DISSERTATION

PHYSICOCHEMICAL AND SENSORY QUALITY OF CHIFFON CAKE PREPARED WITH REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE

Submitted by

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ABSTRACT

PHYSICOCHEMICAL AND SENSORY QUALITY OF CHIFFON CAKE PREPARED WITH REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE

Rebaudioside-A has been used for many years throughout the world as a non-nutritive sweetener in many different food systems (Goyal et al. 2010), however, up until 2008, it was not an approved food sweetener in the United States (FDA 2008). Prior to this approval, stevia extracts were found in herbal and health food stores throughout the United States since the 1970's (Carakostas et al. 2008). Since the passage of the Dietary Supplement Health and Education Act (DSHEA) in 1994, stevia extracts were legally sold as "dietary supplements" in the United States marketplace (Carakostas et al. 2008). However, under the DSHEA, these products were not permitted to be marketed with any packaging or advertising language suggesting that they be used as a sweetener (Carakostas et al. 2008).

Since 2008, Truvia™ brand sweetener produced and marketed by Cargill (2010) has been available on the consumer market in the United States. This sweetener is a mixture of rebaudioside-A and erythritol that is made for a consumer to replace sucrose in applications at a 1:1 ratio (Cargill 2010). The sweetening characteristics of rebaudioside-A appear to be approximately 300 times that of sucrose (Lin and Lee 2005) and digestion, if any, occurs in the large intestine by gastrointestinal microflora, lending a minimal caloric intake to the user.

Truvia™ and other blends of rebaudioside-A and erythritol can be used as a natural non-nutritive sugar alternative and may prove to be an effective and acceptable replacement to sucrose in baked systems such as chiffon cake (Cargill 2010). Rebaudioside-A is an leaf extract of the *Stevia rebaudiana* plant (Cargill 2010) and erythritol is a four-carbon polyol widely found in nature in such food as melons, grapes, pears, seaweeds, fungi as well as naturally occurring in fermented food products (Moon et al. 2010).

The purpose of this research was to determine the physicochemical and sensory effects of replacing a mixture of rebaudioside-A and erythritol for sucrose at varying levels (0, 25, 50, 75 and 100%) in chiffon cake. Analytical testing of specific gravity, texture, volume, water activity, moisture, color and differential scanning calorimetry was conducted on the five cake formulations in addition to nutritional analysis and a consumer sensory evaluation ($n = 40$). Five treatments were prepared using four replications in a randomized complete block one factor design. ANOVA was used to determine significant differences. If so, differences among means were examined using Tukey's honestly significant difference.

There were no differences in instrumental color of crust or crumb among any of the replacement levels with the exception that crumb color of both the control and 25% reduced sucrose samples were "more yellow" than the 100% reduced sucrose cakes ($p < 0.05$). Water activity ranged from 0.86 to 0.91 and some statistical differences were found among treatments. However, there were no differences in percent moisture. Texture analysis showed the 25 and 50% reduced sucrose cakes were more tender than the 100% reduced sucrose cake ($p < 0.05$). Volume and specific gravity tests had no differences among treatments.

Differential scanning calorimetry (DSC) results showed that as the replacement level of sucrose with a mixture of rebaudioside-A and erythritol increased, both the onset and peak temperatures decreased. These results corresponded with DSC testing conducted by Lim and others (1992) on wheat starch, sucrose and water interactions. Enthalpy was consistent for the control, 25 and 50% reduced sucrose samples and increased for both the 75 and 100% samples; however, differences were small and would likely not be detectable by consumers.

A nine-point hedonic scale ranging from "like extremely" to "dislike extremely" was used to measure overall liking of color of crumb, tenderness, sweetness, aftertaste and overall acceptability. Panelists were asked to rank preference of the five samples from one to five with one being the most preferred. Sensory panelists "liked" all of the chiffon cake samples and the highest "overall acceptability" scores were for cakes prepared with 25 and 50% reduction in sucrose ($p < 0.05$). These two samples also ranked highest in preference ($p < 0.05$). Additionally panelists "liked" the tenderness of the 25 and 50% reduced sucrose cakes more than the other samples ($p < 0.05$). For sweetness and aftertaste, panelists "liked" the 25, 50 and 75% reduced sucrose cakes ($p < 0.05$). Chiffon cake prepared with 50% sucrose and 50% rebaudioside-A and erythritol resulted in a product with high overall consumer acceptability and 20% fewer calories than one formulated with 100% sucrose.

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I would like to thank my family and friends for putting up with me during this long journey. My father Robert E. Lothrop tragically passed away before this research was completed. Since he was not able to see this journey end, this is dedicated to him.

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CHAPTER I

INTRODUCTION

The purpose of our research was to determine the physicochemical and sensory effects of replacing a mixture of rebaudioside-A and erythritol for sucrose at varying levels (0, 25, 50, 75 and 100%) in a chiffon cake. Analytical testing on specific gravity, texture, volume, water activity, moisture, color and differential scanning calorimetry was conducted on the five cake formulations as well as nutritional analysis and consumer sensory evaluation. With these tests, we set out to determine what replacement level if any is acceptable both from a physicochemical and sensory perspective.

