THEAPPLICATION OF SWEETENER MIXTURES AS SUGAR REPLACEMENTS AND ITS EFFECTS ON THE CHARACTERISTICS OF BUTTER COOKIES

MR. KEVIN RUDKOFFSKY
ID 563-5117

A special project submitted to the School of Biotechnology, Assumption University in part of fulfilment of the requirement for the degree of Bachelor of Science in Biotechnology

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Title	**:**	**The Application Of Sweetener Mixtures As Sugar Replacements And Its Effects On The Characteristics Of Butter Cookies**
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Level of study	**:**	**Bachelor of Science**
Department	**:**	**Food technology**
Faculty	**:**	**Biotechnology**
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Kevin Rudkoffsky

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ABSTRACT

Butter cookies with different ratios of different sweetener mixtures were produced and analyzed based on their physical diameters, color, texture and their sensory properties. The sweeteners used in this experiment were sucralose, PP (isomaltulose, palatine®), stevia and mogroside. There are three objectives of study in this experiment. Firstly, the determination of appropriate types of sweetener mixtures on the properties of butter cookies was investigated. The selected type of the sweetener mixtures was then further optimized the ratios between them. Lastly, the investigation of the amounts of sweeteners that can be substituted sugar and how this affects the properties of butter cookies and their acceptance was studied. The sweetener mixtures of sucralose/isomaltulose, stevia/isomaltulose, sucralose/mogroside, and stevia/mogroside has been used to study and compare to a control sample (100% sugar). These mixtures were set at a ratio of 1:1 and replaced 50% of the sugar in the original formula. The results showed greatest acceptance for the samples with sweetener mixtures of sucralose/isomaltulose and stevia/isomaltulose. These mixtures were therefore chosen for further improvement in the next step. The different ratios of sweeteners were used at 100:0, 75:25, 50:50, 75:25 and 0:100 and replaced 50% sugar in cookies. The results indicated that the cookies with a ratio of 25:75 of sucralose/isomaltulose had the highest hardness and acceptance which also comparable to the control sample. It was chosen for further study on the maximization of the substitution to sugar. The sugar to sweetener mixture ratio were set at 100:0 (control), 75:25, 50:50, 25:75, 0:100, additionally compared to commercial butter cookies in the sensory evaluation in order to give a more realistic comparison between samples. The results revealed that substitution of sugar up to 50% resulted in a decent acceptance while a higher degree of sugar substitution (more than 50%) lead to less acceptance. Liking score on sweetness was comparable to the control but the preference on texture of the low calorie samples was rather lower behind the control samples. Hence it can be inferred that that the sweetener mixture of sucralose/isomaltulose at a ratio of 25:75 can partially replace sugar in cookies up to 50% without affecting the cookies physical and sensory characteristics too gravely.

INTRODUCTION

In modern society there have been a variety of problems that occur on a regular basis but are difficult to treat or change. One of these problems belongs to the category of nutrition, in a lot of countries overconsumption and unhealthy foods with high amounts of fat and sugar create serious issues (World Health Organization, 2015).
Despite the fact that sugar has been around since the early centuries of AD, problems caused by the overconsumption of sugary products have only started to appear since several decades ago due to the improvements in technology allowing us to produce sugar in greater proportions at a cheaper price compared to the past.

The availability of sugar led to the production of soft drinks and sweets that contain huge amounts of refined sugar affecting the health of the consumers negatively; problems that are related to this may include overweight, diabetes and more (Frank B. Hu, 2010). Especially in Thailand this is a huge concern because the Thai citizens enjoy sweet drinks and sweet foods, causing many health issues that can be directly related to sugar. Regarding the aforementioned issue, the Thai government decided to make changes that may help improve those negative conditions. Therefore, the government implemented a sugar tax, that sugar tax was aimed at drinks with high concentrations of sugar, the specific tax amount would correlate directly with the amount of sugar in the beverage with a higher amount equating to a higher amount of tax. The tax percentage would start at 20% as the maximum value and decreases according the amounts of sugar. With the new tax in place, companies aiming for healthier beverages with less sugar can be more economic. They save money that would have been otherwise spent on said tax. Not only will they become more economical, but they can follow up on popular trends such as the healthy lifestyle that have become more important recently. The influence of social media contributes a lot to the healthy lifestyle since fans see people, they admire such as actors or singers get in shape by eating healthy food, which may in turn motivate the followers and fans to make similar choices. The sugar tax currently applies only to beverages but has potential to be adopted for food products as well.

In light of these changes it is necessary to research the potential of sweeteners in different solid products. Certain types of sweeteners have already been successfully introduced and applied in beverages such as Coke Zero which uses aspartame (Borges et al, 2017). The difference in product type creates barriers and not all sweeteners can be applied the same way, the purpose of sweeteners is to replace sugar without changing the product characteristics too gravely (Van der Sman & Renzetti, 2019). The product type chosen for the research were butter cookies, they are very popular amongst all age groups, are easy to produce and it can be considered a good product for sugar replacement due to the high amount of sugar usually used in the recipes (Van der Sman & Renzetti, 2019).

With successful application of the sweeteners in beverage and dairy product, it should be possible to create a healthier alternative for cookies reducing potential health risks, while simultaneously lessening economic burden. Therefore, the objectives of this study were to determine the appropriate types of sweetener mixtures and how they affect the properties of butter cookies. Moreover, the study aims to analyze the different ratios within the chosen sweetener mixtures and how different ratios may affect butter cookie properties. An investigation of the amount of sugar that can be substituted by using sweetener mixtures without altering the characteristics of the cookie was also conducted.

OBJECTIVES

1. To determine the appropriate types of sweetener mixtures and how they affect the properties of butter cookies
2. To determine the appropriate ratio within the chosen sweetener mixtures and how different ratios may affect butter cookie properties
3. To investigate the amount of sugar that can be substituted by using sweetener mixtures without altering the characteristics of the cookie

LITERATURE REVIEW

Hruby and Hu (2015) clarify in their study that the epidemic of overweight and obesity represents a major problem to chronic disease prevention and health across the world. They explain that due to various factors such as "...economic growth, industrialization, mechanized transport, urbanization, an increasingly sedentary lifestyle, and a nutritional transition to processed foods and high calorie diets over the last 30 years, many countries have witnessed the prevalence of obesity in its citizens double, and even quadruple." (Hruby & Hu, 2015). Obesity is a disease influenced by different sources and increases the chance of affliction by other illnesses and death (Hruby & Hu, 2015). This journal helps in breaking down obesity and analyzing the issues correlated with it. Obesity is a grave problem and should be addressed as soon as possible according to the researchers.

Sylvetsky & Rother (2018) reassessed discoveries concerning non-nutritive sweeteners (NNS) and their influence on metabolism, weight, and obesity-related chronic diseases. Evidence and conclusions that were drawn based on reviews of the relevant scientific literature (Sylvetsky & Rother, 2018). Increased body weight and diseases related to obesity were linked with NNS consumption explain Sylvetsky & Rother. Yet according to Sylvetsky & Rother randomized controlled trials would indicate that NNS may support weight loss. Conclusive proof whether or not NSS has any influence on weight will heavily rely on further studies.

Sylvetsky & Rother (2016) found that consumption of LCS (low-calorie sweeteners) had increased significantly in recent years and continuation of this trend is expected. Sylvetsky & Rother intended to expose trends and differences in LCS consumption across consumer groups with this study. According to Sylvetsky & Rother sweeteners such as aspartame are becoming less popular whereas sucralose experiences increased usage. At the same time natural sweeteners such as stevia expanded in popularity (Sylvetsky & Rother, 2016). The highest consumption of LCS occurred in white, older and better-educated consumers according to the author's observations.

DellaValle (2018) assessed LCS distribution in foods, beverages and food and beverage additions (FBAs), in the US adult diet. DellaValle did this by calculating the number of reported dietary items within 24-h recall and if they had LCS or NS (nutritive sweetener). The results showed that 56.1% were foods, 29.1% were beverages and 14.8% were FBAs (DellaValle, 2018). LCS content was determined as 0.7% in foods, 8.1% in beverages, and 10.4% in FBAs (DellaValle, 2018). So according to DellaValle's calculations FBAs make up the majority of the LCSs.

According to Dunford's (2018) studies, the consumers' interest in reducing dietary sugar has led to more products that make use of non-nutritive sweeteners (NNS). Dunford also mentions that even though the usage of NNS has increased, it is unclear to what degree NNS are used how it is replacing sugar. In order to get a better understanding a branded food composition database from Australia, Mexico and New Zealand and the US was used to make a comparison of total sugar density with products that in and excluded NNS (Dunford, 2018). The results of the study indicate that 5% of the products included at least one NNS; beverages on the other hand had the highest percentage with 22% (Dunford, 2018). The numbers clearly show an inclination of using NNS mainly for beverages whereas food products still see relatively low addition of NNS.

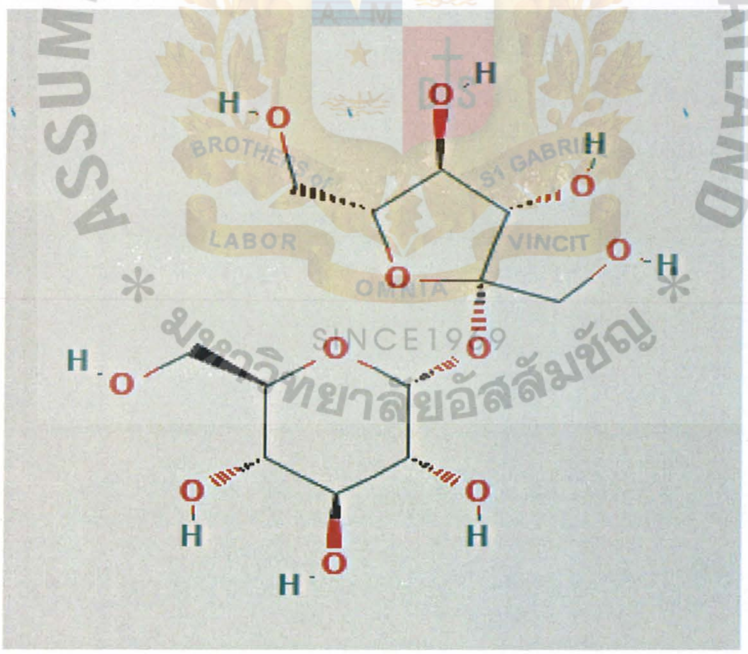

Figure 1: Sucrose structure

(Source: National Center for Biotechnology Information, (2020))

Sweetener

Sensory characteristics such as taste, smell, texture and appearance greatly affect the sensory properties of the food (Sorensen et al. 2003). Food selection is of vital importance seeing that it influences the appetite and intake regulation (Chattopadhyay et al. 2014).

