Applications of Major and Minor Steviol Glycosides of *Stevia rebaudiana* in Complex Food Systems

by

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Abstract

Stevia is an all-natural plant-based high-intensity sweetener comprised of major and minor glycosides. Major glycoside, rebaudioside (Reb) A, is the most widely used glycoside in the food industry but possesses a strong aftertaste. Minor glycosides (Rebs D and M) do not possess as prominent of an aftertaste when compared to Reb A, but little has been conducted on high-sugar product applications. Furthermore, Rebs D and M are only found in minute concentrations in Stevia *rebaudiana*'s leaves, making it impossible to use Rebs D or M as the sole sweetener in a food product as it is cost prohibitive. Therefore, a blend of the major and minor rebaudiosides can be developed to improve the overall taste profile of stevia as a natural high-intensity sweetener. The first study examined the consumer acceptability of stevia's usage in a bakery product despite being a non-browning sugar replacement and investigated the impact of consumer knowledge on product and attribute acceptability (n=114). The following study aimed to determine an optimal blend of these major and minor rebaudiosides using time-intensity analysis in complex food systems such as ice cream and cola beverage. The consumer acceptability study of ice cream and cola beverage (n=42, n=39, respectively) illustrated that minor glycosides could be incorporated into baking applications and found that presenting knowledge to a consumer could improve the overall acceptance of a high-intensity sweetener. The time-intensity study demonstrated that a blend of the major and minor glycosides could produce an optimized taste profile of stevia-sweetened products. However, a food products matrix must be considered when choosing an optimized ratio to use because it was found that

high concentrations of Reb D produced a more optimized taste profile in ice cream, whereas high concentrations of Reb A were preferred in colas.

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List of Abbreviations

AI.............Average intensity

ANOVA...One-way Analysis of Variance

AUC.........Area under the curve

a_w............Water activity

DI.............Discrimination index

E-Nose......Electronic nose

FDA..........United States Food and Drug Administration

GRAS........Generally recognized as safe

HIS............High-intensity sweetener

IMAX........Maximum intensity

N.S............Not statistically significant

PC.............Principal component

PCA...........Principal component analysis

Reb........... Rebaudioside

TIMAX.....Time of maximum intensity

Chapter 1: Introduction

Obesity rates have been steadily increasing in the United States since the 1960's. Where in 1960-1962 only 13.4% of the American adult population was determined to be obese (a person who has a body mass index of 30.0 or higher), whereas in 2016 it was found that 39.8% of American adults were considered obese (Waters & Graf, 2020). During this 50-year period sugar consumption rates per capita have increased, to the levels they are at now. The average American male consumes 72 grams of sugar per day, and the average American female consumes 60 grams of sugar per day (Faruque et al., 2019, Waters & Graf, 2020, Johnson et al., 2009). These consumption rates are double and triple the daily recommended intake allowance by the American heart association (Johnson et al., 2009). These elevated consumption rates have been theorized to be linked to obesity, and it has been directly linked to heart disease, hypertension, strokes, and diabetes (Johnson et al., 2009 CDC, 2022). Just in the United States alone heart disease and diabetes make up almost one quarter of all deaths per year (CDC, 2023).

Consumers have been innately aware of their overconsumption of sugar products and their negative health effects since the late 90's and have been looking for alternative sweeteners to sugar for over 30 years (Waters & Graf, 2020). In 1999 the average American was consuming 111 grams of sugar a day. Post 1999 the consumption of sugar has been slowly declining due to consumers using artificial high-intensity sweeteners (HIS) as their sugar alternative due to their relatively low nutritive value (Faruque et al., 2019, Saraiva et al., 2020). Consumers however were faced with limited choices of these artificial sweeteners as there are only 6 that are Generally Recognized as Safe (GRAS) from the Food and Drug Administration (FDA). These six are aspartame, saccharine, acesulfame-k, neotame, and sucralose (FDA, 2017). Each of these six artificial HIS possess their own unique sweetening properties and relatively little nutritive content to them (Saraiva et al. 2020). However, it has been reported that consumption of these artificial HIS have been linked to obesity and poor overall gut health (Suez et al. 2014, Pearlman et al. 2017).

With the negative consumer perspective on these artificial HIS sweeteners consumers have begun to explore natural HIS options. The FDA has given GRAS status to only two natural high-intensity sweeteners (Stevia and Monk Fruit extract) (FDA, 2019, Gibson et al. 2014, Román et al. 2017). Stevia is a natural non-nutritive HIS that stems from the *Stevia rebaudiana* plant's leaves. These leaves contain steviols and rebaudiosides (Rebs) that are responsible for the stevia plants sweetening properties (Tanaka, 1982). These rebaudiosides are extracted from the plant leaves and produce a powder that on average is 200-300 times sweeter than sucrose (table sugar) (Peteliuk et al., 2021). These rebaudiosides are high-heat stable, non-fermentable, non-browning, pH stable (3-9), glycemic index friendly, non-caloric, anti-diabetic, antioxidant, anti-hypertensive, and anti-microbial (Tanaka, 1982, Peteliunk et al., 2021, Barathi, 2003, Ahmad et al., 2020, Pang et al., 2021, Singh & Rao, 2005.) Thus, making it appealing to commercial food processors and consumers alike due to these properties while still obtaining high sweetening abilities.

Rebaudioside A (Reb A) is the most used Reb in the food industry since its GRAS status in 2009, as it is the most abundantly found Reb in the *Stevia rebaudiana* plant's leaves (FDA, 2019). Reb A can be found in concentrations of up to 3.8% of the leaf's total dry mass, when compared to Rebs D and M which are found in more minute concentrations (0.4-0.5%) (Olsson et al., 2016). However, despite Reb A being the most abundant and widely used Reb. Reb A possesses an intense bitter aftertaste when consumed and is known to be the least sweet of the rebaudiosides (Goyal et al., 2009, Hossain et al., 2017, Muenprasitivej et al., 2022). While Reb's

D and M possess a higher degree of sweetness and a less intense bitter aftertaste when compared to Reb A (Prakash et al., 2014, Muenprasitivej et al., 2022). Thus, despite their minor concentrations in the *Stevia rebaudiana* plant's leaves, Rebs D and M are considered the next generation of stevia sweeteners due to their superior taste profiles (Prakash et al. 2014; Olsson et al. 2016).

Since receiving GRAS status in 2009 Stevia sweetened products have begun to emerge in the marketplace as alternatives to normally high sugar products. Examples of these products include Pepsi True, Coca-Cola Life, Cran Naturals (Old Orchard), and no-added sugar Heinz Hoops (Baker, 2017, Bloom, 2014, Bloom, 2015a, Bloom, 2015b). These products, however, have been met with mixed opinions by consumers, and a few of these products have even been pulled from the market space. Each of these products that launched using Stevia and then were pulled from the market space all sight the significant aftertaste as the reason consumers did not respond well to the products. Most consumers had reported it was the strong bitter aftertaste left in their mouths as the reason they did not repurchase the product (Baker, 2017). Given the consumers response of a strong bitter aftertaste it is reasonable to assume these products were being sweetened with rebaudioside A, which is known to have the most intense bitter aftertaste and is the most abundant rebaudioside in the stevia plants leafs (Goyal et al., 2009, Hossain et al., 2017, Olsson et al., 2016).

In new food product development, it is vital to conduct sensory testing to gauge whether a consumer will purchase the product. Sensory testing in new food product development can take several forms consumer testing, quantitative descriptive analysis, focus groups, and time-intensity to name a few (Lawless & Heymann, 2010). Each of these methods offer a unique insight into the possibility a product could succeed in the marketplace. However, for this manuscript, we will focus on consumer testing and time-intensity testing. Consumer testing allows for sensory scientists to gauge consumer's reactions to new food products using hedonic methods (Lawless & Heymann, 2010). Whereas time-intensity testing offers an insight into the consumers reaction to a product over time for certain attributes (Lawless & Heymann, 2010). Time-intensity testing of the sweetness, bitterness, and lingering aftertaste are essential to new food product development when introducing a zero-sugar or low-sugar version of a normally sugar dense product. With consumers demanding heathier zero-sugar or low-sugar food and beverage options, companies are scrambling to find ways to employ these natural high intensity sweeteners (Waters & Graf, 2020).

However, to the authors knowledge, it is not known whether any commercially available food products are on the market that are being sweetened using these next generation of stevia sweeteners (Rebs D and M) or being sweetened with a blend of all major and minor rebaudiosides (Rebs A, D, and M).

Overall Objectives

- To compare the consumer acceptability of the next generation of stevia (Rebs D and M) to the most widely used Reb (Reb A)
- To determine the role of information in consumer acceptability under blind (non-informed) and Informed conditions
- Investigate Stevia's physiochemical properties once included into normally high-sugar complex food systems such as: ice-creams, pound cakes, and cola-like beverages
- To determine an optimal ratio blend of Rebs A, D, and M to produce a commercially viable sweetener blend through time intensity analysis

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Chapter 2: Consumer Acceptability of No Added Sugar Pound Cakes under Blind and Informed Testing Conditions

Abstract

Stevia is an emerging natural high-intensity sweetener. There are negative perceptions of zero-calorie sweeteners, but studies that provide knowledge of these sweeteners improve their perception. This study evaluated consumer acceptability of a zero-sugar bakery product under blind and informed conditions (n=96). Rebaudioside A (Reb A) and the new types of stevia (Reb D and M) along with sugar as a control, were used to formulate pound cakes. Panelists evaluated the overall hedonic impressions (aroma, texture, flavor, aftertaste, sweetness & bitterness intensity) of the cakes under blind and informed conditions with an enforced 2-week break between evaluations. During the informed session, a document was provided prior to evaluating samples that included stevia's health benefits and the nutritional facts panels for the cakes. The cakes underwent volatile profile (electronic nose) and water activity (a_w) analysis. Overall, stevia cakes showed an increase in flavor and texture liking during the informed session when compared to the blind session, but only Reb A showed a significant difference ($P < 0.05$). The increase in liking scores indicated that the information positively affected the consumer's perception of the stevia-sweetened cakes attributes. The e-nose confirmed differences in aroma among stevia and sucrose cakes. There was a significant difference in a_w of the samples Rebs A, D, M versus sucrose with 0.96, 0.94, 0.95 versus 0.87, respectively ($P < 0.05$). This study illustrates that stevia, despite being a non-browning or fermenting sugar alternative, can be used in a practical baking application, and product-related information impacts consumer acceptability.

2.1. Introduction

Overconsumption of sugar has been associated with obesity, heart disease, and diabetes (Rippe & Angelopoulos, 2016). In the U.S., the average intake of sugar by adults 20 and older is 17 teaspoons a day (68 grams) (CDC 2021). With consumers becoming aware of their overconsumption of sugar, they have begun to look for ways to reduce sugar consumption. Using high-intensity sweeteners is one of the ways to curb sugar habits (Pang et al., 2021). Currently, there are only 8 FDA approved high-intensity sweeteners on the market, with 6 being artificial (aspartame, saccharine, acesulfame-k, neotame, and sucralose) and 2 being natural (stevia, and monk fruit extract) (FDA 2017). However, consumers have harbored a sense of distrust in these high-intensity sweeteners, especially high-intensity sweeteners, stemming from a lack of knowledge of the benefits and risks of artificial high-intensity sweeteners and inaccurate or misleading scientific information (Gardner et al. 2012, Farhat et al. 2021). Due to the mistrust or misinformation of high-intensity sweeteners, consumers want to seek natural sugar alternatives such as stevia to reduce or avoid their sugar intake.

Stevia is a natural non-nutritive high-intensity sweetener, which was first introduced to the Europeans in the 1800s but has long before then been cultivated by the Eupatorieae tribe in South America (Ramesh et al., 2006). The powder or liquid extracts from the plant's leaves have been demonstrated to be on average 200 to 300 times sweeter than sucrose (Bayliak et al., 2021, Peteliuk et al., 2021). The compounds responsible for the sweetness property of stevia leaves are steviol glycosides, including stevioside and rebaudiosides (Rebs) (Tanaka, 1982). Steviol glycosides make up almost 30-40% of the total sweet compounds in the leaves, while the other 60-70% consist of other bioactive compounds such as polyphenols, chlorophylls, carotenoids, and ascorbic acid, which contribute to stevia's anti-hypertensive, anti-diabetic, and anti-oxidant properties (Tanaka, 1982, Bursać Kovačević et al., 2018, Peteliuk et al., 2021)

The Rebs are non-fermentable, non-browning, high heat stable, and stable over a pH range of 3-9 (Barathi, 2003). They do not contain any calories and have been demonstrated to be glycemic index friendly (Ahmad et al., 2020, Pang et al., 2021). Of these compounds, Reb A is the most concentrated rebaudioside in stevia's leaves, making up 3.8% of the total dry weight. It is the most widely used purified Reb in the food industry, followed by Reb D and Reb M in their usage (Goyal et al., 2009, Hossain et al., 2017, Muenprasitivej et al., 2022). Reb A, however, is known to have a strong bitter aftertaste (Muenprasitivej et al., 2022). Reb D has the same sweetness intensity as Reb A but does not have as intense of a bitter aftertaste when compared to Reb A (Prakash et al., 2014, Muenprasitivej et al., 2022). Reb M is the sweetest and least bitter among the three Rebs (A, D, and M) (Prakash et al., 2014, Muenprasitivej et al., 2022). Although found only in trace quantities of the dried leaves (0.4 – 0.5%) (Olsson et al., 2016), Reb D and Reb M are considered to be the next generation of stevia due to their superior taste profiles (Prakash et al., 2014). These two Rebs (D &M) are currently being integrated into more complex high-sugar content food systems such as biscuits, cookies, brownies, and ice creams due to their superior taste profile and decreased aftertaste (Abdel- Rahim et al., 2004, Góngora Salazar et al., 2018, Muenprasitivej et al., 2022, Rungraeng et al., 2022).

Stevia received GRAS (Generally Recongized as Safe) status from the FDA in 2008 but has remained a relatively unpopular choice for a high-intensity sweetener. A Mintel survey in 2009 reported that 70% of consumers surveyed in the United States had never heard of stevia, and a further 62% said they had no interest in trying stevia. With 11% of consumers believed that stevia was unsafe for human consumption (Browne, 2009). Since then, the number of regular consumers of stevia has continued to increase year after year. In 2022 it was reported that 19% of United States consumers who have used a sweetener in the past month have used stevia compared to the 18.6% of consumers who reported using an artificial high-intensity sweetener (Olsen, 2022). Although the survey revealed that stevia was used as often as other artificial sweeteners, most of the consumers surveyed still used sugar or nutritive sugar alternatives such as honey or maple syrup as their preferred sweetener (Olsen, 2022). This leaves a large majority of consumers who may not know enough about stevia to use it, but still look for healthier sugar alternatives to reduce sugar consumption. The motivations for the transition to healthier sugar alternatives include 1) increasing consumer acceptability of the sugar alternatives that have similar taste profiles to sugar and 2) providing accurate scientific information on sugar and sugar alternatives to overcome mistrust or ignorance of sugar alternatives.