Since 2008, Truvia™ brand sweetener has been produced and marketed by Cargill (2010) for the consumer market in the United States. This sweetener is a mixture of rebaudioside-A and erythritol formulated specifically for a consumer to replace sucrose in applications at a 1:1 ratio (Cargill 2010). The sweetening characteristics of rebaudioside-A appear to be approximately 300 times that of sucrose (Lin and Lee 2005). Digestion, if any, occurs in the large intestine by gastrointestinal microflora, lending a minimal caloric intake to the user.

Truvia™ and other blends of rebaudioside-A and erythritol can be used as a natural non-nutritive sugar alternative and may prove to be an effective and acceptable replacement to sucrose in baked systems such as chiffon cake (Cargill 2010). Rebaudioside-A is a leaf extract of the *Stevia rebaudiana* plant (Cargill 2010), and erythritol is a four-carbon polyol widely found in nature in such food as melons, grapes, pears, seaweeds, fungi as well as naturally occurring in fermented food products such as wine, cheese and soy sauce (Moon et al. 2010).

Depending on the plant, *Stevia rebaudiana* contains eight main sweet-tasting steviol glycosides at varying levels—stevioside, steviolbioside, rebaudiosides A, B, C, D, E and dulcoside A (Rajasekaran et al. 2008; Goyal et al. 2010). "The sweetness of rebaudiosides increases with increasing amount of sugar units bonded to the aglycone (steviol) (Kovylyaeva et al. 2007). The sweetest species is *Stevia rebaudiana* Bertoni which contains all eight glycosides in its leaves and with stevioside is the most predominant with about 3 to 8% by weight of the dried leaf (Kinghorn et al. 1984). Leaf extract compositions are dependent on the composition of the leaves influenced by the soil, climate and extraction processes used (Wallin 2004).

Steviosides are considered the most bitter of the most common non-nutritive high-intensity sweeteners, especially at higher concentrations (Schiffman et al. 1995). Rebaudioside-A, although perceived as less bitter, is ranked second in bitterness to stevioside (Schiffman et al. 1995). Sugar alcohols, such as erythritol may provide a bitterness suppressing effect when combined with rebaudioside-A in food systems (Hashimoto et al. 2007). Hashimoto and others (2007) found that sugar alcohols seemed to be effective in suppressing the bitterness of quinine sulfate solution.

Rebaudioside-A has been used for many years throughout the world as a non-nutritive sweetener in many different food systems (Goyal et al. 2010), however, up until 2008, it was not an approved food sweetener in the United States (FDA 2008). Prior to this approval, stevia extracts were found in herbal and health food stores throughout the United States since the 1970's (Carakotas et al. 2008). Since the passage of the Dietary Supplement Health and Education Act (DSHEA) in 1994, stevia extracts were legally sold as "dietary supplements" in the United States marketplace (Carakotas et al. 2008).

However, under the DSHEA, these products were not permitted to be marketed with any packaging or advertising language suggesting that they be used as a sweetener (Carakotas et al. 2008).

The purpose of this research was to determine if an acceptable level of sugar can be replaced by a mixture of rebaudioside-A and erythritol in a chiffon cake. The null hypothesis was that there were no differences among cakes prepared with or without rebaudioside-A and erythritol. Five treatments were prepared using four replications in a randomized complete block one factor design. ANOVA was used to determine if significant differences existed and if so, differences among means were examined using Tukey's honestly significant difference. Differences were determined when the p-value was less than the significance level of 0.05. Sensory analysis was conducted with forty panelists.

CHAPTER II

REVIEW OF LITERATURE

THE STEVIA PLANT AND ITS SWEETENING COMPOUNDS

The plant *Stevia rebaudiana* belongs to the Asteracea family and is native to the Amambay region of northeastern Paraguay as well as Brazil and Argentina (Brandle and Telmer 2007). It is a small perennial plant growing between 60 and 80 centimeters in height. Interestingly, within the Asteracea family of plants, only two of the two-hundred and thirty species produce steviol glycosides; *Stevia rebaudiana and Stevia phlebophylla* with *Stevia rebaudiana* being the sweetest (Kinghorn et al. 1984).

Depending on the plant, *Stevia rebaudiana* contains eight main sweet-tasting steviol glycosides at varying levels—stevioside, steviolbioside, rebaudiosides A, B, C, D, E and dulcoside A (Rajasekaran et al. 2008; Goyal et al. 2010). "The sweetness of rebaudiosides increases with increasing amount of sugar units bonded to the aglycone (steviol) (Kovylyaeva et al. 2007). The sweetest species is *Stevia rebaudiana* Bertoni which contains all eight glycosides in its leaves and with stevioside is the most predominant with about 3 to 8% by weight of the dried leaf (Kinghorn et al. 1984). Leaf extract compositions are dependent on the composition of the leaves influenced by the soil, climate and extraction processes used (Wallin 2004).

