFR 172.515 and 21 CFR 175.105, respectively). The control samples were plain chocolate ice cream. The spiked samples contained varying amounts of sucrose octaacetate, with final concentrations of 10, 20, 40, 80, and 160 µM. For example, to make the 160 µM sample, 3.1513 g of SOA was added to 32 kg of ice cream mix (see below).

**Ice Cream Processing**

The ice cream formulation used was: 14.1% fat (added as cream and whole milk), 10.0% non-fat milk solids (added, in part, as non-fat dry milk, Maryland and Virginia Milk Producers Association, Inc., Reston, VA), 13% sugar (Good Food Inc., Honey Brook, PA), 3.7% corn syrup solids (Tate and Lyle, Decatur, IL), 3% cocoa powder (Forbes, Broadview Heights, OH), 0.5% stabilizer/emulsifier blend (CREMODAN IcePro, Danisco DuPont, St. Joseph, MO). Three samples of each treatment and control were analyzed for total solids and fat on finished product using CEM SMART Trac (Mathews, NC).

Samples were formulated and manufactured to the formulation described above at the Berkey Creamery at the Pennsylvania State University. Ice cream mix was batched
and pasteurized at 178.5°F for 43s on a HTST pasteurizer system and then held at 4°C until mix was frozen. Immediately prior to freezing, ice cream mix was weighed into separate batches and spiked with the appropriate amount of SOA (in 50mL of ethanol for ease of dispersion) into the mix. Ice cream mix was frozen on a Gram GIF 400 continuous freezer (Gram Equipment of North America, Tampa, Florida) with overrun set to 80%. Calculations from ice cream mix and finished product yielded an 82.6% overrun across all samples. Samples were packaged into 4 oz. plastic cups (Airlite Plastics, Omaha, NE) on an automatic filling system (T.D. Sawvel Co. Inc., Maple Plain, MN). All ice creams were then stored at 5°F for one week prior to serving. Before being served to panelists, samples were tested for total coliforms and total aerobic plate count according to standard methods (Wehr, Frank et al., 2004).

**Sensory Testing Procedures**

Individuals were recruited from the Penn State community via email, and screened for their willingness to eat chocolate ice cream. Ninety-six reportedly healthy, non-smokers (thirty-one men) participated in the study. Half of the participants were between 18 and 24 years of age, and the remaining half were between 25-45 years old. Recruitment aimed for equal numbers of participants with a stated preference for solid milk chocolate and solid dark chocolate. Forty-six participants indicated that they preferred milk chocolate and fifty indicated that they preferred dark chocolate when asked at the conclusion of the test (see *Table 4-1*). All participants provided informed consent and were paid for their time. Procedures were exempt from Institutional Review Board review by the Penn State Office of Research Protections under the wholesome
foods/approved food additives exemption in 45 CFR 46.101(b)(6). All sensory data were collected using Compusense *five* software v5.2 (Guelph, ONT).

Rejection Thresholds were measured using a series of Two-Alternate Forced Choice (2-AFC) paired preference tests. Samples were presented as blinded pairs (a control and a sample spiked with SOA), and the participants were asked to indicate which of the two samples they preferred; a ‘no preference’ option was not given (see (Harwood, Ziegler et al., 2012a)). The pairs were presented in order of ascending SOA concentration (increasing bitterness). Within the pairs, the order of the samples was randomized to prevent order biases. All tests were carried out in individual testing booths at the Sensory Evaluation Center at Penn State under white lighting. Each sample pair was presented as needed through a serving hatch by laboratory staff in order to prevent melting of the samples and consistency of tasting temperature. Participants were instructed to rinse with room temperature (21°C) water between samples. All samples were presented between -12 and -15°C in 4 oz. plastic cups labeled with random three-digit blinding codes. At the conclusion of the 2-AFC tests, participants were asked to indicate their preference for solid milk chocolate or solid dark chocolate, chocolate or vanilla ice cream, gender, and age.

*Table 4-1. Breakdown of participants by gender and solid chocolate preference*

<table>
<thead>
<tr>
<th></th>
<th>Total n</th>
<th>Number of Men</th>
<th>Number of Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Participants</td>
<td>96</td>
<td>31</td>
<td>65</td>
</tr>
<tr>
<td>Milk Chocolate Preference</td>
<td>46</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>Dark Chocolate Preference</td>
<td>50</td>
<td>13</td>
<td>37</td>
</tr>
</tbody>
</table>
Statistical Analysis

Rejection thresholds were analyzed using the procedure outlined previously (Harwood, Ziegler et al., 2012a), with participants separated into groups based on their self-reported preference for solid chocolate (milk chocolate or dark chocolate) and ice cream (chocolate or vanilla). Fisher’s exact test was used to investigate effects of gender on preference groups, as well as stated preference for solid chocolate (milk versus dark), and preference for vanilla or chocolate ice cream.

Results and Discussion

As expected, the dark chocolate preferring group had a significantly higher rejection threshold (RjT) for SOA in the chocolate ice cream than the group preferring milk chocolate, with a concentration approximately 2 times higher (see Table 4-2). This result (both the concentration of SOA required for rejection as well as the separation between milk and dark chocolate preferring groups) corresponds with what has been shown previously for the same compound (SOA) in chocolate flavored fluid milk and solid milk chocolate flavored compound coating (Harwood, Ziegler et al., 2012a, b). The rejection thresholds for SOA in chocolate ice cream fall approximately halfway between those observed previously for chocolate milk and compound chocolate; this was expected, as the sugar and fat content of chocolate ice cream falls between the levels in these products (see Table 4-3).
Table 4-2. Group Rejection Thresholds for SOA in Chocolate Ice Cream

<table>
<thead>
<tr>
<th>Preference Group</th>
<th>Concentration of SOA</th>
<th>Fold Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Chocolate</td>
<td>14.17 μM (±1.00 SE)</td>
<td>1.93x</td>
<td>0.0002</td>
</tr>
<tr>
<td>Dark Chocolate</td>
<td>27.42 μM (±1.04 SE)</td>
<td>1.45x</td>
<td>0.0039</td>
</tr>
<tr>
<td>Vanilla Ice Cream</td>
<td>14.6 μM (±1.09 SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate Ice Cream</td>
<td>21.1 μM (±1.03 SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Participants</td>
<td>20.2 μM (±1.01 SE)</td>
<td></td>
<td>n/a</td>
</tr>
</tbody>
</table>