Sensory panels have been used to determine consumer perceptions and acceptance of novel products. One of the methods employed in evaluating consumer acceptance of these novel products is to test the products blind (without information) and reconvene after provided information regarding the product (informed). This method has been employed to prevent bias from influencing the panelists' decisions (Guinard et al., 2012, Kwak et al., 2017, Lee & Lee, 2020). Previous studies have showed that the information regarding the product positively affected consumer perception (Pereira et al., 2019, Schouteten et al., 2016). Thus, the objective of this study was to compare consumer acceptability of the next generation of stevia (Reb D and M) with the most widely used pure Reb (Reb A) in a high sugar application (i.e., pound cakes) under blind and informed conditions. This was to evaluate hedonic impressions of better-tasting stevia and determine the role of stevia information (Appendix A.) provided before tasting in the consumer acceptability of stevia-sweetened products. Physiochemical properties (water activity, textural analysis, and color) were also investigated to determine if stevia-sweetened pound cakes would alter any of these properties.

2.2. Materials and Methods

2.2.1. Ingredients and Samples

All materials required to produce the pound cake samples sweetened with either sucrose or stevia rebaudiosides were acquired locally from a grocery store or ingredient wholesalers; all-purpose flour (Kroger, Cincinnati, OH), baking powder (Kroger, Cincinnati, OH), coarse salt (Salt Morton, Chicago, IL), salted butter (Land O'Lakes, Arden Hills, MN), whole eggs (Kroger, Cincinnati, OH), vanilla extract (Spice Island, B&G Foods Inc, Parsippany-Troy Hills, NJ), lemon juice (Kroger, Cincinnati, OH), fresh lemon zest (Kroger, Cincinnati, OH), granulated white sugar (Kroger, Cincinnati, OH), soy lecithin (Fast Easy Bread, Salt Lake City, UT), sour cream (Daisy Brand, Dallas, TX), Maltodextrin (Tate & Lyle, London, UK) and powdered egg whites (Lesser Evil, Danbury, CT). Powdered egg whites were used in the stevia versions to improve texture and binding. The high-purity stevia rebaudiosides (95% Reb A, D, and M) were used in the formulation of the stevia pound cake samples (Sweegen, Santa Margarita, CA).

2.2.2. Sample Preparation

Reb A, D and M were used at 0.15% (w/w) in the stevia pound cake samples as it was found that 0.15% (w/w) of steviol glycosides had a similar level of sweetness to the 0.22% (w/w) ratio of sucrose used in control (sucrose) pound cakes without providing a high amount of bitter aftertaste (Muenprasitivej et al., 2022). The same concentration of steviol glycosides were used for the Reb A, D, and M to compare their sweetness and bitterness at the same concentration. Table 2.1 shows the lemon pound cake formulations and ingredient functionalities. The difference between the two formulas was 1.8% (900 vs 916.4 g for sucrose and stevia samples, respectively). The difference was to create the stevia-sweetened samples, which would be as close as possible to the sucrose sample.

Table 2.1. Ingredients, ingredient properties, and quantities used to prepare sucrose and stevia pound cake samples.

Ingredients	**Functionality**	**Sucrose (g)**	**Stevia (g)**
Flour	Structure	180	180
Baking Powder	Leavening	4	4
Salt	Fortification	2.8	2.8
Butter	Mouthfeel & Flavor	227	227
Eggs	Binder & Texture	200	0
Liquid Egg Whites*	Binder & Texture	0	200
Vanilla Extract	Flavoring	8.6	8.6
Lemon Juice	Flavoring	57.5	57.5
Lemon Zest	Flavoring	20	20
Sugar	Sweetener	200	0
Stevia (Reb A, D, M)	Sweetener	0	1.4
Maltodextrin	Bulking Agent	0	100
Soy Lecithin	Binding Agent	0	1.5
Sour Cream	Fat Replacer	0	113.4
	Total	900	916.4
	Calories Per Serving (69 g)	260	230

**Reconstituted to total volume of 200 mL using 50 grams powdered egg white plus150 mL of tap water*

First, the powdered eggs were rehydrated using a hand mixer with an immersion attachment (Bella Houseware, New York City, NY) in a Pyrex mixing bowl. Upon completion of the rehydration process, the frothed egg whites were combined with the butter and sour cream in a stand mixer (KitchenAid, Benton Harbor, MI). The dry ingredients (flour, salt, baking powder, maltodextrin, lemon zest, sucrose and/or stevia rebaudioside A, D, or M) were then added along with the lemon juice and vanilla extract, and mixed until the batter became homogenized.

The homogenized cake batter was then placed into a 9.25”x5.25”x2.75” loaf pan that was lined with parchment paper and greased with PAM no-stick cooking spray (Conagra, Chicago, IL) for easier removal post bake. The loaf pans containing the batter were placed into a preheated 177°C Viking professional electric oven (Viking Ranges, Greenwood, MS) for 65 minutes. To ensure the cakes were fully cooked the toothpick method was used. After removing the baked pound cakes from the oven, they were removed from the loaf pans and parchment paper and were allowed to cool on wire racks for 60 minutes before being portioned into 1.25”x 1.25”x 0.50” thick slices. These slices were then put into a 2-ounce serving cup and closed with a lid. The samples were labeled using three-digit random codes. Using the formulations above, nutritional and ingredient statements were prepared using Genesis R&D Supplement Formulation & Labeling Software (ESHA Research, Oak Brook, IL).

2.2.3. Panelists recruitment & testing procedure

Auburn University’s Institutional Review Board approved the protocol (IRB #19-437 EX 1910) (Auburn, AL), and all panelists gave written consent to participate in this study. This study was conducted onsite at the Tony & Libba Rane Culinary Science Center, and took place three times a week, with a two-week enforced break between visits.

A prescreening survey was conducted using Qualtrics (Qualtrics, Provo, UT) to collect demographic information, general dietary habits, and eligibility for this study. Among the total of 378 respondents, participants were included if they consumed sweetened bakery products at least 2-3 times per month, had no food allergies that would put them at risk if participating, and did not avoid consuming any high-intensity sweeteners, leaving 114 panelists for this study.

2.2.4 Blind testing

The panelists evaluated the pound cake samples on their first visit under blind testing conditions. Prior to the evaluation of their first sample, panelists were asked to fill out simple demographic information (age, gender, income, and education). This was to allow the panelists to have a task prior to the blind sample evaluation, as during the informed testing the panelists were given information to read prior to the sample evaluation in the informed testing. Samples were presented in a monadic sequential randomized order for evaluation using a 9-point hedonic scale (1-extremely dislike, 5-neither like nor dislike, 9-like extremely) for the following attributes: appearance, aroma, texture, aftertaste, and overall flavor. Additionally, panelists also evaluated the aftertaste, sweetness, and bitterness intensity using a 15-cm line scale after swallowing their first bite of the samples. The panelists' responses were recorded using RedJade sensory science software (RedJade Sensory Solutions LLC, Redwood City, CA) loaded onto Apple iPads (Apple, Cupertino, CA).

After evaluating the samples, panelists were administered a 10-question knowledge quiz pertaining to high-intensity sweeteners to measure their pre-knowledge level (Cieslinski, 2019). The placement of the pre-knowledge measurement at the end of the blind evaluation was to prevent panelists from forming bias over the samples to be evaluated. The 10 questions (Appendix B) were modified from Cieslinski’s (2019) previous study, including a true or false knowledge test pertaining to high-intensity sweeteners, both natural and artificial. Panelists could select “True”, “False”, or “I don’t know” with their own weighted ranking system. A value of 1 was assigned for every correct answer, a value of 2 was assigned for every wrong answer, and a value of 3 was assigned to a panelist’s answer when they selected “I don’t know”. A score of less than 16 out of 30 meant that panelists had a high knowledge of high-intensity sweeteners, while a score of greater than 16 out of 30 meant that panelists had a low knowledge of high-intensity sweeteners. The value, 16 was chosen as it allowed for no more than 3 missed answers to be selected by the panelists.

2.2.5. Informed Testing

Panelists on their second visit after a 2-week break would evaluate samples under informed testing conditions. Placed at each individual sensory booth was a laminated 8 ½” x 11” one-sided piece of paper containing information pertaining to the naturalness of stevia and its health benefits, as well as a comparison between nutritional information for a slice of a sucrose and stevia pound cake (Appendix A). Panelists were instructed to read the document thoroughly prior to answering an attention check question to ensure panelists had read through the document. Panelists then evaluated the four-pound cake samples as described above and completed eating behavior and consumption questions.

2.2.6. Electronic Nose Analysis

Heracles Neo electronic nose (e-nose, Alpha MOS, Toulouse, France) was used to determine the volatile profiles of pound cake samples by detecting the volatile compounds present in them. At first, all pound cake samples were allowed to cool down in a closed container at room temperature (~ 22°C) for 30 minutes to prevent any unwanted aerial contamination. Two grams of each sample were transferred into 20 mL e-nose vials. The vials containing 2 grams of the pound cake samples were agitated at 500 rpm with 50°C incubation temperature for 20 minutes in the autosampler incubator to generate volatiles for headspace analysis. After the incubation, the autosampler injector inserted 5000 ml of the headspace gas at 125 ml/s to concentrate the odor inside the trap. The trapping condition was maintained at 40°C for 50 seconds. Hydrogen gas was then used at 1 ml/min flow rate to carry the volatile components into the two capillary columns namely MXT-5 (non-polar) and MXT-1701 (slightly polar) for chromatographic analysis with two Flame Ionization Detectors (FID1 and FID2) that work in parallel. Both columns were 180 mm in diameter and 10 m long. The final temperature of the analysis sequence was increased up to 250°C at 1°C/s temperature ingredient from the initial 40°C temperature. The peaks of the chromatogram were identified by comparing the retention time of each compound with their corresponding Kovats retention indices in the AroChemBase database of AlphaSoft software (Version 2021-7.2.8, AlphaMOS). The e-nose analysis was conducted in triplicate.

2.2.7. Water Activity

Water activity was determined using an Aqualab® CX-2 water activity meter (Meter Food, Pullman, WA). Cake samples were broken into small pieces to fit into the sample cups. Approximately 5 grams of the pound cake samples were loaded and read at room temperature. The water activity testing was conducted in triplicate for each sample for more accurate data collection.

2.2.8. Texture Analysis

A sample from the center of each type of pound cake was taken after cooling at 4-5^{O}C for at least 16 hours. Slices were prepared at 5mm thickness using a serrated cake knife. The texture profile analysis (TPA “two-bite” test) of the cake samples were taken with a 36mm diameter cylindrical probe, with 50% compression and a testing speed of 1.0 mm s-1 using a TA-X2I textural analyzer (Texture Technologies Corp. Hamilton, MA). The crust of the pound cake samples was removed to ensure that the textural properties of each slice of cake were as similar as possible to each other. Other parameters include a pre-test speed of 2.0 mm s and a post-test speed of 2.0 mm s, with a trigger force of 5 grams. This test was replicated 7 times for each sample for more accurate data collection.

2.2.9. Color Analysis

The crust of the pound cakes and the inside of the pound cakes were measured separately to see how the absence of a reducing sugar would affect the overall color in the stevia cakes, as well as browning. First, crusts were cut from the cakes, and were put aside for color testing. Then, cubes measuring 1” x 1” (25 x 25mm) were cut from the inside of each cake for internal color readings using a Hunter colorimeter (Hunter Labs, Reston, VA). Data was collected as L*a*b* values. Comparisons were drawn by determining the difference in L*a*b* values between samples containing sugar (sucrose) and the three separate stevia cakes (*Rebs A, D,* and *M*). The analyses were done in triplicates.

2.2.10. Data Analysis

The mean differences from each of the experimental samples (Reb A, Reb D, and Reb M) and the control (sucrose) were compared to one another using one-way analysis of variance (ANOVA) with Tukey-HSD using SAS with a 95% confidence level ($P < 0.05$) (SAS Institute, Cary, NC) for post-hoc pairwise sample comparisons among the samples. Tukey-HSD was also used to compare demographic information and purchasing intent. A t-test was used to compare the high and low sweetener knowledge groups, as well as to compare the blind and informed sessions using SAS with a 95% confidence level ($P < 0.05$) (SAS Institute, Cary, NC). The Principal

Component Analysis (PCA) was conducted to analyze the e-nose data using the AlphaSoft software (Version 2021-7.2.8, AlphaMOS).

2.3. Results and Discussion

2.3.1. Panelist Demographics

Out of 114 panelists (Table 2.2.), a vast majority of panelists were female (69.30%) and were between the ages of 18 and 33 years old. Caucasians made up the majority of panelists at 78.95%, followed by Asian (8.77%), Latino (6.14%) and Black (3.51%). Of these panelists 53.51% held a high school diploma or GED, followed by 23.68% holding a graduate level degree, and 21.93% holding a 4-year college degree. The majority of panelists made under $30,000 a year (82.46%), followed by $30,000 - $49,999 (11.40%), $50,000 - $79,999 (0.75%), and over $80,000 (4.39%).

Table 2.2. Socio-demographic characteristics of panelists (n=114).

Variable	Definition	Panelist (n)	Frequency (%)
Gender	Male	35	30.70%
	Female	79	69.30%
Age	18-25	71	62.28%
	26-33	26	22.81%
	34-41	8	7.02%
	42-49	7	6.14%
	50-57	2	1.75%
Education Level	High School Diploma or GED	61	53.51%
	2-year college degree	1	0.88%
	4-year college degree	25	21.93%
	Graduate degree	27	23.68%
Household Income	Under $30,000	94	82.46%
	$30,000 - $49,999	13	11.40%
	$50,000 - $79,999	2	1.75%
	Over $80,000	5	4.39%
Ethnicity	White or Caucasian	90	78.95%
	Hispanic or Latino	7	6.14%
	Black or African American	4	3.51%
	Asian or Pacific Islander	10	8.77%
	Other	2	1.75%
	Prefer not to say	1	0.88%

The breakdown between panelists knowledge groups is reported in Table 2.3. A majority of panelists (n=59) scored low enough (x<16) to qualify as a high-knowledge panelist. While the remaining 55 panelists scored greater than 16 on their knowledge quiz placing them in the low knowledge group.

Table 2.3. High and low sweetener-knowledge groups of the panelists (n=114).