In order to reproduce the taste of sugar in a food product, sweeteners are used; they are also known as sugar substitutes (Chattopadhyay et al. 2014). It is commonly seen that consumers tend to pick food products that make use of low caloric sweeteners; this allows them to experience a similar sweetness to sugar without having to worry about any calories that may be added through sugar (Chattopadhyay et al. 2014).

There is a variety of sugar substitutes that possess different attributes. Due to the difference in their origin and characteristics we can place them into categories such as artificial sweeteners, natural sweeteners, high intensity sweeteners, nutritive sweeteners and non-nutritive sweeteners. Some of the sweeteners may or may not be part of more than one category according to their attributes.

Sucralose is one of the commercially successful sweeteners and has been categorized as an artificial, high intensity and non-caloric sweetener. It can be produced through a multistep synthesis in which chlorine is added to sucrose in substitution of 3 hydroxyl groups. The sweetness produced from Sucralose is considered 300 to 1200 times greater than sugar and has no effect on insulin levels thus making it a safe option for diabetics. The relative sweetness intensity of Sucralose is related to its concentration in comparison to sugar. Additionally factors such as pH, temperature and food ingredients have to be considered as well as they do affect sweetness as well. Sucralose is said to combine well with other sweeteners and works in a synergistic way with most, therefor enabling the customization of the flavor profile.

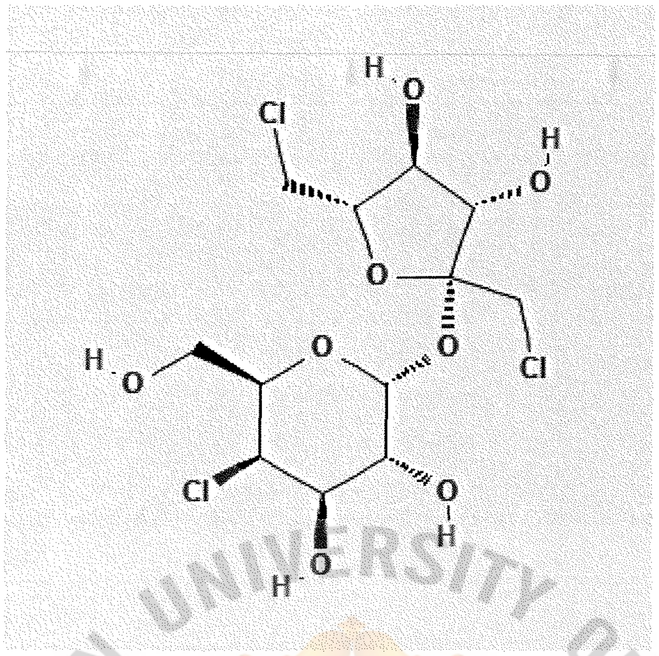

Figure 2: Sucralose structure

(Source: National Center for Biotechnology Information, (2020))

Isomaltulose or palatinose is a natural sweetener and a reducing sugar, it can be found in honey. It is made of glucose and fructose and therefore classified as a disaccharide. Isomaltulose is commercially synthesized through isomerization of sucrose, this process is done through the enzyme sucrose isomerase which catalyzes the rearrangement of the glycosidic (1->2) -linkage between glucose and fructose into an (1->6) –linkage producing isomaltulose (Helmers et al. 2018). The sweetness of isumaltulose is about 50% the sweetness of sugar and can be used as a bulking agent in food products. Furthermore, it has a low Glycaemic Index value of 34, which correlates to a reduced blood glucose and insulin response hence making it a good option for people that suffer from diabetes type 2.

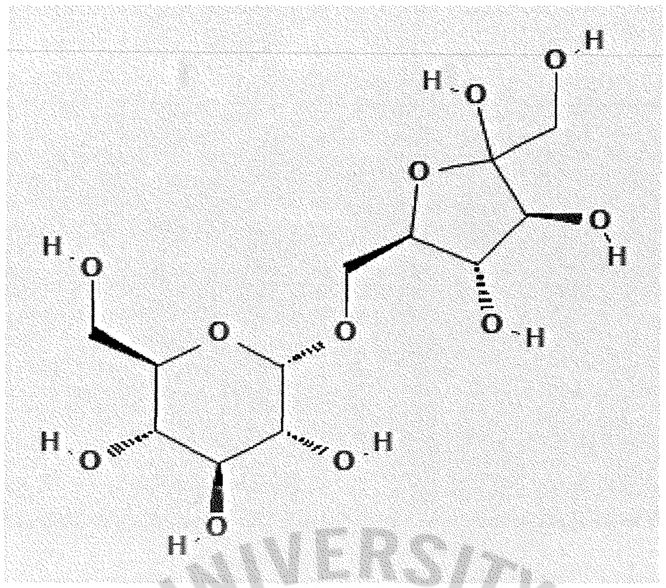

Figure 3: Isomaltulose structure

(Source: National Center for Biotechnology Information, (2020))

Stevia is a natural sweetener that is made from the leaves of Stevia rebaudiana. The chemical compounds in the leaves that provide the sweet taste are known as steviol glycosides. The steviol glycosides have a sweetness of about 30 to 150 times that of sugar. Moreover, these compounds are heat and pH stable, which makes them ideal for the usage in tandem with food products as they can go through processing such as heating without creating variations. Another benefit provided is the inability of the body to metabolize glycosides. This effectively means that stevia is a non-caloric sweetener and may not add any calories upon consumption. Great attribute differences between sugar and stevia can be observed in the sweetness onset and duration, the onset of stevia is slower than that of sugar and perceived for a longer duration, in high concentrations stevia will also carry a type of aftertaste.

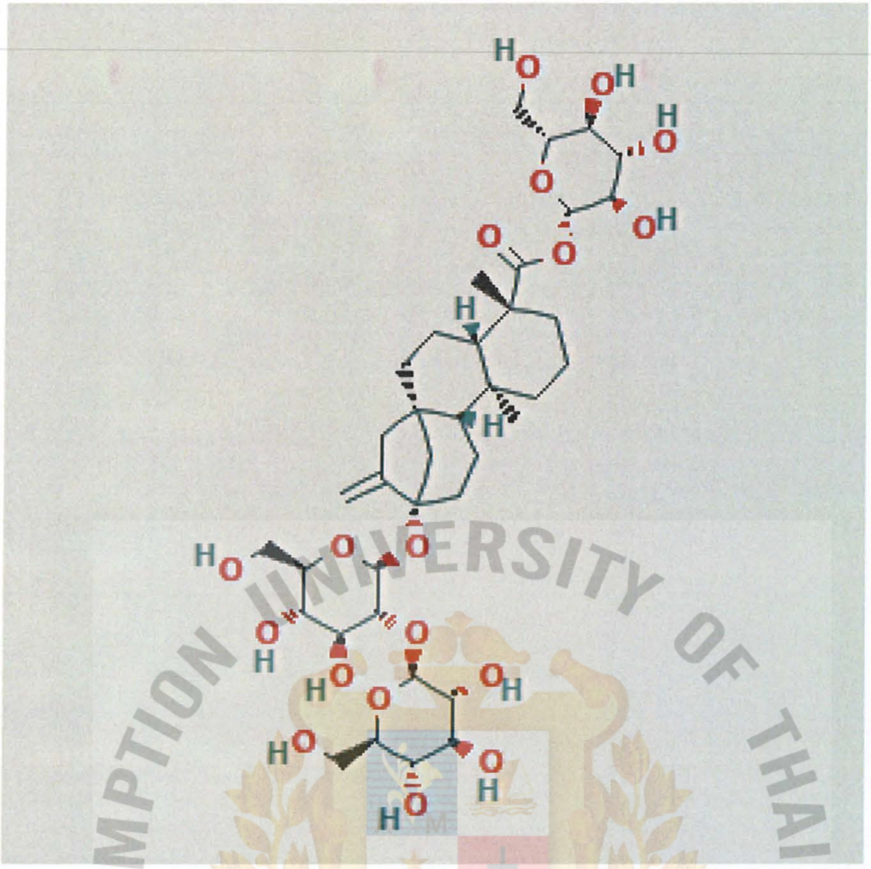

Figure 4: Stevioside structure

(Source: National Center for Biotechnology Information, (2020))

Similar to stevia, a mogroside is a natural and non-caloric sweetener. A mogroside is a glycoside that is found in plants such as Siraitia grosvenorii also known as the gourd vine. It has about 250 times the sweetness of sugar. A study done by Itkin et al. (2016) clarifies that there are mainly five enzyme families involved in the synthesis of mogroside V: squalene epoxidases, triterpenoid synthases, epoxide hydrolases, cytochrome P450s, and UDP-glucosyltransferases.