While we did not design this series of experiments to specifically pull apart the effects of temperature, fat, or sugar, our results are likely due to the differences in sugar content of the different sample matrices. It seems likely that the bitterness of SOA was masked by the sugar via the process of mixture suppression. Mixture suppression is a commonly observed perceptual phenomenon in taste where the sensation of a mixture is less than the sensations that would be elicited from the stimuli when experienced separately (Lawless, 1979; Keast and Breslin, 2003). Alternatively, if the RjTs were a function of temperature rather than sugar content, they would be in a different order than what was observed across these experiments (present data; Harwood et al., 2012a, b). That is, if cold temperature was suppressing the bitterness, the ice cream would have the highest RjT followed by the chocolate milk and then finally the compound chocolate would have the lowest RjT.
Table 4-3. Sugar and Fat Content of Previously Investigated Samples and Corresponding RjTs

<table>
<thead>
<tr>
<th></th>
<th>% Sugar</th>
<th>% Fat</th>
<th>RjT Milk Chocolate Preferring</th>
<th>RjT Dark Chocolate Preferring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate Milk</td>
<td>10%</td>
<td>2%</td>
<td>3.9 μM</td>
<td>9.0 μM</td>
</tr>
<tr>
<td>Chocolate Ice Cream</td>
<td>13%</td>
<td>14.1%</td>
<td>14.2 μM</td>
<td>27.4 μM</td>
</tr>
<tr>
<td>Solid Milk Chocolate Flavored Compound Coating</td>
<td>55%</td>
<td>31%</td>
<td>43.9 μM</td>
<td>113.5 μM</td>
</tr>
</tbody>
</table>

However, this interpretation does not preclude the possibility that we are observing a physiochemical effect rather than a perceptual interaction due to partitioning into the fat phase. Specifically, increasing fat levels can decrease the bitterness of some stimuli (Metcalf and Vickers, 2002). Metcalf and Vickers (2002) demonstrated a reduction in the bitterness of quinine sulfate by oil in water emulsions with a concentration dependent effect; more fat in the system resulted in greater reduction of bitterness. They hypothesized this was due to the lipophilic nature of quinine sulfate, causing a portion of it to partition into the fat phase of the emulsion, thereby rendering it unavailable to taste receptors. Contrary to these findings, however, Keast (2008) demonstrated the opposite effect for caffeine: as milk fat level increased, so did bitterness. This might be explained by the lipophilicity hypothesis of Metcalf and Vickers (2002), as caffeine is predominantly hydrophilic, with a partition coefficient of -0.07 (Biagi, Guerra et al., 1990). When considering our experiments with SOA, it seems unlikely that increasing fat would reduce the bitterness intensity: SOA has a negative partition coefficient (-0.9) according to PubChem (PubChem, 2013), so it would be
expected to partition into the water phase. Whether this may also be influenced by pH is unknown (e.g. Bennett, Zhou et al., 2012).

Another potential explanation for the differences in RjTs between experiments might be differences in viscosity across the matrices. Previous work in liquids of varying viscosity suggest that the bitterness of quinine sulfate decreases as viscosity increases (Moskowitz and Arabie, 1970). The concentration of solids in solution in the saliva would increase from chocolate milk to chocolate ice cream to compound chocolate due to an increase in viscosity across these products; if this also reduces perceived bitterness (which was not directly assessed in our tests), then we might also expect differences in hedonics. That is, as the viscosity increases, more SOA may be required to elicit the same intensity of bitterness that results in rejection of the spiked sample.
Preference for chocolate or vanilla ice cream was tested for association with preference for milk or dark chocolate (see Table 4-4) using Fisher’s exact test, and found to be significant (p <0.02). That is, people who prefer milk chocolate were over-represented in the vanilla preferring group, while the chocolate ice cream preferring group was comprised of 42% milk chocolate and 58% dark chocolate preferring individuals. Secondary analyses of rejection thresholds based on these alternative groupings reflect this distribution of preferences. The RjT for the vanilla ice cream group
was 14.6 µM (similar to the 14.2 µM seen for the milk chocolate preferring group). The RjT for the chocolate ice cream preferring group was 21.1 µM, which is similar to what we see for the average RjT of all the participants (48% milk chocolate preferring, 52% dark chocolate preferring) at 20.2 µM.

Table 4-4. Contingency table for preference group distributions (Fisher’s exact test)

<table>
<thead>
<tr>
<th>Chocolate Ice Cream</th>
<th>Milk Chocolate</th>
<th>32</th>
<th>45</th>
<th>77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla Ice Cream</td>
<td>14</td>
<td>19</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>50</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>

While fatigue is a potential concern with a high fat product like ice cream, Guinard and colleagues previously demonstrated participants could evaluate nine samples without experiencing significant fatigue (1996). In this study, participants each tasted 10 different samples with a minimum of 60 seconds (controlled by timed delays enforced via software) between sample pairs and participants were instructed to rinse with water between each sample. Additionally, all participants were informed of the large amount of samples to be tasted and instructed to only taste one spoonful (approximately 4g) of each. Consequently, we believe that fatigue did not play a major role in the results of our study.

Previously, almost all studies on rejection thresholds have been applied to liquid foods like wine, milk or soup (Prescott, Norris et al., 2005; Lee, Prescott et al., 2008; Saliba, Bullock et al., 2009). We recently demonstrated the use of this method with a solid food (Harwood, Ziegler et al., 2012b), and we extend this work further here, by showing this technique can be applied to an intermediate food matrix like ice cream. This validation should be of interest to those who work with dairy products because of the
broad potential application of the rejection threshold method in dairy products. Chocolate ice cream is a very complex stimulus that can be difficult to evaluate with consumers, and can require trained panels to profile, which are costly and labor intensive. In contrast, the rejection threshold method is simple to carry out for both the researcher and the participant as it is based on straightforward preference tasks. As demonstrated by this experiment, this method could be used to quantify group rejection threshold limits for an assortment of off flavors or attributes that only become objectionable at high intensities in a variety of dairy products. In addition, past and present data indicate it can also be used when considering specific market segments.