Stevioside is the major glycoside found in the stevia plant leaves and can be used as a sweetener in food systems (Tanaka 1997); however, the second major glycoside, Rebaudioside-A is sweeter and less bitter than stevioside. Due to its sweetness and pleasant flavor, rebaudioside-A has the largest potential for replacing sucrose in beverages and baked goods (Tanaka 1997). In May 2008, the United States Food and

Drug Administration Granted GRAS status to rebaudioside-A for use as a general purpose sweetener in foods excluding those containing meat and poultry products (FDA 2008). Prior to this approval, stevia extracts were found in herbal and health food stores throughout the United States since the 1970's (Carakotas et al. 2008). Since the passage of the Dietary Supplement Health and Education Act (DSHEA) in 1994, stevia extracts were legally sold as "dietary supplements" in the United States marketplace (Carakotas et al. 2008). However, under the DSHEA, these products were not permitted to be marketed with any packaging or advertising language suggesting that they be used as a sweetener (Carakotas et al. 2008).

Steviol is the aglycone of all the principle and secondary sweetener components (Wallen 2004). Figure 2.1 below illustrates the chemical structure of steviol in its simplest form and all subsequent glycosides are formed based off this structure with either a hydrogen or glucose molecule attached at R-group one; R-group two either contains two or more glucose molecules or a combination of glucose and rhamnose. Table 2.1 outlines the main steviol glycosides and their respective R-groups. The sweetest of these, rebaudiosde-A, contains one glucose molecule attached at R-group one and three glucose molecules at R-group two which is unique to other steviol glycosides. A complete diagram of each structure can be found in Appendix I.

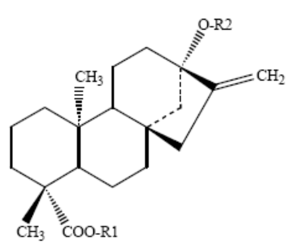

FIGURE 2.1: STEVIOL STRUCTURE

(Carakotas et al. 2008)

TABLE 2.1: STEVIOL GLYCOSIDES AND THEIR RESPECTIVE R-GROUPS

Compound Name	R-Group 1	R-Group 2
Steviolbioside	H	β-Glc- β-Glc (2→1)
Stevioside	β-Glc	β-Glc- β-Glc (2→1)
Dulcoside A	β-Glc	β-Glc-α-Rha (2→1)
Rebaudioside-A	β-Glc	β-Glc- β-Glc (2→1) | β-Glc (3→1)
Rebaudioside B	H	β-Glc- β-Glc (2→1) | β-Glc (3→1)
Rebaudioside C	β-Glc	β-Glc-α-Rha (2→1) | β-Glc (3→1)
Rebaudioside D	β-Glc- β-Glc (2→1)	β-Glc- β-Glc (2→1) | β-Glc (3→1)
Rebaudioside E	β-Glc- β-Glc (2→1)	β-Glc- β-Glc (2→1)

(Wallin 2004)

NUTRITIONAL QUALITIES OF REBAUDIOSIDE-A AND ERYTHRITOL

Stevia has been used for many years throughout the world as a non-nutritive sweetener in many different food systems (Goyal et al. 2010), however, up until 2008, it was not an approved food sweetener in the United States (FDA 2008). Obviously, a benefit of using stevia in food systems is to gain a sweet tasting characteristic; however, because this sweetener provides no nutrients, it is especially of interest to diabetic and weight-loss patients. Hutapea and others (1997) published a study where *in vitro* digestibility of steviosides by various digestive enzymes was examined. It was found that none of the enzymes digested the stevioside. However, it was found that intestinal microflora "hydrolyzed it to both steviol and steviol- 16, 17 alpha-epoxide. Steviol 16, 17 alpha-epoxide was then completely converted back into steviol" (Hutapea et al. 1997). Steviol is excreted from the body in urine as steviol glucuronide (Chatsudthipong 2009).

Diabetes is a leading cause of illness in many countries including the United States. The International Diabetes Federation (Anon. 2010) reported that 285 million of the world's population (6.4%) is currently affected by the disease. North America represents 37.4 million (10.2% of the North American Population) of the world's people suffering from diabetes (Anon. 2010). Interestingly cardiovascular disease is the cause of death in 50% of the diabetic population (Anon. 2010). For this group, controlling blood glucose levels is critical in maintaining their health. Replacing nutritive carbohydrate based sweeteners with non-nutritive sweeteners such as rebaudioside-A gives these individuals another tool to support these efforts (Anon. 2010).

Type 2 diabetes mellitus in most people is directly related to body weight and obesity. Effective weight loss requires individuals to consume fewer calories than they expend. Diabetes in clinically obese patients has been extensively studied in the bariatric medicine community. Shah and others (2010) studied fifteen patients over nine months who elected to undergo bariatric surgery. At the start of the study, eighty percent of them required insulin and twenty percent required an oral hypoglycemic medication. Three months post bariatric surgery, 100% of these patients no longer required diabetic medications and their HgA_{1C} decreased from 10.1% (+/- 2.0%) to 6.1% (+/- 0.6%) $p<0.001$ (Shah et al. 2010). Utilizing non-nutritive sweeteners allows for calorie reduction in a normally sweet food system, thus, assisting with weight loss and weight management.