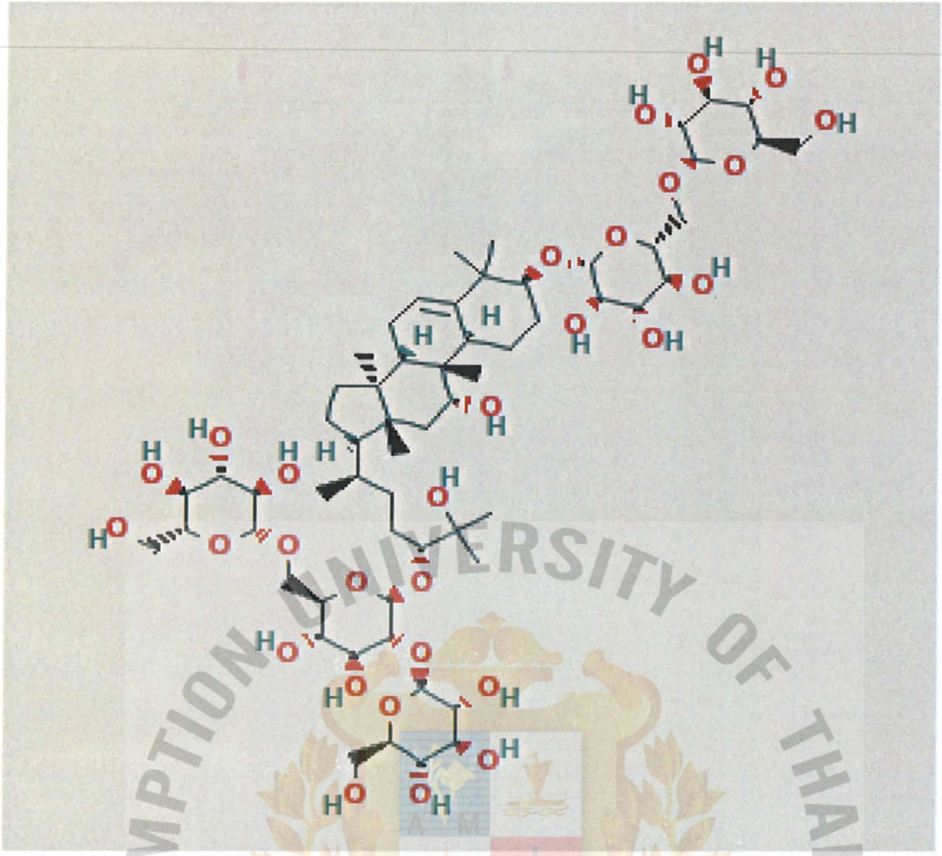

Figure 5: Mogroside v structure

(Source: National Center for Biotechnology Information, (2020))

Function and Formulation

To be able to substitute sucrose within biscuits, it is necessary to understand how it functions and what role it plays in the manufacturing process. Van der Sman & Renzetti (2019) were studying sucrose functionality from the angle of sugar replacements. They express that sucrose has attributes that affect structure and texture of the biscuit. This means that sugar replacements have to have similar attributes and must contribute to the formation of texture and structure (Van der Sman & Renzetti, 2019). Van der Sman & Renzetti state that the Complex Dispersed Systems methodology can depict the structure and texture development produced by sucrose. Additional data can be obtained through the usage of a supplemented state diagram by plotting changes in the system (Van der Sman & Renzetti, 2019). Furthermore, the authors expound that system changes can be determined experimentally, yet this approach is very inconvenient and that developing a numerical model would be of greater assistance.

Bakery Products

Finding a sugar replacement is a very difficult task. The usage of artificial and natural sweeteners has happened mostly in beverages whereas usage of sweeteners in food products have been minimal. Even though the application of sweeteners for food has been rather small there are still experiments conducted that follow this direction of thought.

Already back in 2014 Mr. Handa conducted experiments in which fructoligosaccharide (FOS) was used as a sugar replacement for cookies. In the conducted study FOS in levels of 40%, 60% and 80% replaced the sugar (Handa, 2014). After replacing sugar with FOS characteristics such as diameter, height, spread ratio and hardness, moisture and acidity of the extracted fat were analyzed in order to display the differences that occur by the replacement (Handa, 2014). Handa demonstrates through his collected data that there is a change in hardness in which a higher degree of FOS reflects in less hardness and higher spread ratio. Moreover, according to Handa the sensory data implies that panelists accepted the FOS cookies up to 60% replacement due to color, texture and overall appearance. Handas' study illustrates that a partial replacement of sugar with another sweetener is possible and that even though there are changes to the cookies characteristic it is still within the acceptable range depending on the replacement percentage.

In a similar study Aggarwal, Sabikhi, & Sathish (2016) tried to formulate a low calorie and low fat cookie by making use of sugar and fat replacements. Aggarwal, Sabikhi, & Sathish affirm that the sugar replacement of 100% was successful by using a blend of maltitol and FOS-Sucralose in a 3:1 ratio.

Many bakery products use sugar; this does not only include Cookies but many more as well. Therefore, it is of great interest to observe changes occurring in other products as well and see how they are affected by the replacement of sugar. Parallels can be drawn between the different pastry and bakery products and a successful sugar replacement may be a good way to offer a more healthy alternative to the standard type of bakery products.

Mr. Jingrong (2017) investigated the sugar replacement in muffins with "Stevianna". Removal of sugar has grave impact on the characteristics of the product, namely texture, appearance and mouthfeel (Jingrong, 2017). The goal of Mr. Jingrong was to identify the changes that occur by replacing sugar with Stevianna. The results that obtained by Jingrong show that a replacement of 50% achieved results that were comparable to the control (100% sugar) and thus leading to high acceptance in the sensory category. On the contrary, the replacement of sugar by 100% Stevianna led to poor acceptance due to harder texture and dry mouthfeel. The results of this study align with the results from Handa (2014) which clearly show that a partial replacement is of 50-60% is well within acceptance range, without influencing characteristics too badly.

Regarding the influence of sweeteners in pastry products, Quitral (2019) made a study on the role of non-caloric/non-nutrititve sweeteners in pastry products. Quitrals' experiment determined sensory preference and acceptability through ranking test in addition to 9-point hedonic scale. The results suggest that preference of the control was higher than in other samples due to lower sensory response from the samples with sweeteners (Quitral, 2019). Likewise the control sample showed improved shelf life as well as improved texture, stability, aroma, color and flavor which can be traced back to the Maillard reaction (Quitral, 2019).

Safety

Concerning the potential problems and safety of low and no calorie sweeteners, experts of different fields such as food, nutrition, toxicology etc. have met in Lisbon in order to create a Consensus on the usage of LNCS (Serra-Majem, 2018). To summarize the Consensus, the discussion ended in the establishment of three main points. The first point declares that the evaluation of LNCS, done by World Health Organization, US FDA and the European Food Safety Authority had been done broadly and that LNCS are considered safe (Serra-Majem, 2018). The second point emphasizes consumer education on the topic of LNCS and their usage (Serra-Majem, 2018). The third point mentions the usage of LNCS for weight reduction programs in structured diet plans in order to achieve a sustainable weight reduction (Serra-Majem, 2018). Moreover, there might be potential use for diabetes programs as LNCS may be able to help in glycemic control (Serra-Majem, 2018). In contrast, Vikas (2018) explains in his paper that LCS may or may not be harmful. He states that the long-term risks for consuming artificial sweeteners is unknown due to the lack of long-term studies. His statement aligns itself with the findings and thoughts of Sylvetsky & Rother (2018) who also clarified that there has been no conclusive evidence to whether those sweeteners are harmful. Now according to the FDA LCS and NCS are considered as safe, but due to the lack of data there is no 100% guarantee on whether this is true or not. To identify possible problems studies have to be conducted over an extremely long time with careful observations, which make this a very difficult task.

MATERIALS

1. Sucralose (Eatwell®, Bangkok, Thailand) with 700 x relative sweetness
2. Stevia (Eatwell®, Bangkok, Thailand) with 300x relative sweetness
3. Isomaltulose (Eatwell®, Bangkok, Thailand) with ½ x relative sweetness
4. Mogroside (Eatwell®, Bangkok, Thailand) with 400 x relative sweetness
5. Flour
6. Sugar
7. Vanilla Extract
8. Salt
9. Eggs
10. Butter
11. Baking Soda
12. Evaporated Milk (Carnation)

METHODOLOGY

1. The Study the Effect of Sweetener Mixtures on the properties of butter cookies

The test was used in order to determine a cookie formula that may be used as the gold standard. Different formulas were used and compared to find the one that came closest to the desirable traits that have been set before the experiment (e.g. certain color, aroma, crispiness etc.). Different ingredients were adjusted as needed – if the produced cookie was too sweet sugar was reduced or if the cookie was too buttery, the amount of butter used was changed as well. This continued until all important criterias had been fulfilled and the standard had been set. This standard was then used in further experimental steps in order to compare the changes between cookies with and without sweeteners.

4 different sweetener mixtures had been chosen based on prior studies on sweeteners. Those 4 sweetener mixtures were Sucralose with Isomaltulose, Sucralose with Mogroside, Stevia with Isumaltulose and Stevia with Mogroside respectively. Sugar was set at 50%. The exact amount of sweeteners was calculated based on the relative sweetness of the different sweeteners, the formula used to calculate the exact amounts used was $S = kC^n$. Since 2 sweeteners are used per mixture the 50% of the mixture was composed of 25% sweetness from sweetener A and 25% sweetness from sweetener B. After the production step the cookies was evaluated in terms of color, physical size, and texture and sensory.

Table 1 Cookie formula

- Cake Flour 225g
- Baking Soda 2g
- Butter 125g
- Salt 1.2g
- Sugar 100g

- Eggs 1
- Vanilla 2g
- Evaporated Milk (Carnation) 12.5g

1.1 Preparation of butter cookie

The butter, sugars (and/or sweeteners), salt, baking soda were added and thoroughly mixed. 1 egg was then added and mixed at medium speed. About 50% of the flour was mixed in. Evaporated milk and vanilla was then consequently added while mixing the solid ingredients, and finally the rest of the flour was placed into the mix.

Cookie batter was spread evenly on the baking plate through the usage of an ice cream scooper. An ice cream scooper was used in order to achieve a more consistent result concerning the dough volume. The baking process was done at 165°C for 23 minutes. The cookie had been left on sieve to cool down before measurement. Physicochemical and sensory properties have been determined in triplicates.

1.2 Analysis methods

1.2.1 Diameters and Spread Ratio

The physical diameters were determined with the help of a Vernier caliper. The diameter and thickness (height) of the cookie were measured in order to determine the spread ratio. The spread ratio is calculated based on the formula of diameter divided by height (thickness) (Zoulias et al., 2000). For each treatment 5 samples were measured, and the final data was averaged and then the standard deviation was calculated. A higher number for the spread ratio indicated less spread of the cookie, whereas a lower number signified a greater spread of the cookie.

1.2.2 Color Measurement

In order to perform the evaluation of colors, a HunterLab CIE L*a*b* system (Miniscan EZ, Hunter associates Laboratory, Inc., Thailand) was used with D65/10°. The data obtained was displayed as L*, a*, and b* where L* (lightness; 0 = dark to 100 = bright), a* (+ red to - green), and b* (+ yellow to - blue). Results were obtained by placing samples on the sample cup and recording the readings of L*, a*, and b*. For each treatment 3 samples were used and each sample was analyzed in 3 spots on top and 3 spots at the bottom in order to gain accurate data.