Conclusions

In conclusion, RjTs for SOA in chocolate ice cream are influenced by preference for milk or dark solid chocolate. Existing preferences for solid chocolate appear to generalize to chocolate flavored ice cream, as might be expected. Specifically, those who prefer solid dark chocolate to milk chocolate tolerate substantially higher levels of an added bitterant. This difference in RjT agrees with previous studies on RjTs for SOA in other chocolate flavored products. Whether this approach can be used to make chocolate ice cream with less added sugar to be marketed ‘for dark chocolate lovers’ remains to be determined. Rejection thresholds also have the potential to be applied to other dairy foods in quality control or product optimization applications.
Acknowledgements

The authors would like to thank the staff of the Berkey Creamery at Penn State, especially Bonnie Ford and Todd Gantt, as well as Emily Furumoto for their assistance in sample preparation, Rachel Primrose and the Sensory Evaluation Center staff for their assistance with data collection, and our participants for their time and participation. This manuscript was completed in partial fulfillment of the requirements for a Master of Science degree at the Pennsylvania State University by the lead author. This work was partially supported by funds from the Pennsylvania Manufacturing Confectioners’ Association (PMCA), who were not involved in the study design. The corresponding author receives support from USDA Hatch Project PEN04332 funds and a National Institutes of Health grant from the National Institute of Deafness and Communication Disorders (DC010904).
Chapter 5

Rejection Thresholds in Semisweet Chocolate with Varying Polyphenol Content

Abstract

Indigenous polyphenolic compounds in cacao impart bitter and astringent characteristics to chocolate confections. While an increase in these compounds may be desirable from a health perspective, they are generally incongruent with consumer expectations. Traditionally, chocolate products undergo several processing steps (e.g. fermentation and roasting) that decrease polyphenol content, and thus bitterness. The objective of this study was to estimate group rejection thresholds for increased content of cocoa powder produced from under-fermented cocoa beans in a semisweet chocolate-type confection. The group rejection threshold was equivalent to 80.7% of the non-fat cocoa solids coming from the under fermented cocoa powder. Contrary to expectations, there were no differences in rejection thresholds when participants were grouped based on their self-reported preference for milk or dark chocolate indicating that these groups react similarly to an increase in polyphenol content.

Introduction

Chocolate, a product of *Theobroma cacao*, is naturally bitter (Hoskin, 1994). Chocolate contains phenolic compounds such as catechin, epicatechin, anthocyanins, and other various polyphenols (Drewnowski and Gomez-Carneros, 2000). Polyphenolic compounds are often perceived as bitter and astringent (e.g. Stark, Bareuther et al., 2006), and an increase in polyphenol content has the potential to elicit stronger sensations of
bitterness and astringency. For example milk chocolate confections, which tend to have lower polyphenolic content (Arts, Hollman et al., 1999) (and often higher sugar content) are generally less bitter than their semisweet (‘dark’) counterpart, which is sometimes even referred to as ‘bitter chocolate.’

The process of fermentation reduces total polyphenol content in cacao in a time-dependent manner as these compounds can be oxidized, polymerized, or form complexes with other chemicals (Bonvehi and Coll, 1997; Camu, De Winter et al., 2008). A previous study investigating the relative content of total polyphenols, tannins, and (-)epicatechin has correlated these chemical contents with sensory panel-determined acceptability, indicating that ‘deficiently’ fermented samples can have unacceptably high levels of tannins and (-)-epicatechin (Bonvehi and Coll, 1997). As such it is common practice to ferment cocoa beans to an optimal level for consumer acceptance. However, there is interest in preserving the polyphenol content in foods (e.g. Kealey, Snyder et al., 2011), as these compounds are now being investigated for potential health benefits they may confer to the consumer (e.g. Corti, Flammer et al., 2009). Since bitterness and astringency are generally aversive to most consumers, the challenge now is to balance these oral sensations with phytonutrient content to create acceptable products (Drewnowski and Gomez-Carneros, 2000).

One method for determining acceptable concentrations of compounds that become aversive at high levels is the determination of rejection thresholds. Previous studies investigating rejection thresholds for bitterness in chocolate flavored products (Harwood, Ziegler et al., 2012a, b) have relied on an added bitter ingredient (sucrose octaacetate, or SOA) that is not a naturally occurring source of bitterness in chocolate.
While these studies provide important theoretical groundwork, it remains to be seen if the same clear segmentation will occur in similar populations when the differences between the samples are due to compounds inherent in chocolate. That is, previous studies have found that populations preferring dark chocolate have significantly higher rejection thresholds for the added bitterant (SOA) when presented in chocolate-flavored products such as chocolate milk (Harwood, Ziegler et al., 2012a), milk chocolate-flavored compound coating (Harwood, Ziegler et al., 2012b), and chocolate ice cream (Harwood, Loquasto et al., 2013 under review) when compared to populations that prefer milk chocolate. The primary aim of this study was to determine group rejection thresholds for increased content of cocoa powder produced from under-fermented cocoa beans (and therefore increased cocoa polyphenol content) in a semisweet chocolate-type confection, and to compare rejection thresholds when participants were grouped based on their self-reported preference for milk chocolate or dark chocolate.

**Materials and Methods**

**Test Stimuli**

The test stimuli were semisweet “chocolate” pieces produced from the ingredients described below. High Cocoa Flavanol Natural Cocoa Powder, 10-12% fat content, produced from under-fermented cocoa beans, was provided by Mars, Inc (Elizabethtown, PA). The high flavanol content of this cocoa powder, a result of the reduced fermentation, makes this cocoa powder more bitter than traditionally processed cocoa powder, and gives an overall complex impression of bitterness, astringency, and chocolate character. This cocoa powder will be referred to as ‘high CF cocoa powder’
from here on. NI Natural Cocoa Powder (10-12% fat content) which is less bitter than the under-fermented cocoa powder, was chosen as the ‘control’ cocoa powder, and provided by Blommer Chocolate Company (Chicago, IL). This cocoa powder will be referred to as ‘NI natural cocoa powder’ from here on. Cocoa butter and soy lecithin were donated by the Barry Callebaut Chocolate Company (Pennsauken, NJ). Additional ingredients include sucrose (Good Food Inc., Honey Brook, PA) and canola oil (Wegmans Food Markets, Inc., Rochester, NY). While these samples do not technically meet the US standard of identity for semisweet chocolate (21 CFR 163.123) due to the addition of canola oil and the use of reconstituted liquor rather than liquor ground directly from cocoa beans, we will refer to the samples as semisweet chocolate as they do contain an equivalent amount of reconstituted liquor that exceeds the minimum liquor content requirement.

Semisweet chocolate samples were prepared containing different proportions of the cocoa powders. These samples were prepared from a refined cocoa butter and sugar flake base; reconstituted liquors made from the NI natural and high CF natural cocoa powders with added cocoa butter; canola oil and soy lecithin. In order to slightly soften the samples to make them easier to eat, canola oil was added as an allergen-free fat that is liquid at room temperature. The proportion of components in the samples is outlined in table 5-1. All of the non-fat cocoa solids were added at the conch, with additional cocoa butter, canola oil, and soy lecithin. The different samples contained increasing proportions of high CF natural cocoa powder relative to NI natural cocoa powder (outlined in table 5-2). The cocoa butter/sugar base was mixed, refined, and then divided into the different batches. The batches were conched (uncovered at 65°C for 4 hours),
tempered, moulded, and stored in tightly sealed containers away from light at room
temperature (22°C) for ten days before sensory testing. Each piece weighed
approximately 2.5g, and each participant received one piece of chocolate of each of the
five concentrations, paired with a piece of the control chocolate (containing only NI
natural cocoa powder), resulting in a total of ten pieces per participant. Samples were
presented in clear plastic cups with lids labeled with random, three-digit blinding codes.