Knowledge Level*	Panelist (n)	Frequency (%)	Average Score (± SEM)
High	59	51.75%	15.04 ± 2.61
Low	55	48.25%	20.26 ± 2.61

**Panelists' knowledge levels were obtained through a short 10-question true or false quiz that was administered to participants post-blind sample evaluation.*

The attribute liking scores for low and high knowledge groups are reported as an average for both blind and informed sessions (Table 2.4). The only significant difference ($P < 0.05$) reported between the two groups was in the aroma attribute of the pound cake samples. The lower knowledge group rated the aroma of the cakes significantly higher ($P = 0.013$) than those in the high knowledge group. With the main negative descriptors of artificial sweeteners being reported as mostly negatively associated (bitter, off-flavor, off odor, astringent, metallic, and chemically) it is reasonably assumed that consumer perception of these artificial sweeteners also has been negative (Hanger et al., 1996, Gwak et al., 2012). However, it should also be stated that while there was a significant difference ($P < 0.05$) in aroma liking scores, the total difference between the high and low knowledge groups is small at only 0.31. Additionally, other attribute likings did not show significant differences between the high and low knowledge groups. This lack of statistically significant differences means that panelists’ pre-knowledge level did not affect the liking scores of these stevia-sweetened pound cakes. This was the opposite outcome than what was hypothesized, for it was hypothesized in this study that the higher knowledge group would rate the samples higher in both attribute and intensity liking scores. These findings align with Frøst and Noble (2002), who found that there was no correlation between sensory expertise and the effect of knowledge when evaluating several different wines. They concluded that individual preferences play a larger role in decision-making than consumer knowledge (Frøst & Noble, 2002). However, Hartmann et al. (2021) and Baker et al. (2022) found that consumer knowledge does have an impact on consumer perceptions of the foods they consume. Hartmann et al. (2021) found that the higher knowledge groups were able to better plan a lunch menu with significantly less environmental impact, as well as with a higher nutritional value when compared to the low knowledge groups (Hartmann et al. 2021). Baker et al. (2022) found that there was a small positive relationship that exists between a consumer's level of knowledge and their acceptance of functional foods (Baker et al., 2022). A consumer's familiarity level is another important factor to consider when investigating the effect of knowledge or external factors on the consumer's acceptance (Fandos Herrera & Flavián Blanco, 2011). Thus, in our study we included the sugar version of the pound cakes assuming the consumers acceptance would not be impacted by their knowledge level due to their high level of familiarity with the product.

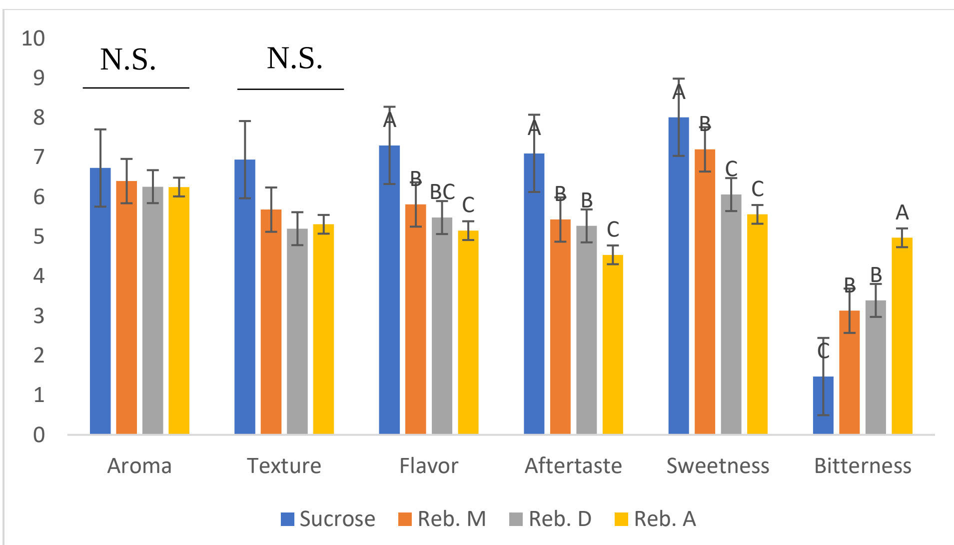
Table 2.4. The influence of sweetener knowledge on attribute liking scores (± SEM) of pound cakes sweetened with stevia (Reb A, D, and M).

Knowledge Level*	Appearance	Aroma	Texture	Flavor	Aftertaste
High (n=59)	6.31 ± 0.12	6.26 ± 0.12[a]	5.75 ± 0.13	5.99 ± 0.13	5.64 ± 0.13
Low (n=55)	6.40 ± 0.12	6.57 ± 0.12[b]	5.83 ± 0.14	5.88 ± 0.13	5.53 ± 0.14

**Panelists' knowledge levels were obtained through a short 10-question true or false quiz that was administered to participants post-blind sample evaluation; A 9-point hedonic scale was used to measure liking scores of the pound cakes; Average liking scores were reported from both under blind and informed sessions (Blind testing conditions were preformed with no information given to the panelists prior to evaluation while informed testing conditions were conducted after panelists had received information regarding stevia's naturalness and health benefits); Different letters indicate statistical differences (P < 0.05)*

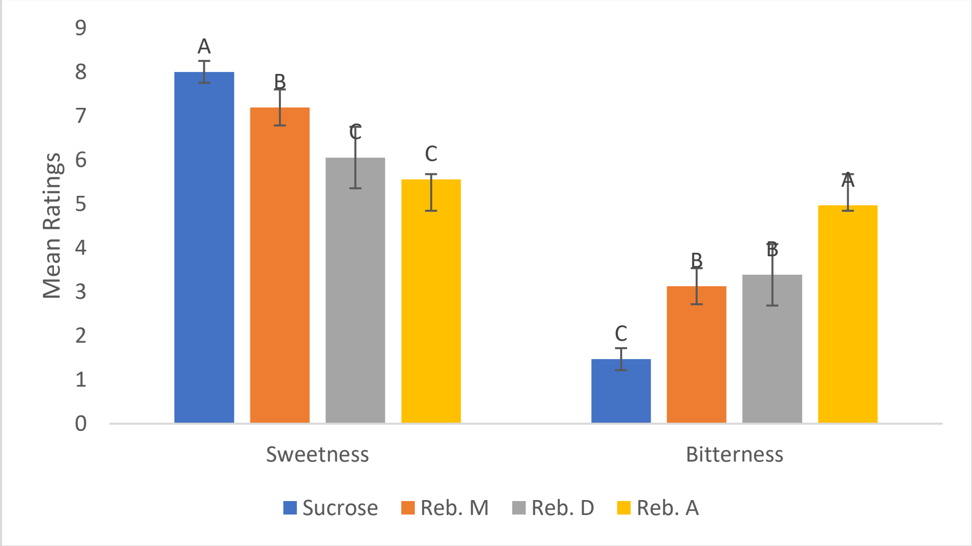
2.3.2. Blind versus informed testing

Figure 2.1. Attribute liking scores (± SEM) of pound cake samples sweetened with stevia and sucrose under the blind condition (n=114).



**Different letters indicate statistical differences (P < 0.05); N.S. means no significance among the samples; Blind testing conditions were preformed with no information given to the panelists prior to evaluation.*

Figure 2.2. Mean intensities (± SEM) of sweetness and bitterness of pound cake samples sweetened with sucrose and stevia under the blind testing condition (n=114).



**Different letters indicate statistical differences (P < 0.05). Blind testing conditions were preformed with no information given to the panelists prior to evaluation.*

There were significant differences (P < 0.05) in texture, flavor, and aftertaste liking among samples (Figure 2.1). The sample evaluation of the hedonic attributes by panelists indicates that consumers will rate sucrose-sweetened products higher than stevia-sweetened ones under blind testing conditions (Figures 2.1 and 2.2). During the informed testing session (Table 2.5) panelists also rated the attribute liking scores of the sucrose sample significantly higher than the stevia samples (P < 0.05) although they read the information about the health benefits, naturalness, and nutritional information of the stevia samples prior to sample evaluation. Sucrose was consistently and significantly (P < 0.05) rated higher than the stevia samples under both blind and informed conditions, as was expected. However, the differences in the liking scores of sucrose and stevia samples were higher than expected. This could be partially due to the stimuli range effect (Mcbride, 2007). One sample (sucrose) yielding a positive hedonic reaction can make the other samples presented in the same evaluation appear mediocre in comparison and, therefore, be rated lower due to the bias introduced from the higher rated sample (Mcbride, 2007, Lawless & Heyman, 2010). Had the sucrose sample not been a part of this study, it may be a reasonable assumption that the stevia samples overall attribute scores would have been rated higher when comparing the blind to the informed testing condition scores. While undergoing the informed sample evaluation, panelists were informed about the sweetener of each sample they were evaluating. However, each sample presented to the panelists was still in a complete randomized block design. Thus, potentially allowing for the familiarity with sucrose and the unfamiliarity with stevia to impact their scores further.

When comparing liking scores of pound cake samples under the blind and informed sessions, the texture and flavor attributes showed a significant increase in liking scores from blind to informed testing sessions (Table 2.5). This significant increase from blind to informed testing conditions was more with the pound cake sample sweetened with Reb A (i.e., both texture and flavor liking). This was important to note that the Reb A sample was the least liked sample during the blind testing condition. This increase in attribute liking scores of the least liked sample from the blind to informed testing sessions hints at a potential link between attribute liking scores and information presented to panelists prior to sample evaluation. The information synthesized by panelists prior to evaluation has been shown to influence rating scores when compared to a blind session (Schouteten et al., 2016, Grasso et al., 2022). Schouteten et al. (2022) showed a similar observation during their increase in liking scores from blind to informed testing sessions where while not a huge jump in attribute or overall liking scores, the panelists did report a more willingness and openness to try hybrid beef burgers based off the information provided to them pre-sample evaluation. Additionally, providing information to panelists to reduce the effect of food neophobia is not a new concept. Several studies evaluating consumer perception of new food products have undergone blind and informed testing conditions in an attempt to remove any perceived bias panelists may have to reduce their distrust and help embrace their acceptance of a new food product or ingredient. Both Gurdian et al. (2021) and Schouteten et al. (2016) used blind and informed sessions to study new food ingredients and products and showed an increase in the liking scores in the informed session when compared to the blind session (Schouteten et al., 2016, Gurdian et al., 2021). A study conducted to evaluate the influence of visual information when comparing samples to one another demonstrated that input of either positive or negative information would impact the panelists ranking and choice of samples (Hurling & Shepherd, 2003). While this study used a dummy sample of either high or low quality before the actual sample evaluation, they too demonstrated that there could be a stimulus introduced prior to sampling that will influence a consumer's behavior (Hurling & Shepherd, 2003).

Table 2.5. Attribute liking scores (± SEM) *for each pound cake sample sweetened with sucrose and stevia (Reb A, D, and M) under blind and informed testing conditions.*

	Appearance		Aroma		Texture		Flavor		Aftertaste	
Samples	Blind	Informed	Blind	Informed	Blind	Informed	Blind	Informed	Blind	Informed
Sucrose	6.44 ± 0.15	6.50 ± 0.16	6.77 ± 0.15	6.69 ± 0.14	6.67 ± 0.17	7.20 ± 0.16	7.25 ± 0.15	7.34 ± 0.17	7.15 ± 0.16	7.01 ± 0.17
Reb M	6.33 ± 0.15	6.36 ± 0.16	6.48 ± 0.15	6.34 ± 0.14	5.48 ± 0.17	5.89 ± 0.16	5.60 ± 0.15[a]	6.04 ± 0.17[b]	5.28 ± 0.16	5.60 ± 0.17
Reb D	6.42 ± 0.15	6.18 ± 0.16	6.40 ± 0.15	6.08 ± 0.14	5.22 ± 0.17	5.15 ± 0.16	5.53 ± 0.15	5.43 ± 0.17	5.35 ± 0.16	5.20 ± 0.17
Reb A	6.43 ± 0.15	6.18 ± 0.16	6.28 ± 0.15	6.21 ± 0.14	4.94 ± 0.17[a]	5.64 ± 0.16[b]	4.89 ± 0.15[a]	5.38 ± 0.17[b]	4.41 ± 0.16	4.68 ± 0.17

Different letters denote a significant difference as reported by a ($P < 0.05$); A 9-point hedonic scale was used to measure liking scores; Blind testing conditions were preformed with no information given to the panelists prior to evaluation while informed testing conditions were conducted after panelists had received information regarding stevia's naturalness and health benefits.

The intensity of sweetness and bitterness are reported using a 15-cm line scale (Table 2.6). While sucrose did remain significantly different ($P < 0.05$) from the stevia rebaudioside samples, there was a significant difference ($P < 0.05$) between Reb M and Rebs D and A in the sweetness attribute. This was to be expected as Reb M is the sweetest of the rebaudiosides used in this experiment (Goyal et al., 2009). However, regarding bitterness, Reb D and M were reported as less bitter than Reb A, which Reb A is known to be one of the most bitter stevia rebaudiosides (Jung et al., 2021).

Table 2.6. Mean intensity (± SEM) of sweetness and bitterness *for each pound cake sample sweetened with sucrose and stevia (Reb A, D, and M) under blind and informed testing conditions.*

	Sweetness*		Bitterness	
Samples	Blind	Informed	Blind	Informed
Sucrose	7.78 ± 0.29	8.22 ± 0.30	1.44 ± 0.34	1.54 ± 0.33
Reb M	6.48 ± 0.29[a]	8.00 ± 0.30[b]	3.31 ± 0.34	2.97 ± 0.33
Reb D	5.71 ± 0.29[a]	6.48 ± 0.30[b]	3.38 ± 0.34	3.43 ± 0.33
Reb A	5.20 ± 0.29	5.93 ± 0.30	4.93 ± 0.34	5.01 ± 0.33

Different letters denote a significant difference as reported by (P < 0.05); Sweetness and bitterness intensities were measured on a 15 cm line scale; *Blind testing conditions were preformed with no information given to the panelists prior to evaluation while informed testing conditions were conducted after panelists had received information regarding stevia's naturalness and health benefits.*

Table 2.7. Gender effect on attribute liking scores of stevia pound cakes under both blind and informed testing conditions.

	Appearance	Aroma*	Texture*	Flavor	Aftertaste*	Sweetness	Bitternes
Males (n= 35)	6.29 ± 0.11	6.31 ± 0.11^{a}	5.66 ± 0.12^{a}	5.85 ± 0.11	5.46 ± 0.12^{a}	6.65 ± 0.23	3.21 ± 0.2
Females (n= 79)	6.48 ± 0.14	6.66 ± 0.14^{b}	6.06 ± 0.16^{b}	6.15 ± 0.15	5.86 ± 0.16^{b}	6.85 ± 0.29	3.27 ± 0.3

A 9-point hedonic scale was used to measure liking scores of the pound cakes; Average liking scores were reported from both under blind and informed sessions (Blind testing conditions were preformed with no information given to the panelists prior to evaluation while informed testing conditions were conducted after panelists had received information regarding stevia's naturalness and health benefits); Different letters indicate statistical differences (P < 0.05).