1.2.3 Texture Analysis

To obtain results concerning the texture of the cookies a Texture Analyzer (TA.XT plus, Stable Micro System, UK) was used with a 4 mm flat-ended cylinder probe, at speed 1 mm/sec. The setting used in the experiments was a three-point bending set up. The cookie samples were placed on the loading platform and compressed. Following the compression procedure, the data was recorded and the attributes of interest included the hardness (maximum force) and fracturability (force at first peak).

1.2.4 Sensory Analysis

Data was obtained through 30 random and untrained panelists that were using a 9-point hedonic scale in which 1=dislike extremely, 5=neither like nor dislike, 9=like extremely. Each panelist was given the samples and water in order to rinse their mouth between sampling. A piece of cookie was served at room temperature.

1.2.5 Statistical Analysis

The data that has been attained through the different experiments has been analyzed to determine statistical significance of treatments. The randomized complete block design (**RCBD**) and R-program version 2.15.3 were used to test the significance of results (Clewer and Scarisbrick, 2001). Significance of $p<0.05$ is mentioned wherever necessary, different letters within the statistics reflect significant difference between means. Non significance ($p\geq0.05$) will be denoted as ns within the data.

2. The determination of the appropriate ratio within the chosen sweetener mixtures and how different ratios may affect butter cookie properties

The standard formula for cookies was still used, the change that studied further concerns the optimization of the sweetener mixture ratios. For this experiment , sugar has been fixed at 50% whereas the sweetener mixtures will vary their ratios where Sweetener A and Sweetener B which were 100:0, 75:25, 50:50, 25:75, 0:100 (sweetener mixture still makes up 50% of total sweetness provided). The butter, sugars (and/or sweeteners), salt, baking soda were added and thoroughly mixed. 1 egg was then added and mixed at medium speed. About 50% of the flour was mixed in afterwards. Evaporated milk and vanilla was then consequently added while mixing the solid ingredients, and finally the rest of the flour was placed into the mix.

Cookie batter was spread evenly on the baking plate through the usage of an ice cream scooper. An ice cream scooper was used in order to achieve a more consistent result concerning the dough volume. The baking process was done at 165°C for 23 minutes. The cookie has been left on sieve to cool down before measurement. Physicochemical and sensory properties have been determined as described earlier.

3. To investigate the amount of sugar that can be substituted by using sweetener mixtures without altering the characteristics of the cookie

In this experiment the ratio between sugar and sweetener mix had been varied whereas the ratio of the sweetener mixtures was set to the one that had been determined in experimental Step 2. The variation of sugar and sweetener mixtures was 100:0 (control), 75:25, 50:50, 25:75, 0:100 (control).

The butter, sugars (and/or sweeteners), salt, baking soda were added and thoroughly mixed. 1 egg was then added and mixed at medium speed. About 50% of the flour was mixed in. Evaporated milk and vanilla was then consequently added while mixing the solid ingredients, and finally the rest of the flour was placed into the mix. Cookie batter was spread evenly on the baking plate through the usage of an ice cream scooper. An ice cream scooper was used in order to achieve a more consistent result concerning the dough volume. The baking process was done at 165°C for 23 minutes. The cookie has been left on sieve to cool down before measurement. Physicochemical and sensory properties have been determined as described earlier.

RESULTS AND DISCUSSION

1. The Effect of Sweetener Mixtures on the properties of butter cookies

With the help of a Vernier Caliper, the samples were measured and the spread ratio was calculated. The obtained numbers are shown in Table 1. The different sweetener mixtures had no significant difference in terms of spread ratio compared to the standard control with the exception of Stevia with Isomaltulose. The spread ratio of Stevia with Isomaltulose was 2.79 ± 0.12 whereas the control had a value of 3.04 ± 0.14. The difference indicates that the form of the Stevia with Isumaltulose sample is more likely to spread to a greater extent when compared to the control given the same environment. The main reason for this occurrence should be linked to a greater stability loss that happens when this sweetener combination is used.

Table 1: Diameters and spread ratio of cookies with sweetener mixtures

Sweetener Mixtures		Thickness (cm)	Diameter (cm)	Spread Ratio (Diameter/Thickness)
Sucralose	Isomaltulose	1.37 ± 0.08^{a}	4.26 ± 0.08^{a}	3.12 ± 0.16^{a}
	Mogroside	1.39 ± 0.02^{a}	4.07 ± 0.08^{b}	2.93 ± 0.07^{a}
Stevia	Isomaltulose	1.53 ± 0.08^{b}	4.26 ± 0.12^{a}	2.79 ± 0.12^{b}
	Mogroside	1.39 ± 0.02^{a}	4.17 ± 0.18^{b}	3.01 ± 0.07^{a}
Normal Cookie (Control)		1.36 ± 0.10^{a}	4.13 ± 0.11^{b}	3.04 ± 0.14^{a}

Remarks: Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

For the color analysis, data was obtained through the usage of the HunterLab CIE L*a*b* system. The results are shown in Table 2. Great differences were shown between the different samples in respect to L*, a* and b*. The samples Stevia with Mogroside and Sucralose with Mogroside had similar values which were also the highest concerning L* which were 67.35 ± 1.96 and 67.66 ± 2.85. These 2 samples were the lightest in color. The darkest sample was Stevia with Isomaltulose with a value of 55.59 ± 3.62. In the aforementioned order the samples became increasingly darker which usually is an indication for a greater degree of browning reaction. As for a* the highest value can be found in the Stevia with Isomaltulose sample with 12.45 ± 2.63 where a higher value reflects a greater tendency to red whereas a negative value indicates a tendency to green. The samples became increasingly less red with the decrease in a* value, furthermore there is no significant difference between the Mogroside samples. The values for b* provide information about the colors yellow (positive value) and blue (negative value). The highest value could be observed in the control with 33.04 ± 2.69 followed by the Stevia with Isomaltulose sample with 32.83 ± 1.96, these 2 samples were not significant different from each other. As results, it is discernable that the samples that included mogroside appeared lighter in color than other samples. Furthermore, it can be identified that isomaltulose induced a darker color and a higher degree of red, leading to a red-brownish appearance. This should be due to a greater degree of browning which most likely stems from the fact that Isomaltulose is readily undergoing browning reaction due to having a free reducing group. Based on appearance it can be determined that the mixture of Sucralose with Isomaltulose resembles the control sample the closest.

Table 2: Color measurement of cookies with various sweetener mixtures

Sweetener Mixtures		**L***	**a***	**b***
Sucralose	Isomaltulose	60.46 ± 5.49^{c}	11.10 ± 3.14^{b}	31.90 ± 2.97^{b}
	Mogroside	67.35 ± 1.96^{a}	5.92 ± 1.84^{d}	28.91 ± 2.51^{c}
Stevia	Isomaltulose	55.59 ± 3.62^{d}	12.45 ± 2.63^{a}	32.83 ± 1.96^{a}
	Mogroside	67.66 ± 2.85^{a}	6.33 ± 2.52^{d}	29.31 ± 3.32^{c}
Normal cookie (Control)		64.55 ± 5.14^{b}	9.93 ± 3.63^{c}	33.04 ± 2.69^{a}

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

Figure 6: Photo of Butter cookies with sweetener mixtures (PP = Isumaltulose)

In order to determine the texture of cookies, the hardness and fracturability were measured using a Texture Analyzer (Table 3). The results showed that the control had the highest hardness out of all samples, whereas the Stevia with Isomaltulose sample came close to it in hardness. All other samples had a lower hardness resulting in them being softer compared to the control. The hardness did not directly correspond to fracturability. This means that fracturability had a lot of variance where some samples with low hardness had a fracturability close to the control. On the other hands, Stevia with isomaltulose with a similar hardness to control had a fracturability of about 10 n/s whereas control had a value of 15 n/s. Thus, there is no identifiable pattern when it comes to the fracturability of the cookie samples.

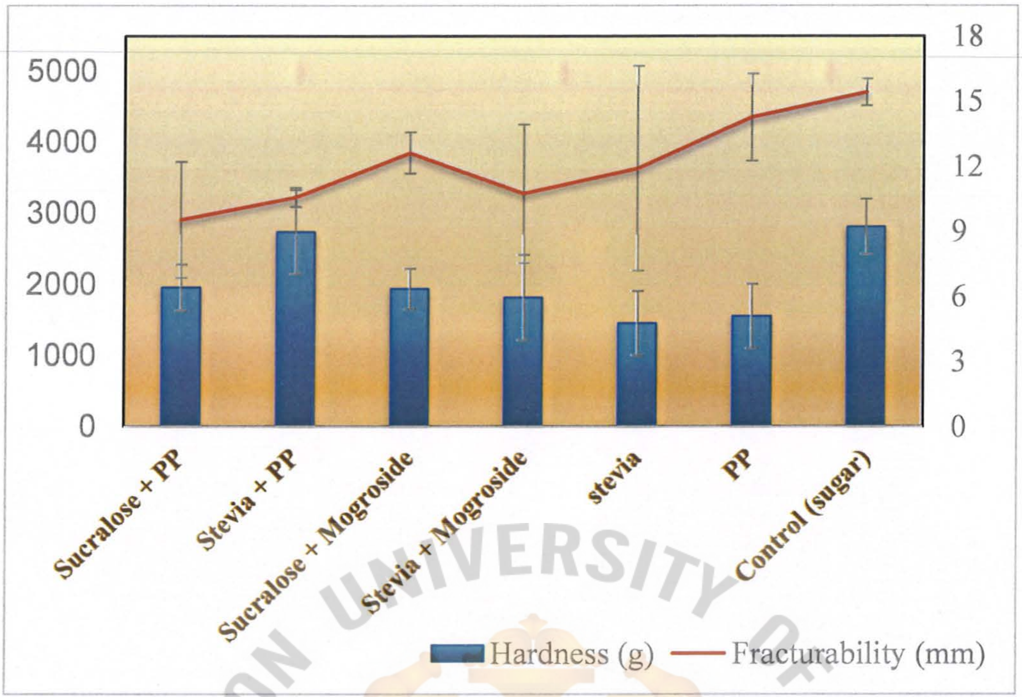

Figure 7: Hardness and Fracturability of cookies at various sweetener mixtures

The sensory analysis of the samples was done by 30 random panelists in order to rate attributes including color, aroma, buttery flavor, sweetness, crispiness, overall texture and overall liking based on a 9-point hedonic scale.