*Table 5-1. Sample Formulation*

<table>
<thead>
<tr>
<th>Components</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Fat Cocoa Solids</td>
<td>44%</td>
</tr>
<tr>
<td>Fat*</td>
<td>32%</td>
</tr>
<tr>
<td>Sugar</td>
<td>23.5%</td>
</tr>
<tr>
<td>Lecithin</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

*29% cocoa butter, 3% canola oil

*Table 5-2. Proportions of cocoa powders in test stimuli*

<table>
<thead>
<tr>
<th>Log concentration</th>
<th>NI Natural Cocoa Powder</th>
<th>High CF Natural Cocoa Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>1.55</td>
<td>65%</td>
<td>35%</td>
</tr>
<tr>
<td>1.70</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>1.80</td>
<td>35%</td>
<td>65%</td>
</tr>
<tr>
<td>1.90</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td>2.00</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Sensory Testing Procedures*

Ninety-nine chocolate consumers (thirty-seven men) were recruited from the Penn
State community via email based on their liking of and willingness to consume chocolate.
All participants were reportedly healthy, non-smoking individuals aged between 18-45
years. Fifty-three of the participants reported preferring milk chocolate and forty-six
reported preferring dark chocolate. Under the wholesome foods/approved food additives exemption in 45 CFR 46.101(b)(6) all procedures were exempt from Institutional Review Board review by the Penn State Office of Research Protections. Participants in this study provided informed consent and were compensated for their time.

All tests occurred in the individual testing booth in the Sensory Evaluation Center at Penn State under white lighting, and Compusense five v5.2 (Guelph, ONT) was utilized for data collection. All data was collected in one testing session with 2-Alternative Forced Choice (2-AFC) tasks to determine rejection thresholds preceding demographic questions (age, gender, milk or dark chocolate preference). Semisweet chocolate samples were presented as pairs containing one control sample (100% NI natural cocoa powder) and one ‘spiked’ sample containing high CF natural cocoa powder for a total of five pairs. The spiked samples were presented in order of increasing content of high CF natural cocoa powder and the presentation order within the pairs was randomized. Participants were instructed to rinse with room temperature (22°C) water between samples.

**Total Phenolic Content**

The phenolic extraction procedure used here was adapted from the protocol used by Hammerstone and colleagues (1999). 20g of each cocoa powder were defatted with hexane three times and left to air dry. Yields of defatted cocoa powder were weighed and calculated as a fraction of the original wet weight. Polyphenols were then extracted from the samples with acetone and water (70:30) twice and methanol and water (50:50) twice. Organic solvents were removed by rotary evaporator under partial vacuum at ~28°C.
Samples were then freeze-dried. Polyphenol content of the freeze-dried extracts was then quantified by the Folin-Ciocalteu method (Singleton, Orthofer et al., 1999).

**Statistical Analysis**

Rejection thresholds were analyzed using the sigmoid method previously described (Harwood, Ziegler et al., 2012a). Group rejection thresholds were calculated for the entire sample population as well as for the group of individuals within this population who reported preferring milk chocolate, the group of individuals who reported preferring dark chocolate, and on the basis of gender. Associations between gender and solid chocolate preference were analyzed using Fisher’s Exact Test (two tailed).

**Results and Discussion**

Defatting the cocoa powder samples with hexane yielded 15.85g fat free cocoa solids from the NI natural cocoa powder and 16.62g fat free cocoa solids from the high CF natural cocoa powder. The final yield was 2.83g of freeze-dried extract from the NI natural cocoa powder and 4.50g of freeze-dried extract from the high CF natural cocoa powder. The total phenolic assay revealed the phenolic content (g phenolic per 100 g of cocoa powder, 10-12% fat) of the NI natural cocoa powder to be 3.4% w/w and the high CF natural cocoa powder to be 7.9% w/w; a 2.3-fold difference (figure 5-1). As bitter and/or astringent taste components increase with polyphenol content (Schwan and Wheals, 2004), this result confirms the decision to use these different cocoa powders to formulate the semisweet chocolate for the sensory evaluation portion of this experiment. While we did not measure the total phenolic content of these samples after processing
(conching and tempering especially as these involve heat) the samples were all subjected to the same processing conditions, so we would expect them to contain the same relative proportions of polyphenols, as any loss would be equivalent across all of the samples.

Figure 5-1. Relative Phenolic Content in 10-12% Fat Cocoa Powder

A significant association was found between gender and solid chocolate preference in this study (p=0.0126). Of the fifty-three participants who reported preferring milk chocolate, twenty-six were male and twenty-seven were female. In contrast, of the forty-six participants who reported preferring dark chocolate, only eleven were male and thirty-five were female. This could potentially represent a limitation in this study, as dark chocolate preferring men were under-represented. However, there were no significant differences found in the rejection thresholds when comparing the men and the women in this study (p=0.803).
The group rejection threshold for the high CF natural cocoa powder in semisweet chocolate for all of the participants together was 80.7% (see figure 5-2), falling almost exactly at the second highest spiked sample. Further analysis revealed no significant differences (p=0.6235) between the rejection threshold for the group preferring milk chocolate and the group preferring dark chocolate (see table 5-3). This suggests that regardless of reported preference for milk or dark chocolate, all of the participants reacted in a similar manner to the increased content of under-fermented cocoa powder in semisweet chocolate. It is interesting to note that contrary to what would be expected, the rejection threshold for those who prefer milk chocolate was slightly higher than the rejection threshold for those who prefer dark chocolate (83% vs. 78%); however, these values fall within almost completely overlapping 95% confidence intervals. Another interesting characteristic of the preference/indifference function in figure 5-2 is the shallow slope of the linear portion. The increase in rejection between concentrations is very gradual when compared to other measured rejection thresholds (e.g. Harwood, Ziegler et al., 2012a, b) obtained from SOA.
Table 5-3. Group Rejection Thresholds for High CF Natural Cocoa Powder in Semisweet Chocolate

<table>
<thead>
<tr>
<th>Group</th>
<th>Rejection Threshold</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Participants</td>
<td>80.7% (±1.05 SE)</td>
<td>n/a</td>
</tr>
<tr>
<td>Milk Chocolate Preferring</td>
<td>83.0% (±1.05 SE)</td>
<td>0.6235</td>
</tr>
<tr>
<td>Dark Chocolate Preferring</td>
<td>78.0% (±1.12 SE)</td>
<td></td>
</tr>
</tbody>
</table>