2.3.3 Male and Female Comparison

As presented in Table 2.7, there were significant differences ($P < 0.05$) in liking scores between male and female panelists in aroma, texture, and aftertaste, with the female panelists rating those attributes significantly higher than males. Females have been noted to have a higher perception of and sensitivity to smell and taste than males impacting their meal choice and intensity ratings (Havlicek et al., 2008). This is due to females having more fungiform papillae than their male counterparts do (Bartoshuk et al., 1994). Males tend not to rely as heavily on aroma but on visual attributes in their meal choice and liking scores (Havlicek et al., 2008). This is evident in the findings (Table 2.7), where the males reported a lower aroma liking score when compared to females). Da Silva et al. (2013) concluded that females have more accurate sensory perception than males and that they have lower gustative detection thresholds for both sweetness and bitterness intensities (Da Silva et al., 2013). However, the intensity ratings did not reveal that females rated higher sweetness or bitterness intensity than their male counterparts.

In addition, it should be noted that research into the consumption of low-calorie sweeteners has revealed that females are more regular consumers of low-calorie high-intensity sweeteners when compared to males (Sylvetsky, A et al. 2012). In the current study, while not statistically significant ($P > 0.05$), there were higher consumption rates of low-calorie products for the female panelists than for the male panelists in this study. This more regular consumption of these sweeteners that females have when compared to males likely explains why their liking scores were significantly higher ($P < 0.05$) in the aroma, texture, and aftertaste attributes (Table 7). This higher rate of consumption and exposure can be liked due to females putting more importance on dietary nutrition than males, as well as females putting a higher preference on weight control and dieting than males (Wardle et al., 2004). The authors do acknowledge that one limitation of this study was the high female gender bias (69.30%). While the authors did try to keep the study as close to a 50% gender ratio, gender was not a primary disqualification factor. High frequency consumers and consumers who did not avoid HIS were the primary decision in recruiting panelists. In future studies gender will be among the primary disqualifying factors to achieve a 50% gender ratio.

2.3.4 E-Nose Analysis Principal Component Analysis (PCA) to Discriminate Samples:

Heracles Neo E-nose (Alpha MOS, Toulouse, France) was used to analyze the volatile compounds of the pound cake samples. To discriminate the samples, a PCA biplot (Figure 2.3) was acquired from the AlphaSoft software (Version 2021-7.2.8, AlphaMOS).

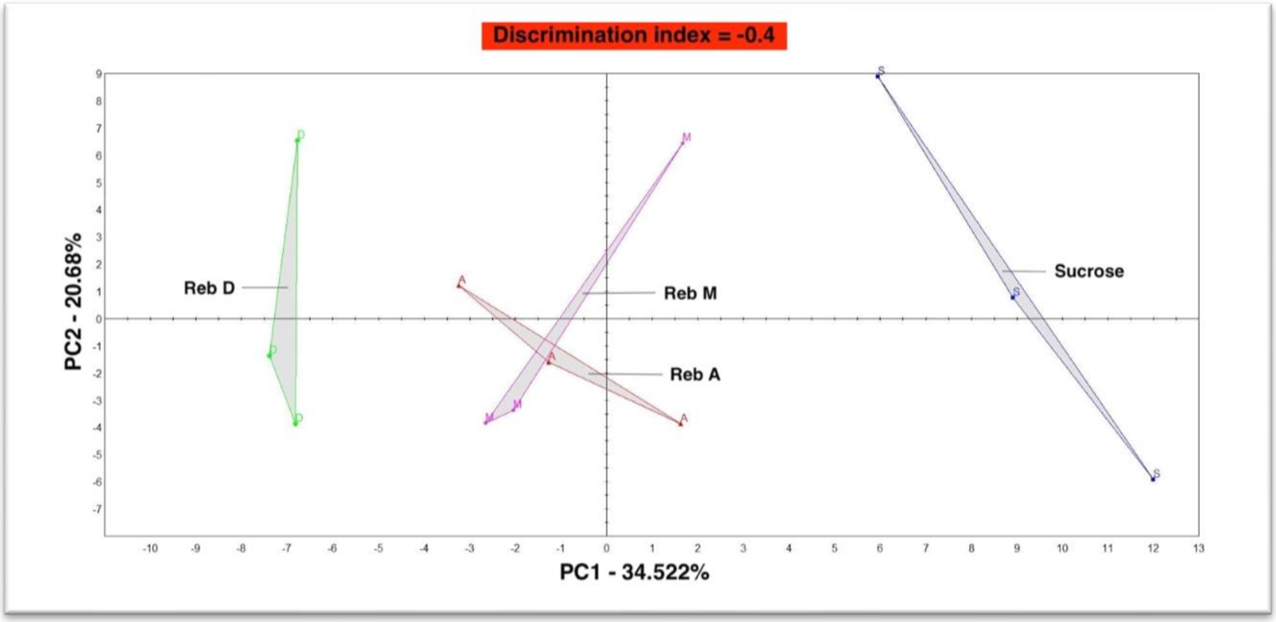


*Figure 2.3. Principal component analysis of the volatile profiles of pound cake samples using Heracles Neo E-nose (Alpha MOS, Toulouse, France). *The three data points of each sample represent three replicates.*

It is evident from the findings that the e-nose could differentiate sucrose and stevia pound cake samples. The Discrimination Index (DI) was negative due to having Reb A and Reb M overlapping, meaning that the e-nose was not able to distinguish Reb A and M samples, but the Reb A, D, and M pound cakes were clearly separated from the sucrose pound cake. The first principal component (PC1) of the PCA plot could explain 34.52% of the variation within the axis, whereas 20.68% variability could be described by the second principal component (PC2) of the PCA plot, explaining 55.20% of total variability within the e-nose responses analyzed for the pound cake samples. Despite having negative D.I. and overlap in Reb A and M pound cake e-nose responses in the PCA plot, these results may have been possible because of the similar backbone in the molecular structures of these steviol glycosides (Ceunen & Geuns, 2013). These results also indicate that the sucrose pound cake has a distinct odor profile. This may have been possible because sucrose reacted with the amino acids of the cake dough through the Maillard reaction to form a molecular complex that produced a more desirable aroma in sucrose-sweetened pound cakes (Liu et al., 2022). Furthermore, the occurrence of caramelization in sucrose-sweetened sugar-made pound cakes may have released greater intensity of common aroma compounds to make it different from the other samples (Martins et al., 2000).

2.3.5 Aroma Profiles of Pound Cake Samples Analyzed by E- nose:

Table 2.8. List of volatile compounds identified in pound cake samples sweetened with sucrose and stevia (Reb A, D, and M).*

Compound Name	**Retention Index**		**Pound Cake Samples**				**Sensory Descriptors**
	MXT						
	#5	**#1701**	Sucrose	Reb A	Reb D	Reb M	
Acetaldehyde	434	493	✓	✓	✓	✓	Fresh, fruity, pleasant
Propanal	451	566	✓	✓	✓	✓	Cocoa, earthy, nutty
Methyl acetate	489	596	X	✓	✓	✓	Blackcurrant, fragrant, fruity, pleasant, sweet
S(+)-2-butanol	591	699	X	✓	X	✓	Oily
Butan-2-one	594	690	X	✓	X	✓	Butter, cheese, chocolate
2-Hexanol	801	900	✓	✓	✓	✓	Cauliflower, Chemical, Fatty, Fruity, terpenic, Winey
Dimethyl sulfoxide	839	1063	✓	X	✓	X	Alliaceous, fatty, garlic, mushroom, oily, sulfurous
Cyclohexanone	896	995	X	✓	✓	✓	Minty, peppermint
1S-()-a-pinene	936	950	✓	✓	✓	✓	Herbaceous, pine, resinous, fresh, sharp

4-Methylhexan-1-ol	953	1064	✓	X	X	✓	Grassy, sweaty
1-Heptanol	970	1077	✓	✓	✓	✓	Aromatic, fatty, fresh, green, nutty, chemical
1,3,5-Trimethylbenzene	994	1011	✓	✓	✓	✓	Aromatic, herbaceous
b-Phellandrene	1031	1059	X	✓	✓	✓	Fruity, herbaceous, minty, pleasant
L-Limonene	1034	1061	✓	X	X	✓	Citrus, minty, orange, pine, woody
Limonene	1048	1061	✓	✓	✓	✓	Citrus, fruity, minty, orange, peely
g-Terpinene	1060	1089	✓	✓	✓	✓	Citrus, fruity, herbaceous, lemon, oily, sweet, woody
Cis-Decalin	1106	1129	✓	✓	✓	✓	Odor, slight

*✓ = Detected; X = Not detect; *Heracles Neo electronic nose (Alpha MOS, Toulouse, France) was used to analyze volatile compounds of pound cakes sweetened with sucrose and stevia (Rebs A, D, and M).*

All the odor compounds identified in the pound cake samples are presented in Table 8, along with their sensory descriptors. As can be seen in Table 8, a total of 12 volatile compounds were identified in pound cake samples made with sucrose, whereas 14 compounds could be found in Reb A-made pound cakes. Similarly, Reb D pound cakes contained 13 volatile compounds. However, the highest number of volatile compounds were detected in Reb M pound cakes consisting of 16 compounds in the e-nose analysis. The odorous sensory descriptors specific to all compounds detected in e-nose analysis are given in Table 2.8.

The common odor compounds that were found in all of the pound cake treatments are listed as Acetaldehyde, Propanal, 2-Hexanol, 1S-()-a-pinene, 1-Heptanol, 1,3,5-Trimethylbenzene, Limonene, g-Terpinene, and Cis-Decalin. In comparison, the presence of 4-Methylhexan-1-ol and L-Limonene was observed only in pound cakes made of Reb A and D. Despite the absence of L-Limonene in Reb A and M made pound cakes, these two cake samples contained Limonene (of which L-Limonene is an Levo isomer) along with pound cakes made of sugar and Reb M. S(+)-2-butanol and butan-2-one were the only two compounds that were present in Reb A and Reb M-made pound cakes. However, their presence was not detected in pound cakes made of sucrose and Reb D. On the other hand, the only volatile compound unique to Reb A and Reb M made pound cakes was identified to be dimethyl sulfoxide, while its presence was not detected in pound cakes made of sugar and Reb D. The commonality of these specific compounds may be the reason why the Reb A and Reb M made pound cakes are overlapped as depicted in Figure 2.3, whereas pound cakes made of sucrose and Reb D are clearly separated in the PCA bi-plot.

Methyl acetate, Cyclohexanone, and b-Phellandrene were the only three compounds that were identified only in pound cakes prepared using steviol glycosides (i.e., Reb A, D, M) but not in the pound cake made with sucrose. One possible reason why these three compounds (Methyl acetate, Cyclohexanone, and b-Phellandrene) were not detected in sucrose pound cake may be explained by the higher water binding capability of sugar which contributes to its humectant property. Along with its higher solubility in water as compared to steviol glycosides (Reb A, D, and M), the Maillard browning reaction that took place in sucrose pound cake during baking may have broken down Methyl acetate, Cyclohexanone and b-Phellandrene into other flavor compounds inside the food matrix (Salar et al., 2020) which may have contributed to better consumer likability of the aroma of sucrose-made pound cakes as compared to pound cakes made of Reb A, D, and M (see Table 2.4). This observation is consistent with the observations made by Quitral et al. (2019), who reported that cupcakes made of sugar exhibited superior aroma as compared to cupcakes prepared with different concentrations of stevia, maltitol, polydextrose, sucralose, tagatose, and isomaltitol.

2.3.6 Water Activity

Figure 2.4 depicts the average water activity (a_w) for the four samples. There were no significant differences ($P > 0.05$) between the three stevia samples. However, there was a significant difference ($P < 0.05$) between the control (sucrose) and stevia samples (Reb A, D, and M). The higher a_w between the three stevia samples was to be expected due to the lack of a water binding agent (sucrose), as well as the increase in liquid-containing ingredients (sour cream, water for egg white rehydration) when compared to the sucrose sample (Ergun & Leitha, 2010). In Figure 2.1 and Table 2.5, the overall texture liking for each of the four samples shows that the sucrose sample is rated higher than the three rebaudioside samples. Water activity has been demonstrated to have a direct impact on crust and crumb formation (Primo-Martín et al., 2008). Therefore, it is likely that with higher water activity, and more dense crumbs were formed in the three rebaudioside samples leading to a lower overall texture rating.

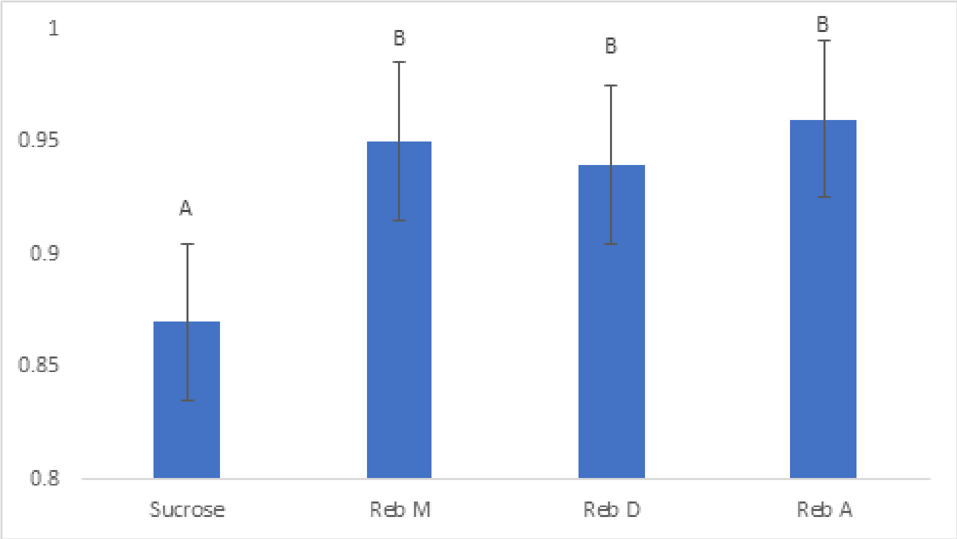
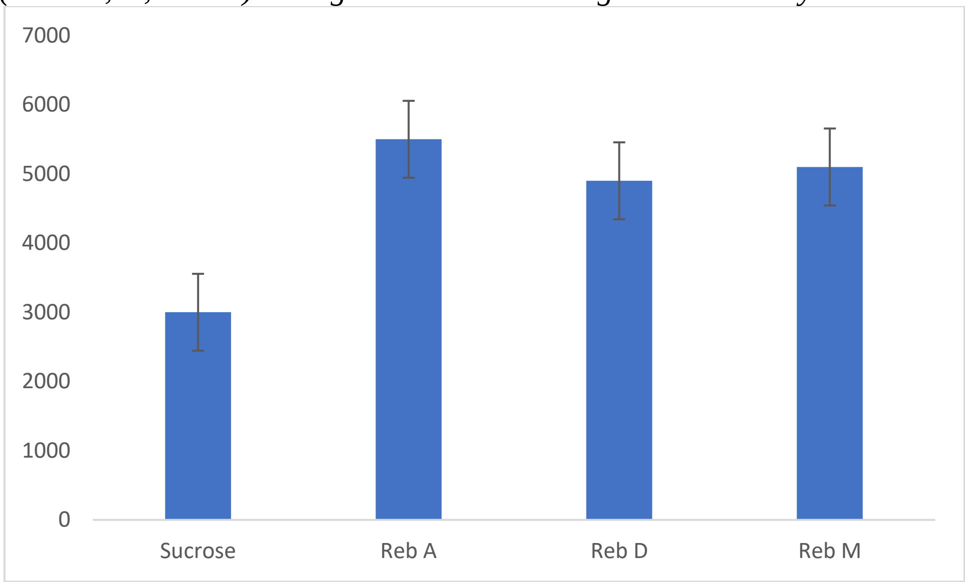


Figure 2.4. Water activity (± SEM) *of the pound cake samples sweetened with sucrose and stevia (Rebs A, D, and M). * Different letters indicate statistical differences (P < 0.05).*

2.3.7 Texture Analysis

In Figure 2.5 the average two bite force test results are reported. There were no significant differences (P > 0.05) reported between the three experimental samples (Rebs A, D, and M) and the control Sample (sucrose). The two-bite compressibility testing shows that all the pound cake samples that contained stevia performed similarly, with a first bite average of ~4928.9N. This is consistent with the data collected in the consumer testing, where panelists reported the stevia samples as denser than the sugar samples.