Erythritol is a four-carbon polyol used as a sweetener in food and pharmaceuticals (Moon et al. 2010). It is also a functional sugar substitute in foods for people with diabetes and obesity. Polyols are carbohydrates in which the carbonyl group has been reduced to a primary or secondary hydroxyl group (Fennema 1996). Erythritol can be widely found in nature in such food as melons, grapes, pears, seaweeds, fungi as well as naturally occurring in fermented food products (Moon et al. 2010). Unlike other polyhydric alcohols, erythritol is unique in that due to its small molecular structure, it is readily absorbed into the bloodstream in the small intestine. Since this absorption occurs early in the digestion process, erythritol is not shown to cause gastric distress like other sugar alcohols (Moon et al. 2010). Systemically, erythritol is not metabolized and is excreted through the urine (Moon et al. 2010). Figure 2.2 illustrates the chemical structure of erythritol or (2*R*,3*S*)-butane-1,2,3,4-tetraol (Anon. 2011) Additional information on this topic is available in the safety section of chapter II.

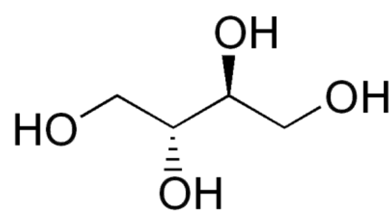

FIGURE 2.2: ERYTHRITOL STRUCTURE

(Anon. 2011)

Erythritol is about 60-80% as sweet as sucrose and can be effectively blended with high-intensity sweeteners as a bulking agent (Lin et al. 2003). Available under the brand name Truvia™ is a blend of rebaudioside-A and Erythritol for household consumers to use as a 1:1 replacement for sucrose. According to Cargill (Cargill 2010) "Erythritol has been used as an ingredient in foods and beverages since 1990 in Japan and has been used in the U.S. since 2000." Erythritol is less sweet than sucrose and combining the ingredient with more intense sweeteners such as rebaudioside-A increases the sweetness to be more like that of sucrose (Cargill 2010).

SWEETENING QUALITIES OF REBAUDIOSIDE-A AND ERYTHRITOL

The main steviol glycosides found in the *S. rebaudiana* Bertoni plant and their relative sweetness on a weight comparison to sucrose, with sucrose equal to 1.0 (Kim and Kinghorn 2002). Table 2.2 outlines the relative sweetness of steviol glycosides on a weight comparison to sucrose. It should be noted that of the eight glycosides, rebaudioside-C (dulcoside B) and dulcoside A are the least sweet, each with a rating of 30; rebaudioside-A is the most sweet with a rating of 242 compared to sucrose with a value of 100 (King and Kinghorn 2002).

TABLE 2.2: RELATIVE SWEETNESS ON A WEIGHT COMPARISON TO SUCROSE

Glycoside	**Relative Sweetness to Sucrose**
Stevioside	210
Rebaudioside-A	242
Rebaudioside B	150
Rebaudioside C (Dulcoside B)	30
Rebaudioside D	221
Rebaudioside E	174
Steviolbioside	90
Dulcoside A	30

(Kim and Kinghorn 2002).

When used in high concentrations, non-nutritive sweetening compounds may be perceived as bitter. Interestingly, the more potent the sweetness compared to that of sucrose, the higher perceived bitterness (Kier 1972). It has been suggested that there may be a relationship between the way in which the human senses perceived bitterness and the potency of sweetness. Kier (1972) stated that high intensity sweeteners often contain lypophilic sites and lypopilicity is known to play a role in perceived bitterness. There have, however, been a number of researchers who suggested that the receptor sites for bitterness and sweetness perception are independent of one another (Cardello 1981). Cardello (1981) reported that some papillae were stimulated by the bitter compound quinine and others were not; the same observation was found with the sucrose.

Steviosides are considered the most bitter of the most common non-nutritive high-intensity sweeteners, especially at higher concentrations (Schiffman et al. 1995). Rebaudioside-A, although perceived as less bitter is ranked second in bitterness to stevioside (Schiffman et al. 1995). Sugar alcohols, such as erythritol may provide a bitterness suppressing effect when combined with rebaudioside-A in food systems (Hashimoto et al. 2007). Hashimoto and others (2007) found that sugar alcohols seemed to be effective in suppressing the bitterness of quinine sulfate solution.

Polyhydric alcohols exhibit a positive enthalpy of solubilization, and can contribute a pleasant cooling sensation (Lin et al. 2010). When mixed within a food system such as a chiffon cake; this cooling effect is masked (Lin et al. 2003). According to Moon and others (2010): "Erythritol is preferred over other polyols because (1) it evokes a very less or no glycemic response. (2) It is produced by natural processes and can well be incorporated into foods that claim to be "natural." (3) It yields 0-0.2 calories per gram while the other polyols yield approximately 2 calories per gram. (4) From the digestive standpoint, it is well tolerated and results in minimum or no gastric discomfort or flatulence" (Moon et al. 2010).