One potential reason for the lack of segmentation observed between milk chocolate and dark chocolate preferring groups could be that the predominant difference in the samples was not the same characteristic that milk or dark chocolate preference is based on. That is, one might assume that the bitterness of dark chocolate is what milk chocolate preferring individuals find aversive about dark chocolate. However it remains
to be seen if bitterness was the predominant character setting apart the spiked samples from the controls in the present experiment. For instance, due to the increase in polyphenolic content it could be that the participants were reacting to an increase in astringency, and it is possible that both chocolate market segments would react similarly to that attribute (astringency). Additionally, it could be that the spiked samples simply had less desirable ‘chocolate flavor’ and hence were less preferred, as the forced choice preference task only implies that one is more preferable compared to the other. It is important to note that with rejection thresholds, the driver of rejection could potentially be the absence of a positive attribute. The 2-AFC task forces preference of one sample over another, mean rejection is always relative to the other sample. In the case of rejection thresholds, a method of constant stimuli, preference is always measured comparing the spike to a control sample. There are many qualities that differentiate milk and dark chocolate, varying from flavor (e.g. sweetness and dairy notes) to melting quality. Training a panel to create a descriptive profile of the spiked and control samples and exploring this further may have shed more light on the reasons for the lack of differences in the rejection of the spiked samples. Unfortunately, this was beyond the scope of the current project.

Additionally, while we can infer tolerance from the rejection threshold measure, it remains to be seen how the participants would rate their liking of the samples. The base formula for the samples is similar to other commercially available products. For example, Lindt Excellence 70% Cacao chocolate, which contains cocoa mass, sugar, cocoa butter, and natural bourbon vanilla beans, contains a similar amount of cacao (70% compared to the 73% cacao content of the study samples), though slightly more fat (40% fat in Lindt
compared to 32% fat in the study samples) and sugar (26.6% sugar in Lindt compared to 23.5% sugar in the study sample) (http://www.lindt.ca/swf/eng/products/bars/dark-chocolate/excellence-70-cacao/). Therefore it may be reasonable to assume that the control sample, which contained traditionally processed cocoa powder, would be rated at least as acceptable.

Conclusions

The rejection threshold for high CF natural cocoa powder in this semisweet chocolate-type confection is 80.7%. No significant difference in rejection threshold was observed when grouping participants based on their self-identified preference for milk or dark chocolate. While solid chocolate preference (milk vs. dark) has been successfully applied to differences in rejection thresholds for a bitter compound previously (Harwood, Ziegler et al., 2012a, b), this segmentation strategy does not appear to be appropriate when considering tolerance for increased content of high CF natural cocoa powder in a semisweet chocolate-type confections, perhaps due to the complexity inherent to chocolate flavor.
Acknowledgements

The authors would like to thank Yeyi Gu and Jong Yung (Sam) Park for their assistance in performing the total polyphenol analysis; Rachel Primrose and the Sensory Evaluation Center staff for their assistance in data collection; as well as Elise Boretz for her assistance with sample preparation; and all of the participants for their time. Additionally we would like to thank Mars, Inc., The Blommer Chocolate Company, and the Barry-Callebaut Chocolate Company for their generous donations. MLH is supported by funds from the Pennsylvania Manufacturing Confectioners’ Association (PMCA) and JEH and GRZ receive support from the Pennsylvania State University.
In order to refine the methodology, analysis, and application of rejection thresholds, a novel sensory tool for determining acceptable concentration/intensity limits, a series of experiments was carried out. These experiments built off one another moving from a model bitter compound in a simple fluid food system to through solid compound, culminating in modified ingredients in a complex solid food system. This includes experiments to determine rejection thresholds for sucrose octaacetate in chocolate milk (chapter 2), milk chocolate flavored compound coating (chapter 3), and chocolate ice cream (chapter 4). Additionally, an experiment to determine rejection thresholds for natural cocoa polyphenols in semisweet chocolate was also completed (chapter 5). A summary of the main findings of these experiments is as follows:

- Rejection thresholds for attributes that become objectionable at high levels form sigmoid shaped preference/indifference functions that should not be analyzed with linear fits.
- When the proportion of respondents rejecting the spiked sample is plotted vs. the concentration/intensity of the attribute of interest, the bottom of the curve will oscillate around chance performance (50%) until the attribute reaches a level that is both noticeable and strong enough to influence the preferences of the participants.
- To an extent, when the concentration range selected for investigation does not capture the entire sigmoid preference/indifference function, the top and bottom
portions of the curve can be constrained to the theoretical minimum (0.5) and maximum (1.0) to successfully estimate rejection thresholds.

- Response to attributes that become objectionable at high levels can reach the theoretical maximum of 100% universal rejection, which had not been previously shown.

- Rejection thresholds can be carried out in complex food systems with varying degrees of solidity, not just in liquid systems.

- It is possible to have significant differences in rejection thresholds that are not associated with differences in the ability to perceive the attribute of interest.

- When investigating rejection thresholds in stimuli that have a milk chocolate background and differ only by the intensity of their bitterness, self-reported solid chocolate preference (milk vs. dark) can be used to group participants. In these instances solid chocolate preference can be used to predict tolerance for bitterness, as those who prefer dark chocolate will tolerate significantly more bitterness (and thus have higher rejection thresholds) than those who prefer milk chocolate.

- When investigating rejection thresholds in semisweet solid chocolate with differing levels of natural cocoa polyphenols, rejection thresholds do not differ as a function of milk or dark solid chocolate preference. This suggests that astringency may not be a major driver of segmentation between milk chocolate and dark chocolate preferring consumers.

While these conclusions represent advancement in the methodology, analysis, and application of rejection thresholds, we find that there are more questions that remain to be
answered as a result of these experiments. Some of these have surfaced as limitations to the studies described, while others should be viewed as experiments necessary to further the refinement of the rejection threshold method.

One limitation to the studies previously discussed is the need to compare results with liking or acceptability scores. Liking was not measured and it cannot be directly inferred from these studies. In the instances of the experiments with sucrose octaacetate, it was assumed that the control samples were well enough liked because they were commercially available food products. However, for the study conducted in semisweet chocolate, the results would be more interpretable if there had been a liking test carried out in conjunction with the rejection thresholds. It remains possible that while the participants showed preference for one sample over the other as concentration of high CF natural cocoa powder increased, they did not necessarily like either of the choices. One way to avoid this would be to carry out a liking test on the control sample prior to running rejection thresholds to ensure that the control sample has some minimal acceptability.