There had been no significant difference between samples in the categories of color and aroma. Therefore, the point of interest lies with the other categories that reflected the differences between the samples. In terms of buttery flavor the samples containing Sucralose with isomaltulose and Stevia with isomaltulose had the highest rating at 6.4 ± 1.5. The Isomaltulose samples had no significant difference between each other, similarly the mogroside samples also didn't have a significant difference between each other, though there is a significant difference when comparing Isomaltulose and Mogroside samples with each other. The same trend of ratings could be observed through all other categories where there were great differences when comparing Isomaltulose and Mogroside samples. The Isomaltulose samples scored higher in all attributes which suggested that these samples were liked considerably more compared to the mogroside samples.

Table 3: Liking score of cookies with different sweetener mixtures

Sweetener Mixtures		Color^{ns}	Aroma^{ns}	Buttery Flavor	Sweetness	Crispiness	Overall Texture	Overall Liking
Sucralose	Isomaltulose	6.1 ± 2.0	5.9 ± 1.6	6.4 ± 1.5^{a}	6.6 ± 1.8^{a}	7.0 ± 1.6^{a}	6.7 ± 1.5^{a}	6.6 ± 1.7^{a}
	Mogroside	6.3 ± 1.9	5.5 ± 1.5	5.0 ± 1.7^{b}	5.2 ± 2.0^{b}	5.4 ±1.8^{b}	5.3 ± 1.6^{b}	5.2 ± 1.5^{b}
Stevia	Isomaltulose	6.1 ± 1.7	6.1 ± 1.1	6.4 ± 1.5^{a}	6.4 ± 1.6^{a}	7.1 ± 1.4^{a}	6.9 ± 1.3^{a}	6.8 ± 1.5^{a}
	Mogroside	6.8 ± 1.5	5.7 ± 1.7	5.5 ± 1.9^{b}	5.2 ± 2.2^{b}	5.8 ± 1.6^{b}	5.7 ± 1.7^{b}	5.8 ± 1.5^{b}
Normal cookie (Control)*		6.7 ± 1.2	6.0 ± 1.7	6.3 ± 1.4	6.7 ± 1.2	7.4 ± 1.1	7.3 ± 1.0	7.1 ± 1.1

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

* Denoted that sensory analysis of control sample were done with different group of panels, therefore, the result in statistic were not performed.

From the Data that has been obtained it became clear that in terms of spread ratio there had been no great differences between the different samples apart from the sample that included a mixture of Stevia with Isomaltulose, which had a slightly lower spread ratio at 2. 79 ± 0.12 cm. Deviations could be explained as part of variation as cookies may not have been shaped identically and slight differences between cookie diameters and shape were to be expected or due to a stability loss that occurred by using these type of sweetener mixtures.

Color measurements indicated significant differences between the 2 samples that have used Isomaltulose and Mogroside respectively as shown in Table 2. The overall appearance for samples that included Mogroside are shown to be paler compared to a standard cookie. The pale color is an indication for the missing of browning reaction and the lack of reducing sugars. The samples containing Isomaltulose were darker in color in comparison to the Mogroside samples and the standard cookie. It can be seen that in terms of color differences that the standard cookie is right in between the different samples whereas cookies with Isomaltulose were darker and cookies with Mogroside were paler. Liking score (Table 4) implied that the difference in color has had no significant influence on how well liked the cookies were.

The hardness and fracturability played a role in determining the texture of a cookie. As can be seen in Table 3 the hardness and fracturability of the samples are all lower compared to the standard. In spite of this fact, the liking score for the samples with Mogroside are seen as the least favorable in terms of overall texture even though they had the lowest hardness amongst the sweetener mixtures. From this it can be inferred that if the hardness of a cookie falls below a certain threshold it will be perceived negatively as they aren't as crispy and hard as other cookies. In terms of Crispiness and overall texture the samples that included Isomaltulose were more liked than the Mogroside samples (Table 4).

Liking Score of the different samples showed that color and aroma were of non-significance by the panelists, buttery flavor and sweetness were all more preferable in samples that included Isomaltulose. According to the liking score, the sweetener mixtures that were chosen for the next experimental were the sweetener mixtures Sucralose with Isomaltulose, as well as Stevia with Isomaltulose.

2. The appropriate ratio within the chosen sweetener mixtures and how different ratios may affect butter cookie properties

For the determination of the physical diameters a Vernier Caliper was used in the same way as in previous experimental steps. The spread ratio for the Stevia with Isomaltulose mixture with different ratios was non-significant. Contrary to Stevia with Isomaltulose the combination of Sucralose with isomaltulose was showing significant differences between spread ratios with changing ratios of the sweeteners. 100% Sucralose and 0% Isomaltulose were showing a spread ratio of 2.84 ± 0.15 whereas 75% Sucralose and 25% Isomaltulose had a ratio of 2.93 ± 0.09 which are the lowest spread ratios for these samples. These ratios had no significant difference between each other, but they had a significant difference to the other ratios of sweeteners. The highest spread ratio can be seen from 50% Sucralose and 50% Isomaltulose with 3.31 ± 0.06 which is comparable to the spread ratio of the control with 3.21 ± 0.12. 25% Sucralose and 75% Isomaltulose and 0% Sucralose and 100% Isomaltulose had slightly lower spread ratio when compared to the control. From these numbers it could be summarized that different ratios would influence the spread ratio to a certain degree. Furthermore, it revealed that the ratio of 50% to 50% of Sucralose and Isomaltulose came close to what a standard cookie would result in. The deviations between samples might have originated from different positions in the oven, if the heat flow was not completely balanced.

Table 4: Diameters and spread ratio of cookies with sweetener mixtures of stevia and isomaltulose at varying ratios

Sweetener Mixtures (Stevia : Isomaltulose)	Thickness^{ns} (cm)	Diameter (cm)	Spread ratio^{ns} (Diameter/Thickness)
100 : 0	1.27 ± 0.08	4.06 ± 0.05^{c}	3.23 ± 0.17
75 : 25	1.26 ± 0.09	4.12 ± 0.09^{b}	3.28 ± 0.16
50 : 50	1.26 ± 0.09	4.26 ± 0.07^{a}	3.28 ± 0.18
25 : 75	1.3 ± 0.07	4.23 ± 0.07^{b}	3.14 ± 0.12
0 : 100	1.35 ± 0.07	4.11 ± 0.06^{c}	3.25 ± 0.18
100% Sugar (Standard)	1.27 ± 0.04	4.29 ± 0.11^{a}	3.38 ± 0.15

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

Table 5: Diameters and spread ratio of cookies with sweetener mixtures of sucralose and isomaltulose at varying ratios

Sweetener Mixtures (Sucralose : Isomaltulose)	Thickness	Diameter	Spread ratio
100 : 0	1.43 ± 0.07^{b}	4.05 ± 0.07^{b}	2.84 ± 0.15^{c}
75 : 25	1.39 ± 0.02^{b}	4.07 ± 0.09^{b}	2.93 ± 0.09^{c}
50 : 50	1.27 ± 0.04^{a}	4.20 ± 0.07^{a}	3.31 ± 0.06^{a}
25 : 75	1.41 ± 0.07^{b}	4.16 ± 0.07^{b}	2.96 ± 0.13^{b}
0 : 100	1.36 ± 0.05^{b}	4.22 ± 0.05^{a}	3.11 ± 0.15^{b}
100% Sugar (Standard)	1.33 ± 0.04^{a}	4.27 ± 0.09^{a}	3.21 ± 0.12^{a}

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

In order to analyze the color characteristics of the cookies, the values of L*, a* and b* were determined using the HunterLab color system. Stevia with Isomaltulose had significant differences in L*, a* and b* with different ratios. 100% Stevia and 0% Isomaltulose had a value of L* as 45.25 ± 1.69 which was similar to the control that had a value of 46.46 ± 1.92, thus it can be seen that there is no significant difference between those 2 samples. 75% Stevia and 25% Isomaltulose had the highest L* value with 47.02 ± 2.06 while there was no significant difference between 50% Stevia and 50% Isomaltulose and 25% Stevia and 75% Isomaltulose. The lowest value for L* was found in 0% Stevia and 100% Isomaltulose (37.83 ± 3.34d) which made it the darkest sample when compared to the others.

Similar to L*, the value for a* for 100% Stevia and 0% Isomaltulose (3.87 ± 1.35) concurred with the value from the control (4.60 ± 1.59) and there was no significant difference between them. 75% Stevia and 25% Isomaltulose had a value of 4.88 ± 1.71b and was significantly different from the other samples. Moreover, the ratios of 50% Stevia and 50% PP, 25% Stevia and

75% Isomaltulose and 0% Stevia and 100% Isomaltulose had no significant differences between each other.

For b*, all values had no significant difference between each other barring 0% Stevia and 100% Isomaltulose. Through the data it can be ascertained that the sample with 100% Stevia and 0% Isomaltulose matched the control sample very closely with no significant difference in color between them. 75% Stevia and 25% Isomaltulose was the sample that appeared to be the lightest in color whilst the sample for 0% Stevia and 100% Isomaltulose had the darkest appearance due to having the greatest amount of Isomaltulose and therefore had the highest degree of browning reaction.

The color characteristics for Sucralose with Isomaltulose showed similar patterns to the characteristics that have been observed in the Stevia with Isomaltulose samples. The value of L* decreased with the increasing of Isomaltulose which showed that samples became darker with higher concentrations of Isomaltulose. There was no significant difference between control (44.23 ± 1.39) and 100% Sucralose and 0% Isomaltulose (44.58 ± 1.87). The value of 0% Sucralose and 100% Isomaltulose was the lowest and was significantly different from other samples. Just as with the values for L*, the values for a* coincided with each other. There was no significant difference between 100% Sucralose and 0% Isomaltulose and the control. There was no significant difference between 75% Sucralose and 25% Isomaltulose, 50% Sucralose and 50% Isomaltulose, 25% Sucralose and 75% Isomaltulose. On the on the other hand, 0% Sucralose and 100% Isomaltulose was different from all other samples and had the highest a* value. The values for b* differed from the patterns that could be seen in L* and a*. 100% Sucralose and 0% Isomaltulose and 75% Sucralose and 25% Isomaltulose had no significant difference. 50% Sucralose and 50% Isomaltulose as well as 25% Sucralose and 75% Isomaltulose had no significant difference while 0% Sucralose and 100% Isomaltulose were significantly different to all other samples (17.72 ± 0.86). Control also had a significant difference from all other samples (19.69 ± 0.74). It was identified that in terms of appearance, the 100% Sucralose and 0% Isomaltulose was comparable to the control whereas the other samples were darker and more brown-reddish in color.