Figure 2.5 Average force (± SEM) *recorded for the pound cake samples sweetened with sucrose and stevia (REbs A, D, and M) during a two-bite test using a texture analyzer*.*

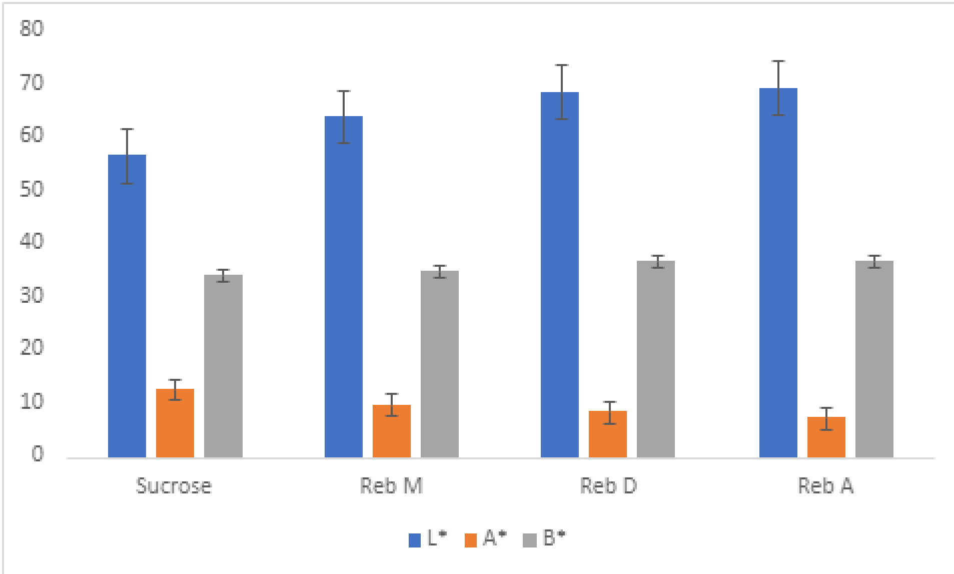


Texture analyses were conducted with a 36mm diameter cylindrical probe, with 50% compression and a testing speed of 1.0 mm s-1 using a TA-X2I textural analyzer (Texture Technologies Corp. Hamilton, MA); The forces are reported in terms of newtons (N)

2.3.7 Color Analysis

In Figure 2.6 the L*A*B* values are reported. There were no significant differences (P > 0.05)observed between the experimental samples (Rebs A, D, and M) when compared to the sucrose control. The experimental samples (Rebs A, D and M) had a higher L* value when compared to the sucrose control. However, the sucrose control held higher values of A* and B* when compared to the three experimental samples. Despite these findings being non-significant they do indicate that rebaudiosides could be used in baking applications.

*Figure 2.6 Hunter L*A*B* colorimeter analysis of pound cake samples with standard error bars.*



2.4. Conclusion

A significant increase in attribute likings of Reb A-sweetened pound cake, the least preferred sample among the three stevia-sweetened pound cakes (Reb A, D, and M) illustrates that information about health and nutritional benefits of high-intensity sweeteners can positively affect sensory perception. However, pre-knowledge levels about sweeteners did not influence consumer acceptability of pound cakes sweetened with stevia. Panelist gender did show that there was a significant difference in females rating the samples higher than their male counterparts in aroma, texture, and aftertaste. The water activity of the three stevia-sweetened pound cakes (Reb A, D, and M) was significantly higher than the sucrose sample. Overall, our study suggests that there is an impact of product-related information on consumer acceptability. By providing educational information (health benefit information, nutritional facts information, and origin information) to consumers, overall consumer perception and acceptability of zero sugar-added products can be improved. The implications of this impact of educational information regarding stevia-based products. Providing similar information on the packaging material of commercially available products could increase the perception and acceptance of these products, as they are relatively novel in the market space. This study also demonstrates that a non-browning HIS can be used in a baking application. Further expanding the range of stevia-based products. This study was limited by a higher female gender bias (69.30%), as well as the cost prohibition to using 100% only of Rebs D & M. This is due to Rebs D& M being found in only minute concentrations in the leaves of the stevia plants currently. Future crop breeding programs could potentially address this by increasing the concentrations of Rebs D & M in the plant's leaves.

Conflicts of Interest

There are no conflicts of interest to declare.

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Chapter 3: Time-intensity Analysis of Six *Stevia Rebaudiana* Rebaudioside Blends in Complex Food Systems

Abstract

Stevia is an emerging natural high-intensity sweetener, with a major rebaudioside (~3.4% of dry mass of stevia leaves), rebaudioside A (Reb A) being the most commonly used in the food industry currently. However, it possesses an intense bitter aftertaste that impacts the consumer acceptability of stevia-sweetened products. Minor rebaudiosides (0.3-0.4% of dry mass of the stevia leaves), Rebs D and M do not possess as intense a bitter aftertaste as that of Reb A and are known as the next generation of stevia. Therefore, it is proposed that an optimized blend of these rebaudiosides could produce an optimal sweetness and bitterness taste profile, while minimizing the production cost associated with using only minor rebaudiosides, Rebs D and M. Through the employment of time-intensity testing in ice creams (n=42) and colas (n=39), six rebaudioside ratios were evaluated to determine this optimal ratio. Panelists evaluated the sweetness and bitterness characteristics of these six ratios for a duration of 90 seconds. This study found that a ratio containing a majority proportion of Reb M delivered a more immediate, intense, and prolonged sweetness sensation, while decreasing the bitterness intensity and duration in ice creams. It also found that a ratio containing equal parts Reb A and D delivered a more immediate, intense, and prolonged sweetness sensation, while decreasing the bitterness intensity and duration in cola beverages. This study illustrates that an optimized blend of stevia rebaudiosides can be employed in complex food systems to optimize the sweetness and bitterness taste profiles.

3.1. Introduction

Stevia is a naturally derived non-nutritive high-intensity sweetener that has been cultivated in South America since the time of the Eupatorieae tribe (Ramesh et al., 2006). Stevia's sweetness properties originate from the plant leaves that produce steviol glycosides. These glycosides once extracted from the leaves on average are 200 to 300 times sweeter than sucrose (Peteliunk et al., 2021, Tanaka,1982). These steviol glycosides include steiviosides and rebaudiosides (Rebs) which are known to make up 30-40% of the total sweet compounds in the leaves. While the other 60-70% are bioactive compounds such as polyphenols, chlorophylls, carotenoids, and ascorbic acids. All of these contribute to stevia's anti-diabetic, antioxidant, and anti-hypertensive properties (Bursać Kovačević et al., 2018, Peteliuk et al., 2021, Tanaka, 1982).

These rebaudiosides once extracted from the leaves are crystalline in structure, odorless, and present as a white powder once the extraction process has been completed (Gasmalla et al., 2014). The stevia rebaudiosides are heat stable (up to 200^{o}C), non-browning, non-fermentable, and stable through a pH range of 3-9 (Barathi, 2003). Rebaudiosides have also been documented to be glycemic index friendly and do not contain any calories (Ahmad et al., 2020, Pang et al., 2021). Stevia's rebaudiosides have also demonstrated antimicrobial properties in which they can inhibit the growth and reproduction of bacteria. These rebaudiosides have been known to prevent *Streptococcus mutants, Pseudomans aeruginos,* and *Proteus vulgaris* from reproducing when exposed to these stevia rebaudiosides (Singh & Rao, 2005). While Reb A is the most abundant rebaudioside in stevia's leaves (3.8% total dry mass) and is the most commonly used rebaudioside in the food industry, it does possess a strong bitter aftertaste (Goyal et al., 2009, Hossain et al., 2017, Muenprasitivej et al., 2022). Rebs D and M possess the similar sweetness intensity that Reb A does, but with a decreased bitter aftertaste. Due to the decrease in bitter aftertaste Rebs D and

M, while making up 0.4-0.5% total dry mass of the stevia plant's leaves, are considered the next generation of stevia (Olsson et al., 2016).

These complex food systems were chosen for this investigation due to the large emergence of no-sugar added or low-sugar versions of these food systems (Olsen, 2022). These complex food systems (ice cream and colas) are known to be high in sugar content and consumed by a majority of the United States population (Scott, 2023). With stevia being a non-nutritive HIS it is a potential replacement for sugar in these two products without sacrificing the flavor characteristics associated with ice creams and colas (Ahmad et al., 2020, Pang et al., 2021). With Reb A being the most used Reb in commercial applications but possessing a strong bitter aftertaste it has driven many consumers away from stevia. However, with Rebs D and M possessing a less intense aftertaste they open the door for their use in these commercially available products. (Goyal et al., 2009, Hossain et al., 2017, Muenprasitivej et al., 2022). Due to Rebs D and M being present in such low concentrations in the stevia plant's leaves, it is theorized that a blend of these three rebaudiosides is possible to produce commercially without imparting a strong bitter aftertaste, unlike a 100% Reb A stevia blend (Olsson et al., 2016).

Time-intensity analysis is a scientific method where quantifiable results are produced through continuous changes in perception in that it continuously monitors panelists' perceived sensations from onset through conclusion (Cliff & Heymann, 1993). Previous studies noted that consumer acceptability of HIS depends on the HIS ability to closely mimic the sweetness time profile of sucrose, making time-intensity a key tool in analyzing panelists' responses over time to reduced or low-sugar versions of products (Lawless & Heymann 2010, Rodrigues et al., 2016). Therefore, in this study, we examined which next generation of rebaudioside sweetener blend in high sugar applications such as ice creams and colas delivers the most optimal delivery of sweetness with the least bitter onset.

3.2. Materials and Methods

3.2.1 Ingredients and Samples

All materials required to produce the stevia-sweetened colas were acquired locally from grocery stores or on Amazon.com; vanilla extract (Kroger, Cincinnati OH), lemon extract (Kroger, Cincinnati, OH), lime extract (McCormick, Hunt Valley, MD), orange extract (Kroger, Cincinnati, OH), star anise extract (Kroger, Cincinnati, OH), ground cinnamon (Kroger, Cincinnati, OH), ground nutmeg (Kroger, Cincinnati, OH), lavender flowers (Kroger, Cincinnati, OH), citric acid (Millard Brands, Lakewood, NJ), and ginger extract (Home Choice Enterprise Limited, St. Catherine, WI).

All the materials required to produce the stevia-sweetened ice creams were also acquired locally from grocery stores; heavy cream (Horizon Organic, Broomfield, CO), non-fat dry milk (Kroger, Cincinnati, OH), vanilla extract (Spice Island, B&G Foods Inc, Parsippany-Troy Hills, NJ), and polydextrose (Litesse®, DuPont, Wilmington, DE).

For both the stevia-sweetened colas and ice cream formulations the high-purity stevia rebaudiosides (95% Reb A, D, and M) were acquired from Sweegen (Santa Margarita, CA). The six Ratios of rebaudiosides (Table1) chosen in this experiment were based on quantitative descriptive analysis conducted by BlueCalifornia (Rancho Santa Margarita, CA) to determine optimal Ratios of Rebs A, D, and M to reduce the cost when compared to 100% Reb A, D, and M when compared to the optimal sweetness.

Table 3.1. Six ratios used in the ice cream or cola formulations.

Sweetener Ratios*	Reb A %	Reb D %	Reb M %
Ratio 1	100	0	0
Ratio 2	0	100	0
Ratio 3	0	0	100
Ratio 4	50	50	0
Ratio 5	16.7	66.7	16.6
Ratio 6	66.7	16.7	16.6

**Rebs A, D, and M were used at the concentrations that were isosweet with 9% sucrose, which were 0.060%, 0.058%, and 0.043%, respectively.*

The ratios mentioned above in Table 3.1 were chosen from a descriptive panel from Blue California Ingredients (Rancho Santa Margarita, CA) that matched the iso-sweetness of sucrose. It is important to note that Rebs A, D, and M do not share the same iso-sweetness level with sucrose. Blue California Ingredients found while conducting their descriptive panel the iso-sweetness level of Rebs A, D and M were 0.060%, 0.058%, and 0.043% respectively when placed in 1000 mL of water. The data acquired from this descriptive panel indicated that ratios 4,5, and 6 exhibited distinct sweetness and bitterness taste profiles while maintaining the iso-sweetness level of sucrose.

3.2.2 Cola Preparation

A concentrated syrup solution of each rebaudioside blend (Ratio 1-6) (Table 1) was first created to be then diluted into the proper syrup to carbonated water Ratio. In table # below the syrup ingredients and weights are reported. To make this concentrated syrup solution of each rebaudioside blend all ingredients except for the rebaudioside blend and caramel coloring were added to a medium-sized saucepot. The caramel coloring and dry stevia blend were placed into a separate Pyrex mixing bowl. The liquid ingredients were heated over medium-high heat until boiling on a Viking professional electric range (Viking Ranges, Greenwood, MS) in the uncovered medium sauce pot. Upon reaching boiling the liquid mixture was placed over low heat and allowed to simmer in the now covered medium sauce pot for 20 minutes. After simmering for 20 minutes, the mixture was removed from the heat and strained through two fine mesh metal sieves with a coffee filter in between to catch any particulates from making it into the mixture. This mixture was strained through these sieves directly into the Pyrex mixing bowl containing the caramel coloring and rebaudioside blend Ratio. The now combined ingredients were then homogenized using a hand mixer with an immersion attachment (Bella Houseware, New York City, NY) for a total time of 2 minutes. Then the now homogenized mixture was transferred to airtight containers and allowed to cool overnight in a refrigerator. To carbonate the water 890 mL of cold (~4 ^{0}C) water (Deer Park 100% natural spring water, Bluetriton Brands, Samford, CT) was placed into a 1L SodaStream plastic bottle (SodaStream, Kefar Sava, Israel). This 1L SodaStream bottle was then placed in a SodaStream Terra carbonator (SodaStream, Kefar Sava, Israel) and carbonated for 10 seconds before immediately being capped and placed back into the fridge for 30 minutes to allow for the bottle to settle. After 30 minutes 110mL of each respective syrup was placed into each bottle before once again immediately being capped and placed back into the refrigerator until it was time to serve the panelists. The afternoon before each day of the testing this process was repeated to ensure that the panelists were served the freshest and most carbonated batch of cola possible. When serving the panelists, a 2-oz sample was placed into a clear 4-oz plastic cup. Each cup was blinded by a 3-digit random code.