Another limitation to the interpretation of the results in chapter six is the lack of descriptive data on the samples. As a group, the participants showed increasing rejection of the spiked samples as the content of high CF natural cocoa powder increased. However, without running a formal descriptive profile of these samples, it remains to be seen which attribute(s) the participants were reacting to, as polyphenol content can effect bitterness, astringency, and potentially other sensory characteristics of the samples. Generating a descriptive profile of these samples could potentially give more insight into
the lack of segmentation observed when comparing individuals who prefer milk chocolate to individuals who prefer dark chocolate.

One area for further work is the concept of group versus individual rejection thresholds. In theory, with repetitive testing, a researcher should be able to determine a rejection threshold for an individual by plotting the proportion of times the respondent chose the control sample at each concentration/intensity. This approach has yet to be tested. This would be very time and material intensive than group rejection thresholds, as the individual participant would likely have to participate in multiple testing sessions. Additionally, while potential differences may exist between individuals, there does not at this point in time seem to be a need to determine these differences as most research involving rejection thresholds is presumably concerned with the preferences of a large consumer base.

Another question that warrants further research is how far off from capturing the entire sigmoid function can the concentration range be, that one could still constrain the minimum and maximum values and still get a reliable result? While we were able to demonstrate successful estimation of group rejection thresholds with a constrained fit and verify the value by adjusting the concentration range (see chapter 3), it remains to be seen how robust the estimation of these parameters can be. It would be beneficial to sensory scientists who would like to add this tool to their toolbox to determine how robust the estimation can be.

Further research should also be conducted to determine if it would be possible to identify market segments post hoc. While there may be groups with significantly different rejection thresholds, it is possible that these effects would be completely washed
out when considering the participants of a study as a whole if groupings are not known prior to analysis. If it is possible to identify these groups from rejection threshold data, this may prove to be a very useful tool for investigating differences in preferences for groups that were not previously known to exist. One potential way to identify these groups may be determination of individual rejection thresholds by repetitive testing.

One last methodological question that should be investigated is the comparison of analysis of rejection threshold data sets for data that captures the entire linear portion by the sigmoid analysis outlined in chapter 3 compared to analysis of the same data sets where the function is cleaved at the x-values surrounding the linear portion of the function and analyzed by linear regression. While linear fits should not be applied to the entire function, as values oscillating around the minimum and maximum y-values have the potential to skew the data, if these points were eliminated it remains to be seen how those values would compare to results fit by the four parameter Hill equation. This may be of interest as it is much simpler mathematically. Of course, this would only be possible when the entire function is available/known and constraints (minimum and/or maximum) are not necessary.
Appendix A: Pilot Study - Effect of Information on Preference for a Functional Chocolate Product by Manipulation of Rejection Thresholds

Introduction

According to the World Health Organization (WHO), cardiovascular disease (CVD) is the leading cause of death accounting for 30% of all deaths (>17 million people) worldwide in 2008 (WHO, 2011). In both their population-based and individual interventions, the WHO recommends that diet can be quite effective in preventing CVD (WHO, 2011), which explains, in part, the current interest in functional foods. Over the last decade, there has been a steady increase in the demand for functional foods in the US and worldwide, as more people are coming to understand the direct impact of diet on health and well-being (Siro, Kapolna et al., 2008). Here, we define functional foods as those that provide “…an additional physiological benefit beyond that of meeting basic nutritional needs (Hasler, 1998).” This would include foods that are currently being investigated for their potential ability to promote circulation and cardiovascular health, such as dark chocolate (e.g. Corti, Flammer et al., 2009; Djousse, Hopkins et al., 2011).

Increasing awareness of the link between healthy eating and well-being is reflected in the current views of the average American consumer. In the International Food Information Council Foundation’s (IFIC) “2011 Food & Health Survey: Consumer Attitudes Toward Food Safety, Nutrition & Health,” it was reported that healthfulness is one of the main drivers of food purchasing behaviors, behind taste and price. The same study also reported that the presence of added beneficial components and fortification do have at least some impact on purchasing decisions for 80% of Americans, with 11%
indicating that it has a great impact, and 88% indicating that these types of foods have a potential impact on overall health (IFIC, 2011). These numbers support the increased demand we see for functional food products.

Unfortunately, there seems to be a gap between these good intentions and actions for Americans. While 58% of the IFIC survey responders reported giving a lot of thought to what they eat, only 25% reported that they thought they have a healthful diet. Additionally, only about 31% of American consumers report that they look for statements about nutrition benefits, and 24% look for statements about health benefits when deciding to purchase or eat a food or beverage (IFIC, 2011). The challenge posed for food manufacturers now becomes developing functional food products that can reach the consumer in as many ways as possible, which may mean a pleasant tasting, reasonably priced products, with specific claims displayed on the package.

It has been suggested that off-flavors (such as bitterness or astringency) may be expected from functional foods, and that a perception of off-characteristics may have the potential to support or even strengthen the cognitive impact of the health claim that the product bears (Tuorila and Cardello, 2002). This is especially relevant, as assumed health benefits may boost acceptance of products, though dependent on the interaction of the information with the background of the consumer (i.e. health consciousness) (Kahkonen, Tuorila et al., 1996). A great deal of research has been focused on how consumers respond to health claims and other information when making food choices and expressing liking, acceptance, or preference e.g. (Kahkonen, Tuorila et al., 1996; Torres-Moreno, Tarrega et al., 2012). While it may depend largely on the priorities and background beliefs of the consumer, labeling packages with health claims will undoubtedly influence
the initial perception of a product. This could potentially negatively impact the way the consumer believes it will taste (e.g. reduced sodium, Liem, Aydin et al., 2012), but also perhaps positively influence their likelihood of purchasing or using the product (e.g. Guinard, Smiciklas-Wright et al., 1996).

In chocolate specifically, the effects of label information regarding brand and percent cacao content was recently explored. Torres-Moreno and colleagues (2012) found that label information did have the ability to influence liking (measured on a 9-point hedonic scale). The primary aim of this study was to examine the potential manipulation of rejection thresholds for increased cocoa polyphenols (particularly increased bitterness and astringency) in solid semisweet chocolate via the presence or absence of information about a potential cardiovascular health benefit. Secondary aims include how the manipulation of rejection thresholds may or may not be influenced by the degree of health consciousness of the participants in the study as well as preference for milk or dark chocolate.

**Materials and Methods**

**Participants**

In the first session, 127 healthy, non-smoking individuals (83 women and 44 men) who prefer dark chocolate were recruited via email from the Penn State campus and surrounding community based on their willingness to eat dark chocolate. In the second session, fifty-four healthy, non-smoking individuals (32 women and 22 men) who prefer milk chocolate were recruited in the same way from the same geographic area.

Procedures were exempt from Institutional Review Board approval by the Pennsylvania
State University Office of Research Protections under the wholesome foods/approved food additives exemption in 45 CFR 46.101(b)(6). All participants were between the ages of 18 and 45, provided informed consent, and were compensated for their time.