Table 6: Color measurement of cookies with sweetener mixtures of stevia and isomaltulose at varying ratios

Sweetener Mixtures (Stevia : Isomaltulose)	L*	a*	b*
100 : 0	45.25 ± 1.69^{b}	3.87 ± 1.35^{c}	18.78 ± 0.96^{a}
75 : 25	47.02 ± 2.06^{a}	4.88 ± 1.71^{b}	19.23 ± 1.15^{a}
50 : 50	40.34 ± 2.18^{c}	6.83 ± 1.12^{a}	19.21 ± 0.39^{a}
25 : 75	40.84 ± 1.29^{c}	6.46 ± 0.93^{a}	19.31 ± 0.94^{a}
0 : 100	37.83 ± 3.34^{d}	7.15 ± 0.87^{a}	18.31 ± 1.03^{b}
100% Sugar (Standard)	46.46 ± 1.92^{b}	4.60 ± 1.59^{c}	19.41 ± 1.00^{a}

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

Figure 8: Photo of Butter cookies with varying ratios of Stevia and Isomaltulose (PP)

Table 7: Color measurement of cookies with sweetener mixtures of sucralose and isomaltulose at varying ratios

Sweetener Mixtures (Sucralose : Isomaltulose)	L*	a*	b*
100 : 0	44.58 ± 1.87^{a}	4.33 ± 1.14^{c}	18.14 ± 0.99^{c}
75 : 25	38.38 ± 1.74^{b}	7.28 ± 1.16^{b}	18.20 ± 0.63^{c}
50 : 50	38.96 ± 2.08^{b}	7.53 ± 0.92^{b}	18.77 ± 0.72^{b}
25 : 75	39.03 ± 2.24^{b}	6.96 ± 1.05^{b}	18.31 ± 0.70^{b}
0 : 100	36.43 ± 2.56^{c}	8.20 ± 1.10^{a}	17.72 ± 0.86^{d}
100% Sugar (Standard)	44.23 ± 1.39^{a}	4.87 ± 1.16^{c}	19.69 ± 0.74^{a}

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

Figure 9: Photo of Butter cookies with varying ratios of Sucralose and Isomaltulose (PP)

The determination of texture had been done through the texture analyzer as in the previous study. The attributes of concern were hardness and fracturability and the corresponding data can be viewed in Table 5 with different ratios of sweetener mixtures (Stevia and Isomaltulose). For the Stevia and Isomaltulose combination, neither hardness nor fracturability had a significant difference between different ratios of sweeteners, which indicated little to no differences between them in terms of texture. The Sucralose and Isomaltulose combination, on the other hands, found changes in terms of hardness but no significantly different changes when it came to the fracturability of the samples. Noteworthy was that 100% Sucralose and 0% Isomaltulose, 75% Sucralose and 25% Isomaltulose and 50% Sucralose and 50% Isomaltulose had no significant difference between each other. Although 25% Sucralose and 75% Isomaltulose as well as 0%

Sucralose and 100% Isomaltulose had differed from the samples with 50% Sucralose or more as they had a greater hardness when compared to them. The hardness of the control was situated between (100%, 75% and 50% Sucralose) and (25%, 0% Sucralose).

Table 8: Hardness and fracturability of cookies with sweetener mixtures of stevia and isomaltulose at varying ratios

Sweetener Mixtures (Stevia : Isomaltulose)	Hardness^{ns} (g)	Fracturability^{ns} (mm)
100 : 0	1455.78 ± 453.20	11.9 ± 4.72
75 : 25	1722.76 ± 445.07	13.74 ± 0.61
50 : 50	1824.45 ± 214.19	14.25 ± 0.69
25 : 75	1573.78 ± 97.37	15.78 ± 0.59
0 : 100	1557.17 ± 451.84	14.25 ± 2.01
100% Sugar (Standard)	1738.04 ± 159.44	14.99 ± 0.79

Different letters within the column denotes significant difference between means (p<0.05), ns denotes non-significance (p≥0.05).

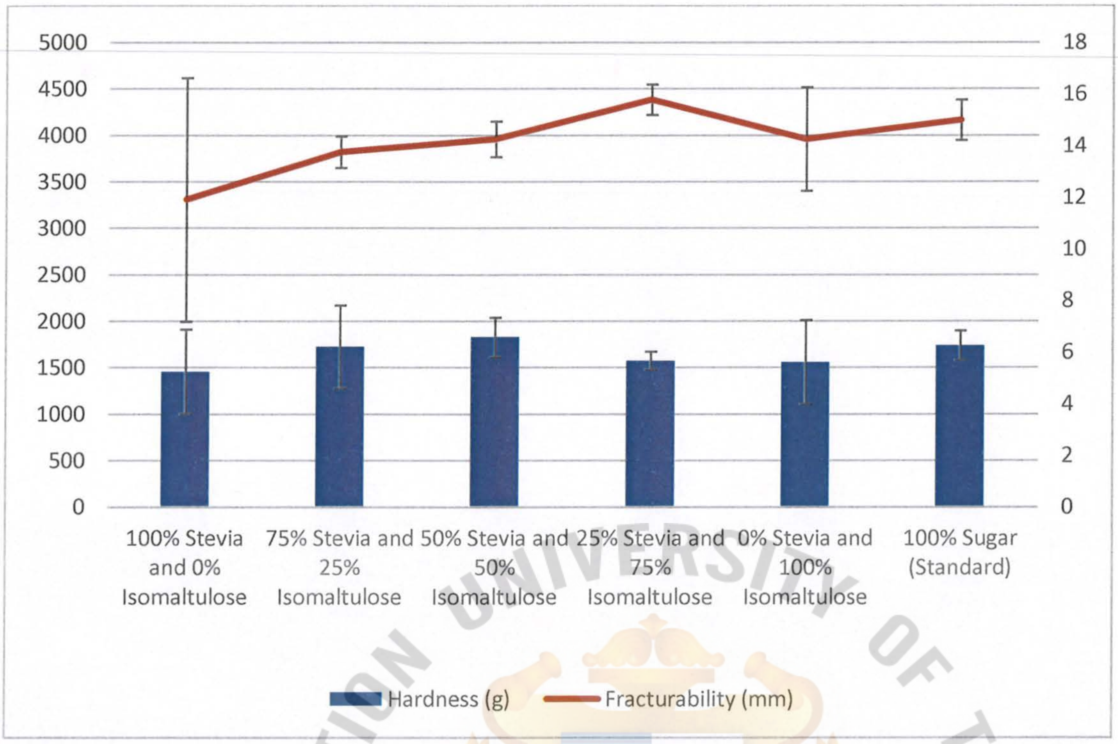

Figure 10: Hardness and Fracturability at varying ratios of stevia and isomaltulose

Table 9: Hardness and fracturability of cookies with sweetener mixtures of sucralose and isomaltulose at varying ratios

Sweetener Mixtures (Sucralose : Isomaltulose)	Hardness (g)	Fracturability^{ns} (mm)
100 : 0	2064.80 ± 289.38c	18.30 ± 2.22
75 : 25	1683.96 ± 263.81c	19.26 ± 0.86
50 : 50	2065.69 ± 344.22c	17.89 ± 0.55
25 : 75	3393.45 ± 381.52a	17.36 ± 0.39
0 : 100	3730.77 ± 779.35a	17.19 ± 0.28
100% Sugar (Standard)	2518.06 ± 231.06b	18.48 ± 0.74

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

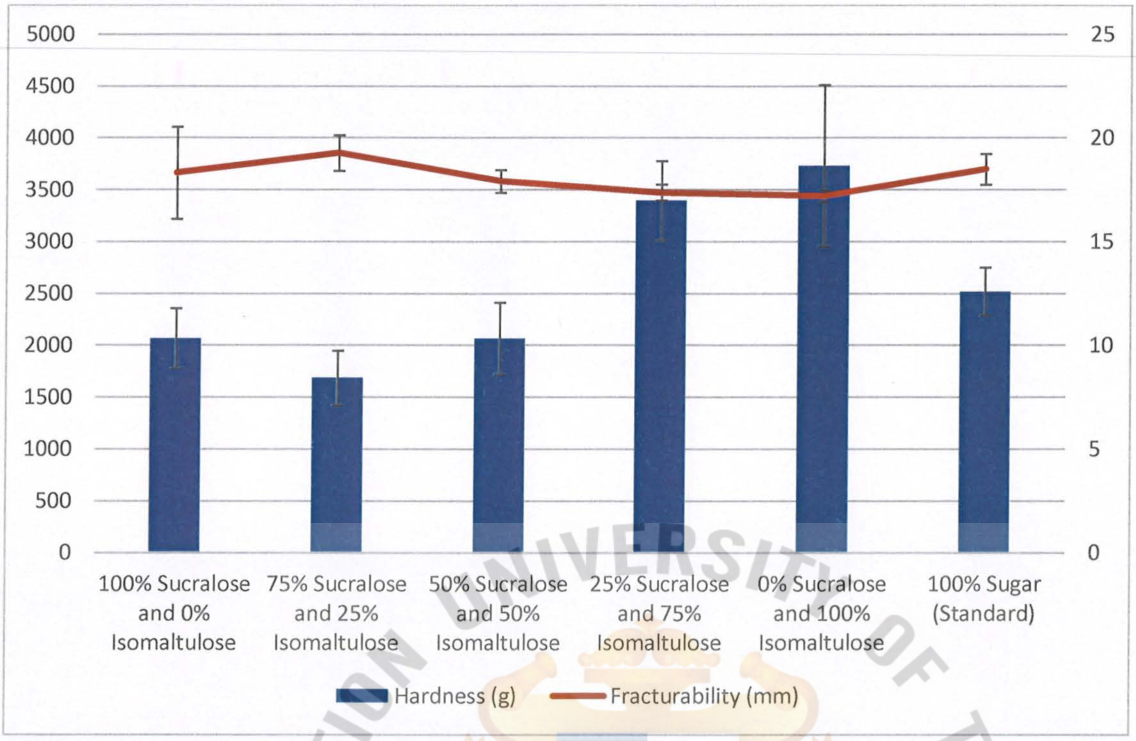

Figure 11: Hardness and fracturability at varying ratios of sucralose and isomaltulose