Table 3.2. Ingredients used to prepare the stevia-sweetened syrups for the cola samples.

Syrup Ingredients	Weight (g)
Vanilla extract	0.3
Lemon extract	0.3
Lime extract	0.3
Orange extract	0.83
Star anise extract	0.05
Ground cinnamon	0.39
Ground nutmeg	0.27
Lavender flowers	0.46
Citric acid	2.83
Ginger extract	0.74
Caramel food color	4.75
Rebaudioside ratio blend	0.5
Water	473

3.2.3 Ice Cream Preparation

The six total ratios used in this experiment (Table 3.1) were used at 0.09% (w/v) in the ice cream formulation. The ice cream formulation was taken from a previous study conducted by Muenprasitivej et al., (2022) (Table 3.3). The dry ingredients (polydextrose, non-fat dry milk, and stevia Ratios 1, 2, 3, 4, 5, 6) were homogenized in a KitchenAid stand mixer (KitchenAid, St. Joseph, MI) with warm water (~43 ^{0}C) added and blended until the dry ingredients were hydrated. Next, the liquid ingredients (heavy cream and vanilla extract) were added under continuous stirring until homogenized once more. The mixture was placed in a refrigerator (4 °C) for one hour to cool, and then placed in an ice cream maker (Cuisinart, Stamford, CT) and churned until the proper texture was achieved. Once the process was completed, the ice cream was transferred into a plastic container and stored at –20 ^{0}C until the day of testing. In table 4 below the formulation for the ice cream is shown. The day before testing began 2 Oz scoops of ice cream were placed into 4 Oz clear plastic ramekin containers and closed with a lid before being stored back in the walk-in freezer. Each sample received a 3-digit blinding code.

Table 3.3. Ingredients used to prepare the stevia-sweetened ice cream samples.

Ice cream ingredients	Weight (g)
Heavy cream	400
Non-fat dry milk	140
Water	650
Vanilla extract	5
Rebaudioside ratio blend	1.3
Polydextrose	245

3.2.4 Panelists Recruitment & Testing Procedure

Auburn University's Institutional Review Board approved the protocol (IRB #23-148 EX 2303) (Auburn, AL), and all panelists gave written consent to participate in this study. This study was conducted onsite at the Poultry Science Buildings Sensory Science Center and took place over a span of two days. Each day the panelists only evaluated a maximum of 4 samples (1 warm-up, and 3 experimental samples). Each day the same warm-up samples were provided to the panelists to familiarize themselves with the time-intensity testing procedures. For the Cola experiment Sam's Choice Diet Cola (Walmart, Bentonville, AR) was used as the warm-up sample, and for the ice cream experiment SimpleTruth (Kroger, Cincinnati, OH) low-carb no-sugar added vanilla ice cream was used as the warm-up sample.

A prescreening survey was conducted using Qualtrics (Qualtrics, Provo, UT) to collect demographic information, general dietary habits, and eligibility for this study. A total of 190 potential panelists responded to this prescreening survey. Only 50, however, were recruited for the cola study, and another 50 separate individuals were recruited for the ice cream study. The following requirements were necessary to be made to be determined eligible for the cola study. Panelists needed to consume cola on a 2-3 times per week basis, have no food allergies and had no avoidance of consuming high-intensity sweeteners. For a panelist to be eligible to participate in the ice cream study, they needed to consume ice cream 2-3 times per month, have no food allergies, and not avoid consuming high-intensity sweeteners.

3.2.5 Sample Evaluation

Upon obtaining consent on the first day, the panelists were handed an informational sheet with instructions on how to perform the time-intensity testing using RedJade sensory science software (RedJade Sensory Solutions LLC, Redwood City, CA) on the Apple iPad (Apple, Cupertino, CA). This instruction sheet (Appendix A) was provided to them once again in the sensory booths, as well as upon arrival on the second day of testing as well.

Panelists would first receive a warm-up sample to familiarize themselves with the time-intensity testing method. This warm-up sample was provided to the panelists prior to the evaluation of the stevia-sweetened ice creams and colas on both days. Panelists were asked to evaluate the cola and ice cream samples for overall liking and flavor liking using a 9-point hedonic scale prior to starting the time-intensity evaluation and were asked to reevaluate the overall liking of the sample after the time-intensity portion had been completed. The panelists were also asked to evaluate the mouthfeel liking of the ice cream using a 9-point hedonic scale prior to the time-intensity evaluation of the ice creams. Panelists were also asked to reevaluate their overall liking of the ice creams after the time-intensity evaluation as well.

Panelists were instructed to take one small sip of cola or a small bite of ice cream and hold this in their mouths while they evaluated these 9-point hedonic questions. Once they had completed these questions, they were asked to swallow the sample and immediately begin the time-intensity evaluation of these samples for sweetness and bitterness for a duration of 90 seconds. The panelists were instructed to move the digital slider along the 10-cm long line scale that best represented their feeling to either the sweetness or bitterness of the samples.

Upon completion of their sample evaluations for both hedonic attributes and for time-intensity. The panelists were asked to fill out simple demographic information of the first day (name and email), and on the second day were asked to fill out more in-depth demographic information (name, age, gender, income, and education).

3.2.6 Data Analysis

Data collected from the time-intensity curves were compared using one-way analysis of variance (ANOVA) with Tukey mean testing using SAS 9.1(SAS Institute, Cary, NC) assuming a 95% confidence level ($P < 0.05$). The time-intensity analysis graphs were prepared using SAS 9.1 (SAS Institute, Cary, NC) and attributes derived from them underwent an ANOVA and Tukey test averages ($P < 0.05$).

3.3. Results & Discussion

3.3.1 Panelists Demographics Ice Cream

Out of 50 recruited panelists, only 42 completed the ice cream study with a gender ratio of 50:50. A majority of the panelists were between the ages of 26-33 years old (40.48%), followed by the 18-25 years old age range (38.10%). Most of the panelists held a graduate-level degree (54.76%) followed by a 4-year college degree (33.33%). A vast majority of panelists earned under $30,000 a year (76.19%). Most panelists were Asian or Pacific Islander (42.86%) followed by Caucasians (35.71%), and Hispanics or Latinos (16.67%).

Table 3.4. Demographical information of the panelists for the ice cream time-intensity testing (n=42).

Variable	Definition	Panelist (n)	Frequency (%)
Gender	Male	21	50%
	Female	21	50%
Age	18-25	16	38.10%
	26-33	17	40.48%
	34-70	9	21.42%
Education Level	High School Diploma or GED	3	7.15%
	2-year college degree	2	4.76%
	4-year college degree	14	33.33%
	Graduate degree	23	54.76%
Household income	Under $30,000	32	76.19%
	$30,000 - $49,999	4	9.52%
	$50,000 - $79,999	4	9.52%
	Over $80,000	2	4.77%
Ethnicity	White or Caucasian	15	35.71%
	Hispanic or Latino	7	16.67%
	Black or African American	1	2.38%
	Native American or Pacific Islander	1	2.38%
	Asian or Pacific Islander	18	42.86%
	Other	0	0%
	Prefer not to say	0	0%

3.3.2 Time-Intensity Analysis of the Sweetness Properties in Ice Cream

In Table 3.5 the area under the curve (AUC), average intensity (AI), maximum intensity (IMAX), and time of maximum intensity (TIMAX) are reported for each of the six different stevia Ratios used in the formulations of the ice creams. There were no significant differences ($P > 0.05$) reported between any of the 6 Ratios for AUC and AI. However, there was a significant difference ($P < 0.05$) in IMAX for Ratio 3 when compared to Ratios 1, 2, 4, 5, and 6. Ratio 3 is comprised of 100% Reb M, which is known as the sweetest rebaudiosde out of the 3 rebaudiosides used in this study (A, D, and M) (Goyal et al., 2009, Muenprasitivej et al., 2022). In terms of TIMAX, there were significant differences ($P < 0.05$) between Ratios 1, 4, 5, and 6 when compared to Ratio 2. Ratio 2 had the shortest time to the maximum point of intensity out of the 6 Ratios. Ratio 2 is comprised of 100% Reb D, and when we examine the next Ratio containing the highest percentage of Reb D (Ratio 5 66.7% Reb D, and Ratio 4 50% Reb D) we see that both Ratios also possessed a shorter time to maximum intensity. Thus, potentially indicating that Reb D has the fastest onset of sweetness out of the 3 rebaudiosides (A, D, and M).

Table 3.5. Mean time-intensity analysis for sweetness properties in ice cream.

Ratios	AUC	AI*	IMAX**	TIMAX**
Ratio 1	320.57	3.60	5.47^{ab}	34.7^{a}
Ratio 2	306.94	3.44	5.43^{ab}	19.5^{b}
Ratio 3	385.80	4.33	6.56^{a}	28.8^{ab}
Ratio 4	331.72	3.72	5.58^{ab}	33.8^{a}
Ratio 5	289.44	3.25	4.53^{b}	40.4^{a}
Ratio 6	344.42	3.86	5.60^{ab}	35.0^{a}

*Means with common letters in the same column do not represent a significant difference (P < 0.05) as determined by Tukey's mean test; Area under the curve (AUC), average intensity (AI), intensity maximum (IMAX), and time of maximum intensity (TIMAX) are reported above; *AI is reported using a 10-point line scale **IMAX and TIMAX are reported in seconds.*

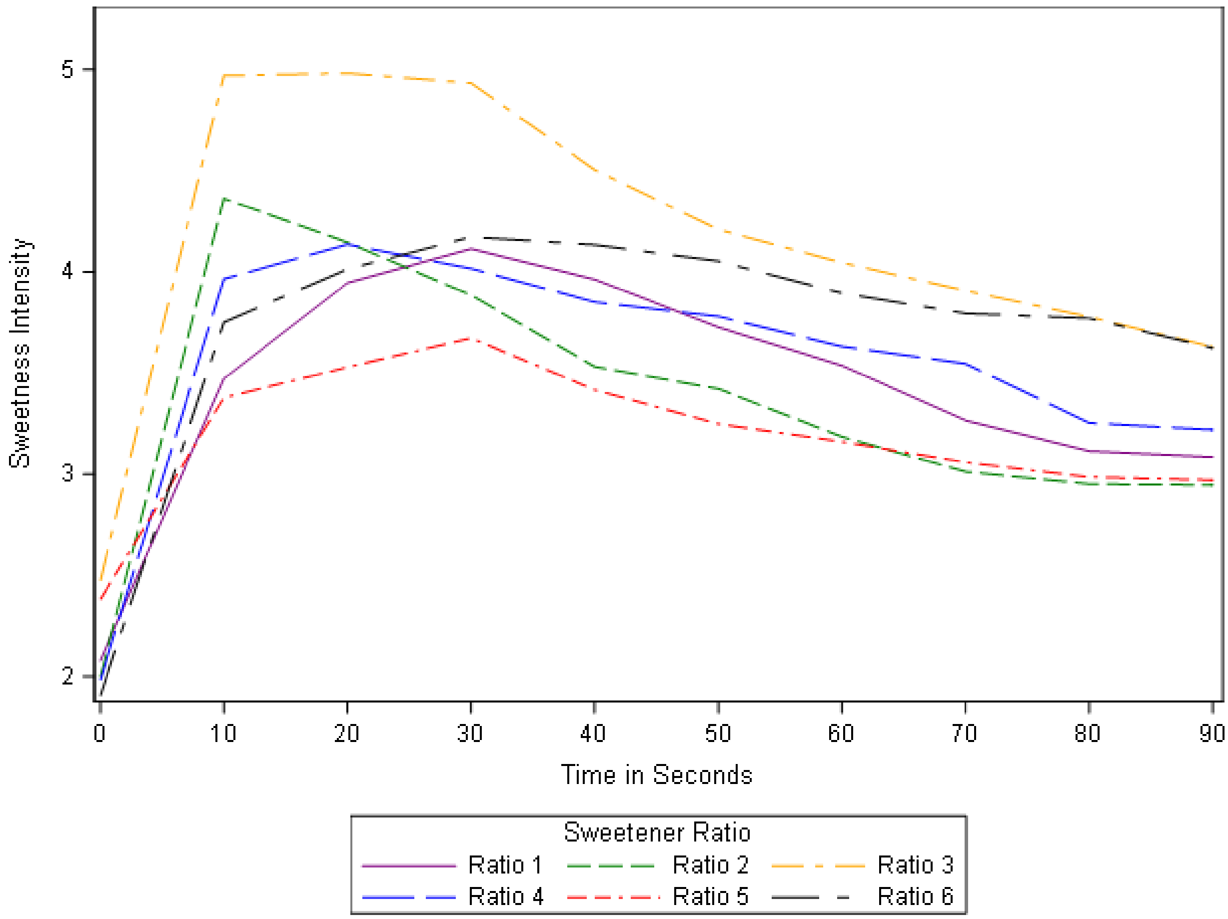

Figure 3.1. Time-intensity profile of sweetness properties of ice creams sweetened with rebaudioside blend ratios as recorded on a 10-cm line scale.

It can be noted that the Ratio 3 (100% Reb M) had the most intense and prolonged sweetness intensity (Figure 3.1). This was to be expected as Reb M is known to have the highest sweetness intensity out of Rebs A, D, and M (Goyal et al., 2009, Hossain et al., 2017). Ratio 5 contained the second most intense and prolonged sweetness intensity out of the ratios. This could be explained by Ratio 5 containing 66.7% Reb D, and equal parts Reb A and M. Reb D and M are sweeter than Reb A and this intense sweetness of Rebs D and M likely masked the bitterness taste of the minute concentration of Reb A (Muenprasitivej et al., 2022). This combination of having a majority portion of the ratio being made of Rebs D and M when compared to Reb A, could explain the second most intense and prolonged sweetness duration. Ratio 5 had the second longest prolonged sweetness intensity during the time measured, but out of the three blended Ratios. However, Ratio 6 did have a more intense initial sweetness intensity. This could be explained by Ratio 6 containing 66.7% Reb A, whereas Ratio 5 contains 66.7% Reb D. This higher inclusion rate of Reb D, which is known to be sweeter than Reb A likely helped to extend the sweetness intensity duration of Ratio 6 (Goyal et al., 2009, Hossain et al., 2017).