STABILITY OF REBAUDIOSIDE-A

In solution, steviosides are stable between a pH of 2-10; however, under acidic conditions of pH 1, decomposition of steviosides can be observed (Kroyer 2010). Moderate baking temperatures (350°F (177°C) or below) are recommended for baking with Truvia™ (Cargill 2010) as well as Pure Via™ (Anon. 2010). Kroyer (2010) performed heat studies which showed degradation of steviosides in model systems at 392°F (200°C) (Kroyer 2010). Rebaudioside-A has been shown to be stable to sunlight exposure as well as in stability studies performed over a four-month period (Clos et al. 2008). Kroyer (2010) studied the interaction of rebaudioside-A in binary aqueous solutions with other non-nutritive sweeteners including saccharin, sodium cyclamate, aspartame, acesulfame potassium and neohesperidin dihydrochalone. He indicated there was no interaction between the individual sweeteners at 176°F (80°C) up to four hours or incubated for four months at room temperature.

HUMAN SAFETY OF REBAUDIOSIDE-A

On May 15, 2008, the United States Food and Drug Administration granted GRAS Status under GRAS Notice GRN 000253 to *Stevia rebaudiana* for use as a general purpose sweetener in foods excluding meat and poultry products (FDA 2008). Rebaudioside-A has been used for years throughout the world as a natural non-nutritive sweetening alternative to sucrose and other nutritive variants (Goyal et al. 2010). The lethal dose of this compound is interesting to explore. Tolstikova and others (2009) reported that the LD_{50} of rebaudioside-A is 8000 mg/kg. The researchers explored rebaudioside-A (and other stevioside) toxicity; therefore, carcinogenicity, digestion and metabolism have been reported. Literature reviewed during this study revealed rebaudioside-A as a compound that is non-toxic, non-carcinogenic and safe for human consumption. It appears that rebaudioside-A is not absorbed or digested in the small intestine, however, it is metabolized by human microflora in the large intestine and is found to be excreted in urine and fecal matter (Tolstikova et al. 2009).

There are several studies in the literature covering research related to animal and human safety of rebaudioside-A (Wingard et al. 1980; Nunes et al. 2007; Carakostas et al. 2008; Chatsudthipong 2009; Williams and Burdock 2009). Williams and Burdock (2009) used a number of methods to assess the toxicity of rebaudioside-A. When subjected to a bacterial reverse mutation test (Ames test) using standard *Salmonella typhimurium* as well as *Escherichia coli*, there was no statistically significant increase in the number of relevant colonies exposed to rebaudioside-A at concentrations up to 5000 µg/plate. In the Ames test, rebaudioside-A was found to be non-mutagenic in these two bacterial strains.

When subjected to *in vitro* mammalian chromosome aberration tests, rebaudioside-A was evaluated for mutagenic potential in cultured human lymphocytes. This test "did not induce a statistically significant increase in the incidence of chromosomal aberrations or polyploidy in cultured Chinese Hamster V79 cells after 4- and 20-h treatments at any of the doses tested with our without S9 Metabolic Activation" (Williams and Burdock 2009). Mutagenic potential of rebaudioside-A on mouse bone marrow cells tested using a mammalian erythrocyte micronucleus test revealed that the highest dose of 5000µg/ml did not produce any statistically significant increase in the incidence of polychromatic immature erythrocytes (Williams and Burdock 2009). In addition, it was found to be non-toxic to cells in the mice that were administered the rebaudioside-A at doses of 150, 275 or 750 mg/kg of body weight compared to the plain saline control (Williams and Burdock 2009). This indicates that rebaudioside-A was non-mutagenic. (Williams and Burdock 2009) Additionally, rebaudioside-A was shown not to cause any signs of toxicity in male Wistar rats after being administered a single dose of 2000 mg/kg of body weight and observed for sixteen hours post dosing (Williams and Burdock 2009).

Rebaudioside-A has a relatively high molecular weight (967.013 g/mol) and, like stevioside (804.9 g/mol), is unlikely to be absorbed during transit in the small intestine (Nunes et al. 2007). In digestive studies using human volunteers receiving dosages of 750 mg/day, stevioside was not detected in fecal matter of any subject, however, free steviol was present (Nunes et al. 2007). This helps confirm that bacteria in the large intestine have the ability to convert stevioside into steviol. This has also been confirmed in rat studies as well (Gardana et al. 2003). Tests with stevioside compounds and the effect of gastric juices and digestive enzymes on them show their failure to degrade or rearrange the compounds (Wingard et al. 1980).

It appears that steviol is the main metabolite stevioside compounds after oral ingestion. Researchers have shown that there are two main routes of excretion, in urine as well as bile (Chatsudthipong 2009). In radiology studies performed in rats, it appears that significant accumulation occurs in the liver, intestine and kidneys (Chatsudthipong 2009). Analysis of bile shows a substantial amount of steviol; whereas urine contained a high amount of steviosides (Chatsudthipong 2009). In human studies urine seems to play the predominate role in excretion (Chatsudthipong 2009). In a study performed in 2008, after 72 hours of ingestion, “steviol glucuronide excreted in urine and free steviol in feces account for 62% and 5.2% of the total dose of stevioside administered respectively" (Chatsudthipong 2009). Research conducted by Carakostas and others (2008) contains a comprehensive illustration of the absorption, metabolism and excretion of rebaudioside-A. This illustration can be found in Appendix II.