The liking score for the various attributes was determined and then statistically analyzed. Results are shown in Table 8. With different ratio of sweetener mixtures (Stevia – Isomaltulose), it was seen that color and aroma was of no significance to the panelists. Buttery flavor showed differences. 100% Stevia and 0% Isomaltulose, 50% Stevia and 50% Isomaltulose and 100% Sugar (Standard) were liked the most whereas 75% Stevia and 25% Isomaltulose, 25% Stevia and 75% Isomaltulose as well as 0% Stevia and 100% Isomaltulose were liked the least. For the attribute of sweetness 100% Sugar (Standard), 100% Stevia and 0% Isomaltulose and 50% Stevia and 50% Isomaltulose were liked the most with no significant difference between them. 75% Stevia and 25% Isomaltulose and 0% Stevia and 100% Isomaltulose scored the least points. As for the crispiness, the 100% Sugar (Standard) had the highest value (7.37 ± 1.07) but was not significantly different from 100% Stevia (6.97 ± 1.00). 75% Stevia and 50% Stevia had similar scores for crispiness, which were a bit lower when compared to the standard. 25% and 0% Stevia also had similar liking scores to each other and these 2 samples were liked the least. For the overall texture, it could be seen that the standard scored the highest with 7.30 ± 0.95[a] .The next 2 samples below the standard were 100% Stevia and 50% Stevia and 50% Isomaltulose while 75% Stevia and 25% Isomaltulose and 0% Stevia and 100% Isomaltulose had the worst liking score amongst the samples. Overall liking showed that the standard was liked the most and this was followed by the 100% Stevia and 0% Isomaltulose in tandem with 50% Stevia and 50% Isomaltulose sample which was worse than the standard but still liked more than all the other samples that included the sweetener mixture. From these results, it could be inferred that the 100% Stevia and 0% Isomaltulose was liked the most among all sweeteners.

The results for the liking score for the Sucralose and Isomaltulose mixture can be seen from Table 8. The first difference that can be seen when compared to Stevia and Isomaltulose mixture was that there was a significant difference in color and aroma with varying ratios of Sucralose and Isomaltulose. The best liking score was given to the 50:50 sample while 100:0 Sucralose to Isomaltulose had the least liking score and thus was perceived as the sample with the darkest color and least preferred amongst the panelists. As far as aroma was concerned the values which were quite close to another while samples for 100% Sucralose and standard had the least aroma (lowest liking score). 50:50, 25:75 and 0:100 of Sucralose to Isomaltulose were perceived as having the best buttery flavor and there was no significant difference between them. 75:25 and the standard had a lower liking score whilst 100% Sucralose had the least rating for buttery flavor. The highest rating for sweetness was seen in 25:75 and 0:100, in comparison 100:0 and the standard had the least liking score on sweetness across the different samples. When it came to crispiness the samples for 50:50, 25:75 and 0:100 were found to be liked the most, while the standard was liked a bit less. As far as overall texture was concerned it was well liked in the samples for 50:50, 25:75 and 0:100 yet 100:0 was perceived as the least. Overall liking showed that the samples with 50:50, 25:75 and 0:100 were liked the most and 100:0 was liked the least. The overall liking for this sweetener mixture was received better than the Stevia and Isomaltulose mixtures.

Table 10: Liking score of cookies with sweetener mixtures of stevia and isomaltulose at varying ratios

Sweetener Mixtures (Stevia : Isomaltulose)	Color[ns]	Aroma[ns]	Buttery Flavor	Sweetness	Crispiness	Overall Texture	Overall Liking
100 : 0	6.1 ± 1.5	6.3 ± 1.0	5.7 ± 1.5[a]	6.4 ± 1.2[a]	7.0 ± 1.0[a]	6.7 ± 1.1[b]	6.6 ± 0.9[b]
75 : 25	6.2 ± 1.4	5.7 ± 1.3	5.0 ± 1.5[b]	5.0 ± 1.5[c]	5.7 ± 1.4[b]	5.8 ± 2.5[c]	5.4 ± 1.4[c]
50 : 50	6.9 ± 1.2	6.2 ± 1.3	6.3 ± 1.5[a]	6.2 ± 1.5[a]	5.9 ± 1.5[b]	6.3 ± 1.6[b]	6.0 ± 1.4[b]
25 : 75	6.4 ± 1.3	5.9 ± 1.3	5.4 ± 1.6[b]	5.8 ± 1.5[b]	4.7 ± 1.8[c]	5.4 ± 1.7[c]	5.5 ± 1.3[c]
0 : 100	6.1 ± 1.5	5.6 ± 1.1	5.3 ± 1.4[b]	5.6 ± 1.3[c]	4.7 ± 1.3[c]	5.4 ± 1.6[c]	5.5 ± 1.4[c]
100% Sugar (Standard)	6.7 ± 1.1	6.0 ± 1.7	6.3 ± 1.4[a]	6.7 ± 1.1[a]	7.4 ± 1.1[a]	7.3 ± 0.9[a]	7.1 ± 1.1[a]

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

Table 11: Liking score of cookies with sweetener mixtures of sucralose and isomaltulose at varying ratios

Sweetener Mixtures (Sucralose : Isomaltulose)	Color	Aroma	Buttery Flavor	Sweetness	Crispiness	Overall Texture	Overall Liking
100 : 0	5.5 ± 1.9^{c}	5.6 ± 1.2^{b}	4.7 ± 1.4^{c}	5.5 ± 1.5^{d}	4.2 ± 1.6^{c}	4.6 ± 1.7^{c}	4.7 ± 1.2^{c}
75 : 25	6.7 ± 0.9^{b}	6.3 ± 1.1^{a}	5.7 ± 1.4^{b}	6.2 ± 1.3^{b}	4.9 ± 1.2^{c}	5.3 ± 1.1^{b}	5.8 ± 0.9^{b}
50 : 50	7.4 ± 1.0^{a}	6.2 ± 1.5^{a}	6.5 ± 1.3^{a}	6.8 ± 1.2^{b}	6.8 ± 1.1^{a}	6.9 ± 1.1^{a}	6.9 ± 1.0^{a}
25 : 75	6.4 ± 1.3^{b}	6.5 ± 1.2^{a}	6.8 ± 1.0^{a}	7.1 ± 0.8^{a}	6.7 ± 1.3^{a}	6.7 ± 1.2^{a}	7.0 ± 0.8^{a}
0 : 100	6.7 ± 1.3^{b}	6.6 ± 1.1^{a}	6.7 ± 0.9^{a}	6.9 ± 1.0^{a}	6.7 ± 1.4^{a}	6.3 ± 1.4^{a}	6.6 ± 0.9^{a}
100% Sugar (Standard)	6.2 ± 1.4^{b}	5.7 ± 1.5^{b}	5.5 ± 1.6^{b}	5.7 ± 1.5^{d}	5.7 ± 1.6^{b}	5.8 ± 1.5^{b}	5.7 ± 1.3^{b}

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

The physical characteristics in the experimental step 2 showed that the spread ratio for the sample at different the ratios of Stevia and Isomaltulose saw no significant differences between each other. The spread ratio in the sample of Sucralose and Isomaltulose showed differences, 100:0 and 75:25 Sucralose to Isomaltulose presented similar spread ratios and showed a spread ratio similar to the standard cookie.

The results the Hunter Lab displayed illustrated that the sample that used Stevia and Isomaltulose showed significant difference in color values. Stevia with Isomaltulose at 100:0 had a similar color value to the standard, 75:25 was slightly different from the standard and 50:50, 25:75 and 0:100 were significantly different from the standard but similar to each other in color appearance.

Similar to the results of the Stevia with Isomaltulose sample, the sample that used Sucralose with Isomaltulose showed differences among the samples with varying ratios. With a ratio of 100:0 Sucralose to Isomaltulose its color was similar to the standard with the exception of the b* value (18.14 ± 0.99) which was lower compared to the standard. The ratios of 75:25, 50:50 and 25:75 had similar color values to each other and were slightly different compared to the standard. The greatest difference in color values was observed at a ratio of 0:100 where the color was disparate compared to the standard. As mentioned before the difference in color strongly correlated to the concentration of Isomaltulose since it readily undergoes browning reaction. Therefore, with higher Isomaltulose % we will see a greater darkening in the appearance of the cookie which may or may not be desirable depending on the manufacturer's needs.

The texture properties of hardness and fracturability in sample Stevia with Isomaltulose showed no significant differences between varying ratios. It could be seen that the change in ratios has very little to no effect on these attributes. The sample that used Sucralose and Isomaltulose had significant differences occur in hardness with varying ratios, the fracturability on the other hand had no significant differences between each other. 100:0 and 50:50 Sucralose to Isomaltulose had similar hardness values to the standard cookie while the other ratios such as 25:75 and 0:100 had a significantly higher value (greater hardness) and 75:25 had a significantly lower value (less hardness).

In respect to the liking scores it became obvious that the sweetener mixture of Sucralose and Isomaltulose was more liked when compared to its Stevia and Isomaltulose counterpart. With the help of the liking score it was determined that the sweetener ratio that was liked the most, was the 25% Sucralose and 75% Isomaltulose mixture. Therefore, it was concluded that in terms of sweetener mixture optimization the ratio of 25:75 had been the most successful out of the given samples and was chosen for experimental step 3 in which the ratio of sugar and sweetener mixture was optimized.

3. The substation of sugar using sweetener mixtures without altering the characteristics of the cookie

As seen from the data of the physical diameters the spread ratio of the standard is 3.06 ± 0.15. The sample with 75% Sugar + 25% Sweetener and 50% Sugar and 50% Sweetener both retained a similar spread ratio to the standard. Samples with 25% Sugar + 75% Sweetener and with 0% Sugar +100% Sweetener showed a significant difference compared to the standard with a spread ratio of 2.70 ± 0.29 and 2.50 ± 0.14. With a decrease of sugar and an increase in sweeteners, the spread ratio changed based on the degree of substitution. The more sugar was substituted for the sweetener mixture the greater the spread of the cookie became, which resulted in cookies less likely to retain their intended shape/size when compared to the standard. These results showed that sugar was very likely acting as a stabilizing agent in the cookies. This meant that if sugar was removed to a great degree another stabilizing agent had to be added in order for the cookies to retain their intended shape.