3.3.3 Time-Intensity Analysis of the Bitterness Properties in Ice Cream

AUC, AI, IMAX, and TIMAX are reported for each of the six different stevia Ratios used in the formulations of the ice creams (Table 3.6). There was a significant difference ($P < 0.05$) reported in the AUC of Ratio 1 (100% Reb A) compared to Ratios 2 (100% Reb D) and 3 (100% Reb M). This difference was to be expected as Ratio 1 has the most significant bitter aftertaste when compared to Ratios 2 and Ratios 3 (Olsson et al., 2016). This significant difference ($P <$ 0.05) was also seen between Ratios 1 and Ratios 2, and Ratio 3 in the AI. However, this significant difference ($P < 0.05$) was only seen between ratios 1 and Ratios 2 in IMAX. While Ratios 1 and 3 were significantly different ($P < 0.05$) than Ratios 2, 4, 5, and 6 in terms of TIMAX. While no significant difference ($P >$0 .05) was observed between Ratios 4,5, and 6 in terms of AUC, and AI. While Ratios 3,4,5 and 6 did not show a significant difference ($P >$0.05) in bitterness attributes for IMAX. Ratios 2, 4, 5, and 6 did show a significant difference (P < 0.05) from Ratios 1 and 3 in TIMAX.

Table 3.6. Mean time-intensity analysis for bitterness properties in ice cream.

Ratios	AUC	AI*	IMAX**	TIMAX**
Ratio 1	328.01[a]	3.68[a]	5.36[a]	35.8[b]
Ratio 2	192.72[b]	2.16[b]	3.50[b]	26.5[a]
Ratio 3	186.75[b]	2.09[b]	3.79[ab]	34.6[b]
Ratio 4	230.10[ab]	2.58[ab]	4.41[ab]	27.1[a]
Ratio 5	245.11[ab]	2.75[ab]	4.20[ab]	29.1[a]
Ratio 6	229.01[ab]	2.57[ab]	4.05[ab]	28.8[a]

*Means with common letters in the same column do not represent a significant difference (P < 0.05) as determined by Tukey's mean test; Area under the curve (AUC), average intensity (AI), intensity maximum (IMAX), and time of maximum intensity (TIMAX) are reported above; *AI is reported using a 10-point line scale **IMAX and TIMAX are reported in seconds.*

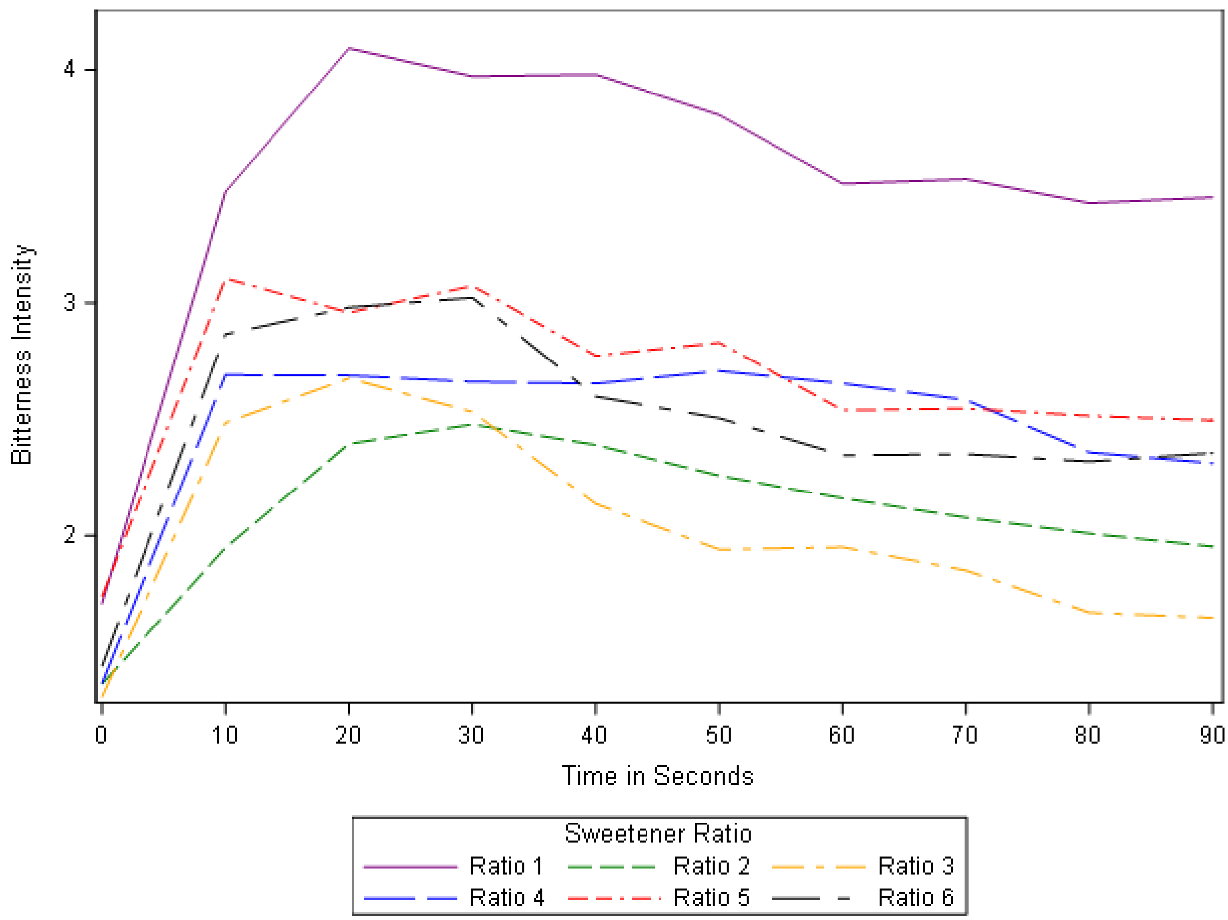

Figure 3.2. Time-intensity profile of bitterness properties of ice creams sweetened with rebaudioside blend ratios as recorded on a 10-cm line scale.

Ratio 1 (100% Reb A) had the most intense and prolonged bitterness intensity, and Ratio 3 (100% Reb M) had the shortest and least prolonged intensity as seen above (Figure 6). This was to be expected as Reb A is known as having the most bitter aftertaste of the Rebs, while Reb

M is known to have the least bitter aftertaste (Goyal et al., 2009, Hossain et al., 2017, Muenprasitivej et al., 2022). While Ratio 6 had the least bitter intensity out of the 3 combined Ratios. This is due to the makeup of Ratio 6 containing 66.7% Reb D, and equal parts Rebs A and M. Ratio 2 (100% Reb D) was found to be less bitter than Ratio 6, despite the minute amounts of Reb A in that Ratio. The bitterness of Ratio 6 was still significantly higher than either of Ratio 2 or 3 (100% Reb D, 100% Reb M). It could be justified despite this that despite the elevated bitterness intensity and duration of Ratio 6, it did produce a longer sweetness duration than that of Ratio 1 and 2 (100% Reb A, 100% Reb D) Figure 3.2.

3.3.4 Panelists Demographics Cola

Out of 50 recruited panelists, only 39 completed the cola time-intensity testing (Table 8), most panelists were female (64.10%) and were between the ages of 18-25 (43.59%) or 26-33 years old (35.90%). Caucasians were the majority ethnicity (58.97%) followed by Asian (17.95%), and Hispanics or Latinos (12.82%). Most panelists earned less than $30,000 a year (61.54%) and were followed by the $30,000 - $49,999 a year earner (23.08%). Most panelists held a graduate level degree (46.15%) followed by a 4-year college degree (41.03%).

Table 3.7. Demographical information of the panelists for the cola time-intensity testing (n=39).

Variable	Definition	Panelist (n)	Frequency (%)
Gender	Male	14	35.90%
	Female	25	64.10%
Age	18-25	17	43.59%
	26-33	14	35.90%
	34-50	8	20.51%
Education Level	High School Diploma or GED	5	12.82%
	2-year college degree	0	0%
	4-year college degree	16	41.03%
	Graduate degree	18	46.15%
Household income	Under $30,000	24	61.54%
	$30,000 - $49,999	9	23.08%
	$50,000 - $79,999	3	7.69%
	Over $80,000	3	7.69%
Ethnicity	White or Caucasian	23	58.97%
	Hispanic or Latino	5	12.82%
	Black or African American	3	7.69%
	Asian or Pacific Islander	7	17.95%
	Other	0	0%
	Prefer not to say	1	2.56%

3.3.5 Time-intensity Analysis of the Sweetness Properties in Cola Soft Drinks

AUC, AI, IMAX, and TIMAX are reported for each of the six different stevia Ratios used in the formulations of the cola soft drinks (Table 3.8) A significant difference (P < 0.05) was reported in AUC and AI between Ratios 1 and 6, while no significant difference (P > 0.05) was observed between IMAX for any of the 6 Ratios. A significant difference (P < 0.05) was observed in TIMAX when comparing Ratios 1 and 4 to Ratios 2, 3 ,5 and 6. As well as a significant difference in TIMAX when comparing Ratio 3 and Ratio 5.

Table 3.8. Mean time-intensity analysis for sweetness properties in cola soft drinks.

Ratios	AUC	AI*	IMAX**	TIMAX**
Ratio 1	101.64^{b}	1.14^{b}	2.22	20.8^{a}
Ratio 2	193.96^{ab}	2.17^{ab}	3.04	36.5^{bc}
Ratio 3	110.91^{ab}	1.24^{ab}	2.52	29.5^{b}
Ratio 4	186.56^{ab}	2.09^{ab}	3.49	21.9^{a}
Ratio 5	141.04^{ab}	1.58^{ab}	2.64	39.3^{c}
Ratio 6	203.95^{a}	2.28^{a}	3.43	33.0^{bc}

*Means with common letters in the same column do not represent a significant difference ($P < 0.05$) as determined by Tukey's mean test; Area under the curve (AUC), average intensity (AI), intensity maximum (IMAX), and time of maximum intensity (TIMAX) are reported above; *AI is reported using a 10-point line scale **IMAX and TIMAX are reported in seconds.*

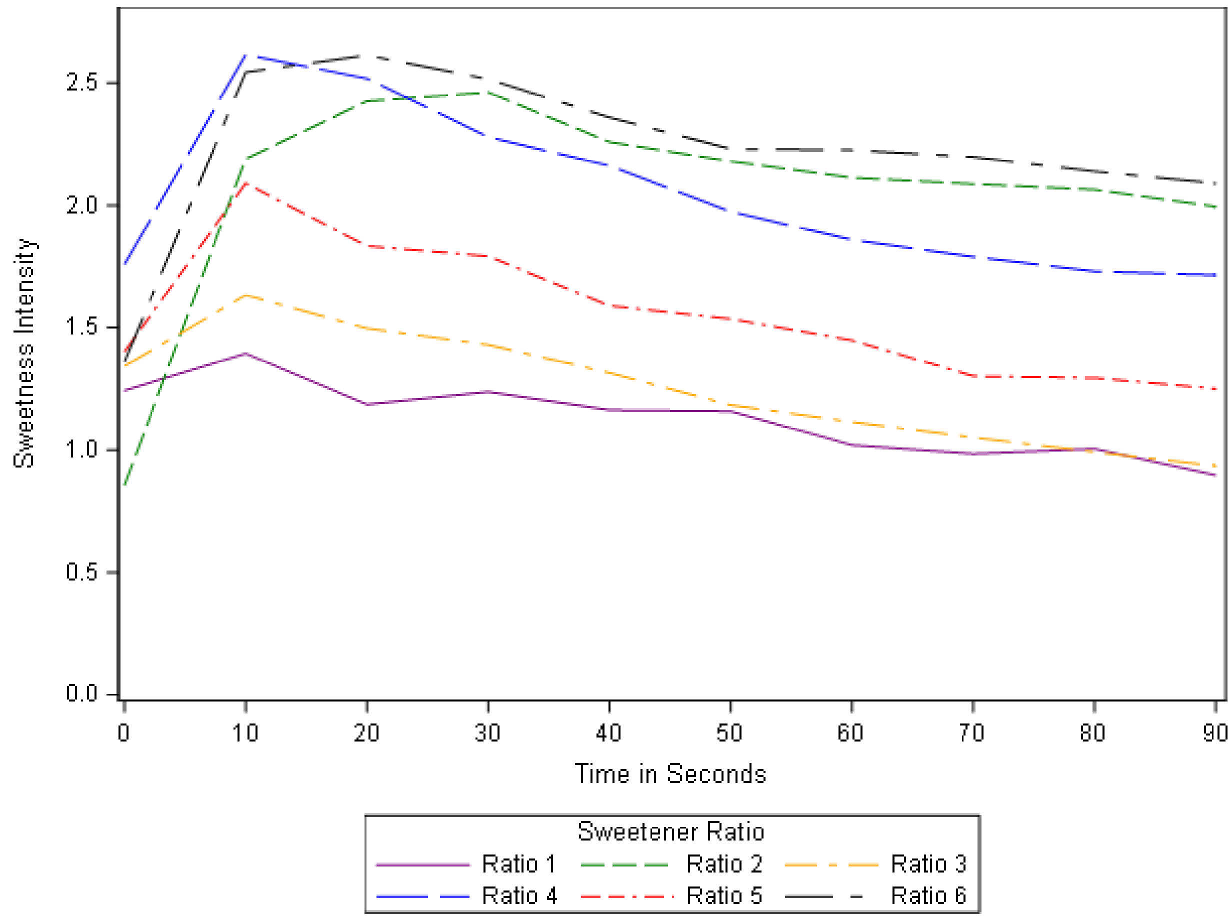

Figure 3.3. Time-intensity profile of sweetness properties of cola soft drinks sweetened with rebaudioside blend ratios as recorded on a 10-cm line scale.

In Figure 3.3 it should be noted that while Ratio 6 had the quickest sweetness onset the sweetness intensity decreased at a faster rate than Ratio 5. This could be explained by Ratio 6 containing 66.7% Reb A compared to 66.7% Reb D of Ratio 5. This could be further compounded when looking at Ratio 2 (100% Reb D) had a more prolonged sweetness intensity than that of Ratio 1 (100% Reb A). With Reb D being significantly sweeter than Reb A the higher inclusion rate of Reb D when compared to Reb A will result in a more prolonged sweetness intensity (Goyal et al., 2009, Hossain et al., 2017, Muenprasitivej et al., 2022)

3.3.6 Time-intensity Analysis of the Bitterness Properties in Cola Soft Drinks

In Table 3.9 the AUC, AI, IMAX, and TIMAX are reported for each of the six different stevia Ratios used in the formulations of the cola soft drinks. No significant differences ($P >$ 0.05) were observed between Ratios for AUC and AI for all the 6 Ratios. There was a significant difference ($P < 0.05$) observed between Ratio 4 and Ratio 5 for IMAX. There were significant differences ($P < 0.05$) observed between Ratio 1 and 2 when compared to Ratios 3, 4, 5, and 6. This could be explained by Ratio 1 containing 100% Reb A, and Ratio 2 containing 100% Reb D which are known to have slightly higher bitterness properties when compared to Ratios 3-6.