**Stimuli**

Semisweet chocolate pieces were produced from the ingredients described below.

High Cocoa Flavanol Natural Cocoa Powder, 10-12% fat content, was provided by Mars Inc. (Elizabethtown, PA). The high flavanol content of this cocoa powder, which is a result of its processing, makes this cocoa powder more bitter than typical cocoa powder, and gives an overall impression of bitterness, astringency, and chocolate flavor. This cocoa powder will be referred to as ‘high CF natural cocoa powder’ from here on. NI Natural Cocoa Powder (10-12% fat content), which is less bitter than the under-fermented cocoa powder, was provided by Blommer Chocolate Company (Chicago, IL). This cocoa powder will be referred to as ‘NI natural cocoa powder’ from here on. Cocoa butter and soy lecithin were donated by the Barry Callebaut Chocolate Company (Pennsauken, NJ). Finally, sucrose (Good Food Inc., Honey Brook, PA) was used as the sugar source in these samples.

Semisweet chocolate samples were prepared containing different proportions of the cocoa powders. These samples were prepared from a refined chocolate flake base (containing sugar, cocoa butter and NI natural cocoa powder); reconstituted liquors made from the NI natural and high CF natural cocoa powders with added cocoa butter; and soy lecithin. The proportion of components in the samples is outlined in *table A-1*. All of the samples contained 50% of the non-fat cocoa solids from the NI natural cocoa powder,
which was added at the refining step. Of the remaining 50% of non-fat cocoa solids, which were added at the conch, the samples contained increasing proportions of high CF natural cocoa powder to NI natural cocoa powder: 20:80, 40:60, 60:40, 80:20 and 100:0. The samples were mixed, refined, conched (uncovered at 65°C for 4 hours), tempered, moulded, and stored in sealed containers away from light at room temperature (22°C) for 3 weeks before the test. Each piece weighed approximately 2.5g, and each participant received one piece of chocolate of each concentration paired with a piece of the control chocolate (containing only NI natural cocoa powder), for a total of ten pieces. Samples were presented in clear plastic cups with lids labeled with random, three-digit blinding codes. Pairs were presented in order of increasing high CF natural cocoa powder content and presentation order within these pairs was randomized.

Table A-1. Sample Formulations

<table>
<thead>
<tr>
<th>Sample Composition</th>
<th>45%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td></td>
</tr>
<tr>
<td>Cocoa Butter (fat)</td>
<td>30.5%</td>
</tr>
<tr>
<td>Non Fat Cocoa Solids</td>
<td>24%</td>
</tr>
<tr>
<td>Lecithin</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ratio of High CF Natural Cocoa Powder to NI Natural Cocoa Powder Added at Conch</th>
<th>% High CF Natural Cocoa Powder of Total Cocoa Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:100 (Control)</td>
<td>0%</td>
</tr>
<tr>
<td>20:80</td>
<td>10%</td>
</tr>
<tr>
<td>40:60</td>
<td>20%</td>
</tr>
<tr>
<td>60:40</td>
<td>30%</td>
</tr>
<tr>
<td>80:20</td>
<td>40%</td>
</tr>
<tr>
<td>100:0</td>
<td>50%</td>
</tr>
</tbody>
</table>
Sensory Testing Procedures

Rejection thresholds were measured in individual booths under white lighting in the Sensory Evaluation Center at Penn State. Participants were randomly divided into two groups with equal proportions of men and women in each group to investigate the influence of information about perceived healthfulness of the samples on tolerance for the high CF natural cocoa powder. In one group, in addition to instructions for the test the participants were provided with additional information about the samples (“Some of the samples you will taste today contain increased amounts of natural cocoa polyphenols. These compounds are currently being investigated by other researchers for their potential cardiovascular health benefits”). This information was meant to cue the participants that some of the samples could potentially be healthier than others. The second group simply received the test instructions. In a longitudinal experiment on deceptive advertising (Olson and Dover, 1978), subjects were exposed to deceptive advertising explicitly stating that a ground coffee product did not have bitterness. The subjects then tasted the actual product and rated its attributes including bitterness. Their ratings of bitterness were significantly lower than controls that had not been exposed to the advertisements. These results support the top-down processing of food perception, as product evaluations are based on the actual sensory experience as well as outside information and beliefs (Olson and Dover, 1978). Similarly, we believe that information given to participants before our study will have the potential to influence their expectations and subsequent ratings of preference for the product. Samples were presented in pairs and participants were asked to perform a 2-Alternate Forced Choice (2-AFC) preference task for each pair of samples.
Participants were instructed to rinse with room temperature (22°C) water between samples.

In other previous studies, the effects (or lack thereof) of nutritional information on impressions of and willingness to try foods has been thought to be connected to differences in concern about nutritional attributes, whereas almost all people agree that they would like for food to taste good (Pelchat and Pliner, 1995; Martins, Pelchat et al., 1997). For this reason we decided to measure health consciousness by means of a questionnaire following participation in the rejection threshold test. Participants were asked to answer a 10-item concern scale that had been used previously by Kahkonen and colleagues to measure basic health consciousness (1996). Concern for the ten items were rated on 9-point scales anchored with “I am not concerned at all” and “I am extremely concerned (Kahkonen, Tuorila et al., 1996).” Participants were also asked to indicate their age, gender, and chocolate eating style (see (Harwood, Ziegler et al., 2012b). All data was collected using Compusense five software v5.2 (Guelph, ONT).

Statistical Analysis

Group rejection thresholds were determined using the sigmoid analysis approach described previously (Harwood, Ziegler et al., 2012a). Results were analyzed with participants grouped based on their “health consciousness” which was determined by the means of the scores reported in the health consciousness questionnaire. Participants were either deemed high concern or low concern based on a median split of the mean scores. That is, out of a possible 10-90 points, those who scored >50 were considered high concern while those who scored ≤ 50 were considered low concern. Results were also
analyzed with participants grouped by presence and absence of additional information, milk or dark solid eating chocolate preference, gender, and eating style.