Researchers have analyzed the effect of consuming stevia extracts on both male and female laboratory animals. Research conducted by Carakostas and others (2008) dosed hamsters at 2500 mg/kg body weight per day and showed no effect on reproduction. Additionally, there was no reported effect on fertility, number of offspring or on reproductive tissue of either male or female hamsters.

There are some reported health benefits to supplementing a diet with rebaudioside-A. In a previous section, the role of using the compound to replace sucrose in a diet for subjects with type 2 diabetes mellitus was discussed. Although it is a suitable compound for removing sugar from food systems and suitable for use by individuals suffering from diabetes, Maki and others (2008) researched the effects of rebaudioside-A on blood glucose levels in a single blind placebo controlled study. Test subjects were given a diet supplemented with 1000 mg/day of rebaudioside-A for sixteen consecutive weeks. The researchers found no statistical effect on HbA_{1C}, blood pressure or blood lipids in the patients (Maki et al. 2008). This showed that simply supplementing a diet with rebaudioside-A would have no negative or positive effects on glucose homeostasis (Carakostas et al. 2008; Maki et al. 2008).

Decrease in postprandial glucose and glucagon levels as well as insulinotropic effects in several cellular and animal studies have been conducted (Chatsudthipong 2009). Some of these researchers have concluded that there may be a relationship between rebaudioside-A consumption in controlling glucose absorption, glucose synthesis and insulin secretion and sensitivity (Chatsudthipong 2009). However, others found the compound does not have a direct effect on glucose absorption or insulin (Chatsudthipong 2009). Due to the contradiction in scientific findings, more research has to be conducted in these areas of interest to determine the effect, if any, of the compound. Additionally, some evidence suggests that steviosides may have an anti-inflammatory effect (Chatsudthipong 2009). Yasukawa and others (2002) researched the effect of steviol glycosides on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice. They found that an inhibitory dose of the steviol glycosides was 54.1-291.6 mcg/ear. Additionally, they reported at 1.0 and 0.1 mg/mouse of the mixture inhibited the promoting effect of TPA (1 mcg/mouse). At the time of study, however, they reported "the mechanism by which these compounds exert these effects remains to be elucidated" (Yasukawa et al. 2002).

SUCROSE FUNCTIONS IN BAKING

In baked systems, sucrose contributes to sweetness, color, flavor, tenderness and structure. Primarily, the substance provides sweetness and bulk to baked goods and is one of the most common sweetening compounds used in cake products (Figoni 2008). Under baking temperatures, sucrose undergoes inversion to glucose and fructose. Once this process occurs, the two monosaccharaides can participate in the Maillard reaction along with amines found in milk and other protein-containing foods (McWilliams 2008). This process "is a series of reactions involving the condensation of a reducing sugar and an amine" (McWilliams 2008). It results in developing process and reaction flavors such as pyrazine compounds, which have pleasant aromas generally associated with baked goods (Murano 2003). The last step in the Maillard reaction produces brown melanoidins which results in significant darkening of food products (Murano 2003). Gallagher and others (2001) found that browning significantly decreased when sugar was removed from baked biscuits. This is further supported by findings reported by Lin and others (2008). The researchers found that browning of Danish cookies significantly decreased when sugar was removed from formulation.

Sugars are used as humectants in the food industry and provides for moisture retention in baked goods such as cakes. Moisture retention contributes to the crumbs' overall tenderness. Competition between sugars and other substances for water during baking also affects texture and tenderness. Due to this, starch gelatinization is delayed and allows for a cake to set when baked without fully hydrating gluten strands. This delay due to the presence of sugar contributes to a tender texture in the final product (Hoseney 1998; Murano 2003).

In foam cakes such as chiffon, angel food and sponge, sugar acts as a whipping aid for egg whites. The foam serves as a basic structure for these types of cakes by retaining air pockets within the batter during baking. Creaming of sugar with a fat such as shortening also contributes to providing air pockets that undergo expansion during baking (Figoni 2008). During baking and as the fat melts, air cells are released into continuous phase and assist with leavening (Hoseney 1998).

SYNTHETIC AND NON-NUTRITIVE SWEETENERS IN BAKING

Synthetic and non-nutritive sweeteners can be used as sugar replacers in baked goods. Their use may have an effect on color, flavor and tenderness of the final product. According to Mariotti and Alamprese (2012) "the reduction of sucrose can cause detectable losses in appearance, texture, flavor and mouthfeel." Sweeteners such as rebaudioside-A can be used in baked systems to replace the sweetness of sucrose. Since this compound is considerably more intense than sucrose, it is used in conjunction with erythritol, a natural polyol, as a bulking agent (Cargill 2011). Compared to sucrose, polyols have a reduced sweetness, lower solubility and higher crystallization effect (Gohosh and Sudha 2012). According to Lin and others (2010) erythritol does not attract water and tends to crystallize during baking. Their research using erythritol in Danish cookies found erythritol crystallization in their cookie using 100% erythritol as a replacement for sucrose.