Table 3.9. Mean time-intensity analysis for bitterness properties in cola soft drinks.

Ratios	AUC	AI*	IMAX**	TIMAX**
Ratio 1	383.94	4.30	5.95^{ab}	30.30^{a}
Ratio 2	343.29	3.85	5.05^{ab}	29.10^{a}
Ratio 3	401.49	4.50	5.91^{ab}	39.30^{bc}
Ratio 4	281.86	3.16	4.31^{b}	37.80^{bc}
Ratio 5	394.23	4.42	6.11^{a}	41.90^{c}
Ratio 6	324.17	3.63	5.14^{ab}	40.80^{c}

*Means with common letters in the same column do not represent a significant difference (P < 0.05) as determined by Tukey's mean test; Area under the curve (AUC), average intensity (AI), intensity maximum (IMAX), and time of maximum intensity (TIMAX) are reported above; *AI is reported using a 10-point line scale **IMAX and TIMAX are reported in seconds.*

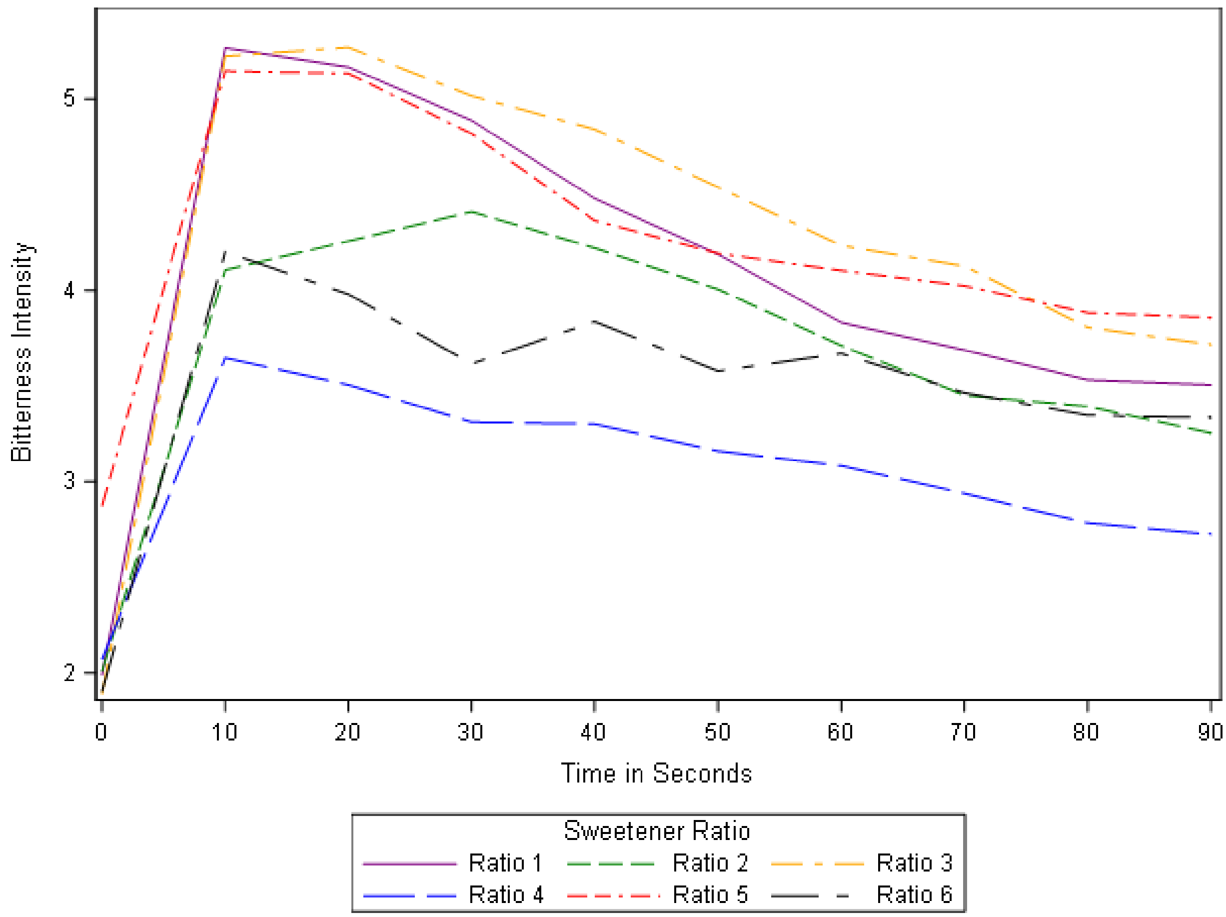

Figure 3.4 Time-intensity profile of bitterness properties of cola soft drinks sweetened with rebaudioside blend ratios as recorded on a 10-cm line scale.

Figure 3.4 shows that Ratio 1 (100% Reb A) had the most intense initial bitterness intensity; it was quickly followed up by Ratio 4 (50% Reb A). However, the bitterness intensity of these two Ratios decreased at a more rapid rate than that of Reb M. While Ratio 6 (66.7% Reb A) had the least intense bitterness intensity out of any Ratio. Followed up by Ratio 5 (66.7% Reb D) which had the second least intense bitterness. This could stem from the inclusion of the equal parts Reb D and M, which do not contain as strong bitter flavors as that of Reb A (Goyal et al., 2009, Hossain et al., 2017, Muenprasitivej et al., 2022).

3.3.7 Comparison of the Effect of Stevia Blends on Different Food Matrices

In complex food systems, each ingredient can have a different impact on the make-up of the food matrix, and within complex food systems, each ingredient plays a role either positively or negatively in the make-up of the food matrix. For instance, with the ice creams of this study fat provided a key role in enhancing the flavor and overall perception of the ice creams despite them being sugar-free. Fat's role in this complex food system was to enhance the overall mouthfeel of the ice cream as well as enhance the ice cream's texture by reducing the size of ice crystal formation (Drewnowski, 1992). It has been directly reported that the fat content in ice cream is proportional to the overall quality of the finished product (Akbari et al., 2019). In the ice creams, it was found that a Ratio consisting of a higher concentration of Reb D (Ratio 5 - 66.7%) elicited a longer and more intense sweetness sensation when compared to that of Ratios consisting of higher concentrations of Reb A (Ratio 4 - 50%, Ratio 6 - 66.7%). This same blend of Reb D elicited a less intense bitter taste, and this was not seen in the colas, another food matrix used in this study. This could stem from the fact that ice cream is a more complex food matrix than that of cola soft drink, where fat in ice cream might elicit a different response to sweetness and/or bitterness of the rebaudiosides.

In contrast to ice creams, colas are a simpler food matrix consisting of flavoring, coloring, and carbonation. Carbonation is used in beverages to increase the overall sensory perception of the beverage itself (Kappes et al., 2006). Carbonation has been documented to have an impact on flavor and mouthfeel by the appearance of a foam head in beer (Zampini & Spence, 2005). In carbonated fruit juices it was documented that carbonation improved flavor and taste (Kaushal et al., 2004). Carbonation has also been reported that if a beverage is under- or over-carbonated it can present an unbalanced flavor profile (Ashurst, 1998). In this study, it is possible that the carbonation levels used were not optimized due to the use of a non-commercial beverage carbonating system. Therefore, regardless of the sweetener ratio used in the cola beverages, the flavor profiles presented to the panelists in an unbalanced manner, impacting their sweetness and bitterness intensities. This might be the reason why responses to the rebaudioside ratios in ice cream and in cola samples were different in that Ratio 6 (66.7% Reb A) elicited a lesser bitterness intensity than that of Ratio 5 (66.7% Reb D). While no significant differences ($P < 0.05$) exist between these two Ratios in terms of bitterness intensity, it could be argued that Ratio 6 (66.7% Reb A) should be used in further studies involving colas as Reb A is the most abundant rebaudioside in the stevia plant and could be supported by the other minor rebaudiosides.

3.4. Conclusion

In this study both the ice creams and cola soft drinks were used as a testing medium to determine which stevia rebaudioside blend delivers the most optimal sweetness and bitterness taste profile with the use of time-intensity testing. Time-intensity testing was used to track the sensations onset, intensity, and duration of both the sweetness and bitterness perceived by the panelists in these complex food systems. In the ice cream samples, ratios containing a larger percentage of Reb M presented with a more immediate, intense, and prolonged sweetness sensation. The samples containing larger proportions of Reb M did not see as intense, or prolonged bitterness sensation. Ice cream samples containing large portions of Reb D shared this bitterness intensity, but had a shorter and delayed and onset. Ice cream samples with Reb D also presented with a more intense and prolonged sweetness sensation. However, in the cola samples an equal proportion of Reb A and D produced a more immediate, intense, and prolonged sweetness sensation. The equal proportion of Reb A and D also did not produce as intense or prolonged bitterness sensation than that of the other Ratio blends. Therefore, this study suggests that there is not a single ratio blend out there that could be used in both ice creams and colas for an optimal sweetness and bitterness taste profile. However, in ice creams a stevia sweetener ratio containing large proportions of Reb D could be used to deliver an optimal sweetness and bitterness taste profile. This study also suggests that in cola beverages a ratio blend containing greater proportion of Reb A can be used to deliver an optimal sweetness and bitterness taste profile. However, future studies could be conducted to further optimize these ratio blends to find a ratio blend that is further optimized to work in both ice cream and cola applications.

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5. Conclusion

These experiments were conducted to determine the viability of the minor steviol glycoside (Reb) D and M in complex food systems, as well as the potential development of a rebaudioside blend combining the major steviol glycoside (Reb A) with the minor steviol glycosides. This study chose to use high sugar-containing products (pound cake, ice cream, cola beverage) to understand the use of different rebaudiosides as sugar substitutes in these products by determining sensory profiles and consumer acceptability.

The first study explored the relationship between consumers' knowledge and acceptance of a food product through blind and informed conditions, with the objective to compare the consumer acceptability of major (Reb A) and minor (Rebs D and M) glycosides It was demonstrated that the information presented to a consumer regarding the naturalness and health benefits of using stevia impacted their overall product acceptability. This was demonstrated in the significant increase in liking scores of the Reb A sweetened pound cake in the informed session compared to the blind session. This significant increase in liking scores in the informed session illustrates that overall consumer acceptability of stevia-sweetened products can be impacted in a positive manner when information regarding naturalness and the health benefits of stevia is given to consumers (e.g., information on the front-of-pack labels).

Through the use of time-intensity analysis, it was discovered that blending the rebaudiosides together improved the overall sweetness and bitterness taste profile. Deliver a more rapid sweetness onset, intense, and prolonged duration, while decreasing the bitterness intensity and duration. This demonstrates that an optimized blend of the old (Reb A) and new generation of stevia rebaudiosides (Reb D and M) is possible and even potentially improves the product's overall quality. However, it should be considered that currently there is no one ratio that fits all food matrices. Within ice creams, a ratio containing more Reb D produced an optimized ratio that enhanced the sweetness and bitterness taste profiles. However, within the cola beverages a ratio containing a greater proportion of Reb A produced a more enhanced sweetness and bitterness taste profile.

From these studies, it was demonstrated that stevia and its use as a sugar substitute in high-sugar food products are a viable option for formulating zero-sugar or low-sugar versions. This study also demonstrated that an optimized blend of the major and minor stevia rebaudiosides is possible to improve the overall sweetness and bitterness taste profile. However, further research is needed to produce a single optimized blend. Future research could focus on the optimization of these ratio blends to produce a single blend that fits all complex food applications, and explore the consumer acceptability of these ratio blends.

These studies did face several potential limitations that should be addressed in future research. The pound cake study was limited by the use of 100% ratios of Rebs A, D, and M. These 100% ratios are cost-prohibitive and therefore not likely to be used in the food industry. The pound cake study was also limited by a high female gender bias. The time-intensity analysis study was also limited in the scope of carbonation. The carbonation of the sodas tried to remain consistent, it is possible that under or over-carbonation occurred due to human error. If a commercially available carbonation machine had been used, this variable could have been eliminated.

Appendix A *Informational brochure panelists received prior to their sample evaluation during the informed session.*

Stevia Cake

Nutrition Facts	
9 servings per container	
Serving size	**1 Slice (69g)**
Amount per serving	
Calories	**230**
	% Daily Value*
Total Fat 16g	**21%**
Saturated Fat 10g	**50%**
Trans Fat 0.5g	
Cholesterol 40mg	**13%**
Sodium 240mg	**10%**
Total Carbohydrate 19g	**7%**
Dietary Fiber 1g	**4%**
Total Sugars 1g	
Includes 0g Added Sugars	**0%**
Protein 5g	
Vitamin D 0mcg	0%
Calcium 19mg	2%
Iron 1mg	6%
Potassium 124mg	2%

*The % Daily Value tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Sugar Cake

Nutrition Facts	
9 servings per container	
Serving size	**1 Slice (69g)**
Amount per serving	
Calories	**260**
	% Daily Value*
Total Fat 16g	**21%**
Saturated Fat 9g	**45%**
Trans Fat 0.5g	
Cholesterol 95mg	**32%**
Sodium 180mg	**8%**
Total Carbohydrate 27g	**10%**
Dietary Fiber 1g	**4%**
Total Sugars 16g	
Includes 15g Added Sugars	**30%**
Protein 3g	
Vitamin D 0mcg	0%
Calcium 77mg	6%
Iron 1mg	6%
Potassium 92mg	2%

*The % Daily Value tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Appendix B *Questions Used to Measure Sweetener Knowledge Levels of the Participants*

Q1. True or False?

All zero-calorie sweeteners are artificial.

True

False

Don’t Know

Q2. True or False?

Sucrose is a zero-calorie sweetener.

True

False

Don’t Know

Q3. True or False?

All natural sweeteners contain calories

True

False

Don’t Know

Q4. True or False?

Stevia is extracted from plant leaves.

True

False

Don’t Know

Q5. True or False?

Stevia is a zero-calorie sweetener.

True

False

Don't Know

Q6. True or False?

Aspertame is an artifical sweetener.

True

False

Don't Know

Q7. True or False?

Zero-calorie sweeteners have different sweetness intensities.

True

False

Don't Know

Q8. True or False?

Sugar alcohols (ex. Xylitol, Mannitol, Sorbitol...ect.) contain calories.

True

False

Don't Know

Q9. True or False?

Stevia is safe for diabetics.

True

False

Don’t Know

Q10. True or False.

Table side sweetener packets (pink, blue, or yellow in color) contain less calories than table sugar.

True

False

Don’t Know