**Results and Discussion**

None of the groups analyzed achieved a statistically significant level of rejection of the spiked samples. The group preferring milk chocolate showed the most promising results trending in the expected direction by the highest concentration of high CF natural cocoa powder (see figure A-1). That is, the concentration range of the samples was apparently too low to elicit rejection. This suggests that the relative intensity of bitterness in the spiked samples was probably not that much stronger than the control samples. This is supported by the preference/indifference function for the group that prefers dark chocolate, where rejection of the spiked samples tended to oscillate around chance at all of the concentrations. If the samples were in fact similar in bitterness to the control samples, that would explain why the dark chocolate preferring group failed to show a strong preference. While not statistically significant, it is also interesting to note that both the milk and dark chocolate preferring groups showed a very low level of rejection of the first sample, only about 35% (see figure A-1).
When considering the groups with high and low health concern, the dark chocolate preferring group again failed to show preference regardless of degree of health concern. The milk chocolate group showed some effects of high and low health concern with those who were grouped as low concern actually trending more quickly towards rejection than those who were highly health conscious (see figure A-2). However caution should be taken when interpreting these results as the milk chocolate preferring group with low health concern has a very small sample size of just eighteen.
When considering the groups with and without additional information we see very little evidence of differences in preference (see figure A-3). It is possible that the effects of information are washed out when considering the milk and dark chocolate preferring groups together. When considering these groups separately, both the milk chocolate preferring groups (with and without information) start to show preference at the highest concentration (see figure A-4) with the group preferring milk chocolate with additional information showing the most consistent results. Again this indicates that the samples were formulated on the low end of the concentration range needed to see rejection. The results illustrated in figures A-3 and A-4 suggest little effect of additional information. This is similar to what was seen in a study carried out investigating the effects of...
information about a health claim on consumers’ pleasantness ratings and purchase intent for chocolate bars (Di Monaco, Ollila et al., 2005). Focus group research done in the same study suggested that the group being interviewed found healthfulness to be irrelevant when considering their impressions of chocolate products, explaining why the presence/absence of the health claim had no effect on the positive hedonic response to the chocolate bars. Another study (Norton, Fryer et al., 2013) investigated the effects of information about fat content in chocolate and found that while expectations were lowered significantly for the chocolate labeled as low fat, actual liking scores were not affected, again demonstrating the often robust quality of hedonic response to chocolate. While in this study we were only measuring preference and not liking, it seems likely that the responses of our participants to these samples were not significantly affected by the presence or absence of health information. Though it has been previously demonstrated that it is possible to achieve rejection of chocolate products (Harwood, Ziegler et al., 2012b).
**Figure A-3.**

Preference/Indifference Function of Those With and Without Additional Information

- **With Information**: Orange line and dots.
- **Without Information**: Black line and squares.

The graph shows the proportion preferring control based on the log percentage of high CF natural cocoa powder. The x-axis represents the log percentage, while the y-axis shows the proportion preferring control.
It should be noted that the relatively small sample size of this study might represent a limitation in terms of the main group of interest. Specifically, highly health conscious women who prefer milk chocolate. They are of interest here because they typify those most likely to be influenced by the presence of information about a health claim. In this study, 115 women participated, thirty-two of who preferred milk chocolate (see table A-2 for the breakdown of participants into groups for analysis). Of those thirty-two milk chocolate preferring women, twenty-four were highly health conscious. Since the measure of health consciousness occurs during the test, we were unable to screen for highly health conscious females to receive the additional information. The best possible distribution for comparison would be a 50/50 split of with and without the information. If
that were the case we would end up with a total of twelve women per group, which is far too small of a sample size to draw conclusions. In this study, only nine of the highly health conscious milk chocolate preferring women received the additional information and the other fifteen were sorted by chance into the group without additional information. Most likely, in order to see the effect that would we expect to see here, we would need a significantly larger sample size. Additionally, it would probably be best to pre sort participants by their degree of health consciousness in order to balance the size of the groups for comparison. However this comes with added complications, as the participants might thus be ‘primed’ prior to the test that degree of health consciousness was being assessed, specifically for the group without added information. The questionnaire was placed at the end of this test to avoid such possible bias.

Table A-2. Breakdown of Participants into Groups for Analysis (Number of People per Group)

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
<th>High</th>
<th>Low</th>
<th>With</th>
<th>Without</th>
<th>Milk</th>
<th>Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>45</td>
<td>94</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>19</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With</td>
<td>34</td>
<td>57</td>
<td>66</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>32</td>
<td>58</td>
<td>73</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>22</td>
<td>32</td>
<td>36</td>
<td>18</td>
<td>27</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>44</td>
<td>83</td>
<td>103</td>
<td>24</td>
<td>64</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>66</td>
<td>115</td>
<td>139</td>
<td>42</td>
<td>91</td>
<td>90</td>
<td>54</td>
<td>127</td>
</tr>
</tbody>
</table>

Interestingly, men and women had almost identical response patterns to the samples, and contrary to what might be expected from the literature, we saw the men
with the additional information actually approaching significance more rapidly by the highest concentration than any other group (see figure A-5). One possible explanation for this trend is that the proportion of highly health conscious women in the group with added information (~75.4%) is higher than the proportion of highly health conscious men in the group with the added information (~67.6%). This could possibly indicate that the information did have a subtle effect, increasing women’s tolerance of the bitterness so that they were less likely to reject the spike, compared to the men in the same informational condition.

*Figure A-5.*

A second potential limitation in this study is that we did not quantify the difference in polyphenol content between the two different cocoa powders, which is thought to be the main driver of differences in bitterness between the two cocoa powders.
Formulation decisions were made based on lab-bench tasting of the powders suspended in a small amount of flavorless vegetable oil. This caused us to underestimate the amount of the under fermented cocoa powder that would be needed to achieve significant rejection of the spiked samples, especially in the presence of sugar. Additionally, concentration of high CF natural cocoa powder that was used was based on round number proportions within the total cocoa powder content. However, for the sigmoid analysis it would have made more sense in hindsight to use log spacing of the concentrations to capture the entire dose/response relationship.

Further, the level of sugar used in the formula could also have been problematic. Sugar (sucrose) and other sweet tasting ingredients have the ability to suppress bitterness in the central nervous system (Lawless, 1979). The formula that we used to produce these samples contained 45% sugar. While this level of sugar is not unusually high for chocolate confections, it was counteractive to our goal of pulling to the forefront the inherent bitterness of cocoa in the samples. Repeating this experiment using a lower level of sugar in the formula would help to ensure that the bitter compounds present in the cocoa powder are more pronounced to enable us to capture the linear portion of the preference/indifference function to effectively investigate rejection.
Conclusions

The main conclusion that can be drawn from this study is that the samples used here were not offensive (bitter) enough to elicit a significant amount of rejection from the participants, regardless of milk/dark chocolate preference, gender, degree of health consciousness, or presence/absence of a health claim. Additionally, in order to study the group rejection thresholds the intensity of bitterness of the samples needs to be increased either by reducing the sugar content, increasing the non-fat cocoa solids content, or a combination of the two. Finally, in order to study the effects of added health information, there would need to be a much larger sample size in order to successfully capture the required number of high health conscious milk-preferring females, as these represent the participants who would be likely to change their preferences due to the given information.

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