Polyols such as erythritol do not participate in Maillard reaction resulting in food products being considerably lighter in color after baking. Gallagher and others (2001) reported a significant lighter surface color of biscuits with the highest level of sugar replacement. In addition, Lin and others (2010) reported a significant increase in lightness of Danish cookies prepared with erythritol as replacement for sucrose.

Sugar alcohols have low humectancy when compared to sugar. This may have an effect on decreased tenderness of baked cakes since moisture retention has a significant effect on this sensory attribute (Murano 2003). Since polyols have a high heat of solution, they produce a cooling effect in the mouth when consumed (Ghosh and Sudah 2012). Used in high percentage in formulation, they have the potential to yield a cooling effect to the sensory experience of a baked product (Ghosh and Sudah 2012).

Sweeteners such as sucralose, acesulfame-K and aspartame have been successfully used in baked systems to partially or fully replace sucrose. Since these compounds alone are substantially sweeter than sucrose, a bulking agent is typically used in conjunction when replacing the weight of sucrose in formulation (Baeva et al. 2000). Depending on the application and sweetener, bulking agents can include dextrins, non-digestible carbohydrates and sugar alcohols (Figoni 2008).

Sucralose is considered 600 times sweeter than sucrose (Fennema 1996). According to Fennema (1996) “sucralose exhibits a high degree of crystallinity, high water solubility and very good stability at high temperatures, thus making it an excellent ingredient for baking applications.” Color is an important quality attribute which sucrose contributes to. Esteller and others (2006) reported replacement of sucrose with sucralose in hamburger buns had an effect on L, a and b values. In hamburger buns, those prepared with sucralose were lighter in color with more red and yellow tones (Esteller et al. 2006). Sensory testing on muffins formulated with varying levels of sucrose replaced with sucralose found that “sucrose can be replaced up to a level of 50% with good sensory acceptability” (Martinez-Cervera et al. 2012). The researchers also found muffin samples prepared with 100% sucralose as replacement for sucrose had a significant decrease in panelist acceptability (Martinez-Cervera et al. 2012).

Acesulfame-K is approximately 200 times sweeter than sucrose (Zoulias et al. 2000). The compound, potassium salt of 6-methyl-1,2,3-oxythiazine-4(3H)-one-2,2-dioxide is heat stable and suitable for baking (Bullock et al. 1992). Zoulias and others (2000) reported cookies prepared with various polyols supplemented with acesulfame-K in substitution for sucrose improved flavor and general acceptance when compared to cookies prepared without acesulfame-K. Researchers also reported changes in cookie texture prepared with various polyols (Zoulias et al. 2000). Cookies formulated with sucrose, fructose and maltitol were significantly harder than those prepared with lactitol, sorbitol, xylitol and mannitol (Zoulias et al. 2000). The addition of acesulfame-K did not have any effect on texture (Zoulias et al. 2000). Though differences were not significant; "the addition of acesulfame-K decreased water activity of the cookies in all cases" (Zoulias et al. 2000).

Aspartame (L-aspartyl-L-phenylalanine methyl ester) is about 200 times sweeter than sucrose and is fully digested, therefore, considered a caloric sweetener (Fennema 1996). Unlike sucralose and acesulfame-K, aspartame is unstable under acidic conditions and degrades when exposed to prolonged high baking temperatures (Fennema 1996). Encapsulated aspartame was developed to maintain the compound's sweetness when exposed to baking conditions (Wetzel et al. 1997). Researchers reported the sweetness of a no-sugar-added cupcake formulated with encapsulated aspartame was not significantly different from a cupcake prepared with 100% sucrose (Wetzel et al. 1997).

CHAPTER III

MATERIALS AND METHODS

Five cake formulations were prepared containing varying amounts of rebaudioside-A and erythritol as replacement for sucrose and used for testing. Prior to baking, specific gravity was measured on the batter. After baking, the cakes were allowed to cool in the pan for two hours before measuring rapeseed displacement for volume. Cakes were wrapped with polyethylene film (Fisherbrand™ clear plastic lab wrap) and kept at room temperature overnight (68°F (20°C) to 71.6°F (22°C)). Further tests for color, water activity, moisture content and texture were completed on the day following baking. Four major replications of the baking and testing days were completed. Differential scanning calorimetry, nutritional analysis and sensory analysis were conducted as separate experiments. Table 3.1 illustrates the sample baking and testing schedule as well as the number of major replications completed for each.

TABLE 3.1: BAKING AND TESTING SCHEDULE FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Testing Schedule	**Test/Analysis**	**Number of Major Replications**
MEASUREMENT DAY 1	Baking Specific gravity Rapeseed displacement (volume)	Four major replications done in triplicate
MEASUREMENT DAY 2	Color Analysis Water activity Moisture analysis Texture analysis	Four major replications done in triplicate
DIFFERENTIAL SCANNING CALORIMETRY DAY 1	Baking Freeze drying	One major replication
DIFFERENTIAL SCANNING CALORIMETRY DAY 2	Differential scanning calorimetry	One major replication
SENSORY DAY 1	Baking	One major replication
SENSORY DAY 2	Sensory testing	Forty replications (panelists)

The chiffon cake formulation in this study was adapted from the work of Lin and Lee (2005). Table 3.2 contains the formulations for cakes at 100% sucrose (control; 0% reduced) and then sucrose reduced by 25%, 50%, 75% and 100%. The weight of the reduced amount of sucrose was replaced by a mixture of erythritol (Zerose™ erythritol; Cargill, Minneapolis, MN) and rebaudioside-A (Truvia™ rebaudioside-A; Cargill, Minneapolis, MN). The relative sweetness of the rebaudioside-A and erythritol mixture is calculated at 300x sweeter than sucrose. Relative percentage of rebaudioside-A to erythritol was 0.33% to 99.67%.