Table 12: Diameters and spread ratio of cookies with varying ratios of sugar and sweetener mixtures of sucralose and isomaltulose

Ratio (Sugar : Sweetener)	Thickness (cm)	Diameter (cm)	Spread ratio (Diameter/Thickness)
Normal cookie (100% sugar)	1.44 ± 0.09^{b}	4.40 ± 0.07^{a}	3.06 ± 0.15^{a}
75 : 25	1.36 ± 0.05^{c}	4.27 ± 0.12^{b}	3.14 ± 0.13^{a}
50 : 50	1.44 ± 0.09^{b}	4.35 ± 0.21^{a}	3.03 ± 0.26^{a}
25 : 75	1.54 ± 0.13^{b}	4.13 ± 0.09^{c}	2.70 ± 0.29^{b}
0 : 100	1.68 ± 0.11^{a}	4.18 ± 0.11^{c}	2.50 ± 0.14^{b}

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

In terms of color changes, the results presented evidence that the standard appears significantly different in appearance from the other samples. The difference between standard and samples was significant whereas the difference between samples was insignificant which implied that there wasn't a great disparity in appearance. All samples appeared darker and more reddish-brownish compared to the standard. The degree of browning correlated with a greater degree of sweetener mixture. This was due to the presence of isomaltulose, isomaltulose undergoes the browning reaction readily because it had a free reducing group.

Table 13: Color measurement of cookies with varying ratios of sugar and sweetener mixtures of sucralose and isomaltulose

Ratio (Sugar : Sweetener)	L*	a*	b*
Normal cookie (100% Sugar)	64.23 ± 2.02^{a}	7.94 ± 1.50^{b}	30.53 ± 1.68^{b}
75 : 25	57.30 ± 5.04^{b}	11.79 ± 2.52^{a}	32.29 ± 1.47^{a}
50 : 50	55.88 ± 4.14^{b}	12.16 ± 2.68^{a}	32.96 ± 1.65^{a}
25 : 75	57.37 ± 5.07^{b}	12.39 ± 3.22^{a}	32.94 ± 2.35^{a}
0: 100	56.07 ± 5.63^{b}	12.52 ± 3.39^{a}	32.89 ± 2.11^{a}

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

Figure 12: Photo of Butter cookies with varying ratios of Sugar and Sweetener mixture

The data obtained for hardness and fracturability showed that the fracturability between all samples was considered as insignificant. The hardness in contrast showed significant differences between samples. The standard of 100% sugar had a hardness of 4617.30 ± 823.16 (g). Curiously, the ratio of 75% Sugar and 25% Sucralose had a hardness that differed from 100:0 and 50:50 who were not significantly different from each other.

Table 14: Hardness and fracturability of cookies with varying ratios of sugar and sweetener mixtures of sucralose and isomaltulose

Ratio (Sugar : Sweetener)	Hardness (g)	Fracturability[ns] (mm)
Normal cookie (100% Sugar)	4617.30 ± 823.16[a]	13.16 ± 1.09
75 : 25	2906.14 ± 178.95[b]	13.51 ± 0.71
50 : 50	4253.40 ± 860.74[a]	13.25 ± 0.62
25 : 75	1993.37 ± 490.99[c]	12.82 ± 1.7
0: 100	1742.81 ± 225.50[c]	13.91 ± 0.47

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

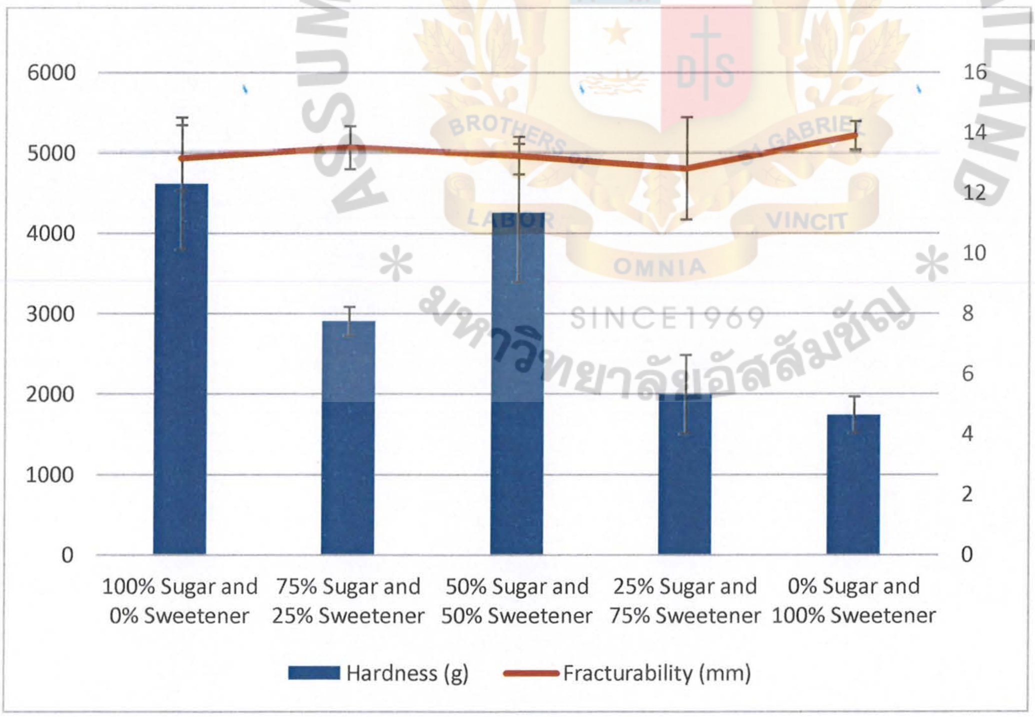

Figure 13: Hardness and Fracturability at varying ratios of sugar and sweetener mixture

The results for the liking score in experimental step 3 can be found in the Table 12. Furthermore commercial butter cookies were included in the sensory tests to draw a better comparison between all the different samples and give a more conclusive end result. In regard to color it was obvious that samples with 100:0, 75:25 and 50:50 of sugar to sweetener had the highest liking scores. On the contrary, 25:75, 0:100 as well as commercial sample scored slightly lower. As for the liking score involved with aroma, the standard of 100% sugar and the commercial sample had the highest liking score while the other samples containing sweeteners received a lower liking score. For buttery flavor the 100:0, 75:25 and the commercial sample got the highest liking scores. The highest liking scores for sweetness were given to 100:0 and 50:50 whereas the commercial sample and 25:75 got the least liking scores. For crispiness 100:0 and the commercial had the greatest liking scores. Overall texture was highest in 100:0 and the commercial. Followed by these 2 samples came 75:25 and 50:50. Overall liking is one of the most important attributes and from this a trend was noticed in how well received the different samples were. The highest liking scores were given to 100:0 and the commercial, however, 75:25 and 50:50 scored comparably to them. It became clear that the standard and commercial sample were liked the most while the liking score was reduced in descending order depending on the degree of sugar substitution.

Table 15: Liking score of cookies with varying ratios of sugar and sweetener mixtures of sucralose and isomaltulose

Ratio (Sugar : Sweetener)	Color	Aroma	Buttery Flavor	Sweetness	Crispiness	Overall Texture	Overall Liking
Normal cookie (100% Sugar)	7.2 ± 1.2^{a}	6.4 ± 1.2^{a}	6.6 ± 1.4^{a}	6.9 ± 1.4^{a}	6.6 ± 1.2^{a}	6.6 ± 1.1^{a}	6.7 ± 1.0^{a}
75 : 25	7.0 ± 0.9^{a}	6.3 ± 1.1^{b}	6.7 ± 1.4^{a}	6.7 ± 1.2^{b}	5.9 ± 1.3^{b}	6.2 ± 1.3^{b}	6.3 ± 1.0^{b}
50 : 50	7.3 ± 1.1^{a}	6.1 ± 1.2^{b}	6.3 ± 1.4^{b}	6.8 ± 1.1^{a}	6.0 ± 1.2^{b}	6.2 ± 1.1^{b}	6.5 ± 0.9^{b}
25 : 75	6.9 ± 1.0^{b}	6.0 ± 1.1^{b}	5.9 ± 1.1^{b}	5.9 ± 1.3^{c}	5.4 ± 1.2^{c}	5.7 ± 1.2^{c}	5.9 ± 1.2^{c}
0 : 100	6.6 ± 1.0^{b}	6.2 ± 1.3^{b}	6.0 ± 1.1^{b}	6.2 ± 1.3^{b}	5.9 ± 1.6^{b}	5.8 ± 1.5^{c}	5.9 ± 1.1^{c}
S & P	7.0 ± 1.4^{b}	6.9 ± 1.1^{a}	6.8 ± 1.3^{a}	6.1 ± 1.4^{c}	6.8 ± 1.6^{a}	6.9 ± 1.2^{a}	6.9 ± 1.2^{a}

Different letters within the column denotes significant difference between means ($p<0.05$), ns denotes non-significance ($p\geq0.05$).

CONCLUSION

This experiment was to study and represent the possibility to substitute sugar content in butter cookie products. The data gave clear trends and indications where the optimization of sweeteners and sugar leads to. As seen from the results, it became distinct that the standard and the commercial samples were liked and accepted the most. However, it was discernable that although samples with sweetener mixtures weren't liked as much as the standard cookies, they weren't disliked either. Agreeable acceptance was found in samples with a sugar substitution of up to 50% by adding the combination of sucralose and Isomaltulose at a ratio of 25:75. Although a complete substitution of sugar might not be possible yet, there is a good chance that the optimized sweetener mixture can readily substitute 50% of the sugar content without affecting the quality or liking score of the cookie to gravely thus making it a good alternative for a more healthy option.

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Sensory Evaluation Ballot:

Low Sugar Butter Cookie:

Instruction: Please taste the samples from left to right; remember to rinse your palate with water before and in between tasting the Cookies. Rate the samples based on your preference using a 9 – point hedonic scale.

1 – Dislike extremely	2 – Dislike very much	3 – Dislike moderately
4 – Dislike slightly	5 – Neither like nor dislike	6 – Like slightly
7 – Like moderately	8 – Like very much	9 – Like extremely

Attributes	Sample Number					
Color						
Aroma						
Buttery Flavor						
Sweetness						
Crispiness						
Overall Texture						
Overall liking						

Which samples do you like the most?

Sample number: ____________________