TABLE 3.2: CHIFFON CAKE FORMULATIONS CONTAINING VARYING AMOUNTS OF REBAUDIOSIDE-A AND ERYTHRITOL

INGREDIENTS	**Control** 0% Reduced Sucrose	**RS-025** 25% Reduced Sucrose	**RS-050** 50% Reduced Sucrose	**RS-075** 75% Reduced Sucrose	**RS-100** 100% Reduced Sucrose
BATTER:	(g)	(g)	(g)	(g)	(g)
Sucrose	75.00	41.25	7.50	0.00	0.00
Erythritol (Zerose)	0.00	33.64	67.28	74.75	74.75
Rebaudioside-A	0.00	0.11	0.23	0.25	0.25
Egg yolk	50.00	50.00	50.00	50.00	50.00
Distilled water	65.00	65.00	65.00	65.00	65.00
Sodium chloride	2.00	2.00	2.00	2.00	2.00
Cake flour	100.00	100.00	100.00	100.00	100.00
Baking powder	4.00	4.00	4.00	4.00	4.00
NF dry milk solids	8.00	8.00	8.00	8.00	8.00
Soybean oil	50.00	50.00	50.00	50.00	50.00
FOAM:					
Egg white	100.00	100.00	100.00	100.00	100.00
Sucrose	60.00	60.00	60.00	33.75	0.00
Erythritol (Zerose)	0.00	0.00	0.00	26.16	59.80
Rebaudioside-A	0.00	0.00	0.00	0.09	0.20
Cream of tartar	0.60	0.60	0.60	0.60	0.60

METHOD OF PREPARATION

The batter and foam portions of the cake were prepared separately and then combined by folding.

BATTER:

- Step One: Combine egg yolk, sucrose, erythritol, rebaudioside-A, sodium chloride and water. With wire whip attachment in place, set mixer (Kitchen Aid Model KSM90, St. Joseph, Michigan) on setting 4 to whip ingredients for 2 minutes.
- Step Two: Add the flour, baking soda and non-fat dried milk powder to the ingredients mixed in step one. With the wire whip attachment in place, continue to whip ingredients on setting 4 for one minute.
- Step Three: Add the soybean oil to the ingredients mixed in step two and continue to mix, with the wire whip attachment, on setting 4 for 1 minute.

FOAM:

- Step One: Combine egg whites, sucrose, erythritol, rebaudioside-A and cream of tartar in electric mixer bowl, mix gently to incorporate all ingredients for approximately 15 seconds (20 strokes).
- Step Two: With wire whip attachment in place, set mixer (Kitchen Aid Model KSM90WH, St. Joseph, Michigan) on setting 8 to beat step one for 3.5 minutes.

BATTER + FOAM:

- Step One: Gently fold foam into batter using a rubber spatula. Ensure all foam is thoroughly incorporated into batter by folding for 1 minute (90 strokes).
- Step Two: Pour 450 grams of batter into each square 20.32 cm (8") wide by 20.32 cm (8") length by 5.08 cm (2") depth non-stick cake pan. Reserve remaining batter for analytical testing.

BAKING AND COOLING:

Calibrated thermometers placed in each oven and were used to ensure temperature was maintained at 350°F (177°C). Treatment replications were baked in different ovens and in random positions.

- Step One: Bake each cake at 350°F (177°C) in a conventional oven for 30 minutes.
- Step Two: After cake is baked, remove from oven and allow cooling in pan on rack for 2 hours prior to testing.

TESTING:

- Testing was conducted according to schedule found in Table 3.1. Differential scanning calorimetry and sensory testing were conducted independently from specific gravity, volume, texture, color, moisture and water activity analysis. For these, cakes were made on one day with testing for specific gravity of batter and baked cake volume conducted on this same day. Moisture, water activity, color and texture analysis was done on the day immediately following baking.

MEASUREMENTS

SPECIFIC GRAVITY

Specific gravity is defined as the ratio of the density of a substance to the density of a reference substance at the same temperature (Nielsen 2003). Standardized 100 ml capacity specific gravity cups were used for this testing. The specific gravity of the prepared chiffon cake batter was calculated by dividing the weight of the filled 100 ml specific gravity cup of cake batter at 68°F (20°C) by the weight of the same standardized cup filled with 100 ml of water at 68°F (20°C). This test was performed in triplicate.

$$\text{Specific Gravity} = \frac{\text{wt. 100 ml cake batter}}{\text{wt. 100 ml water}}$$

CAKE VOLUME

Rapeseed displacement was used to determine volume. The empty cake pan was filled with rapeseed and that amount of rapeseed was measured in a graduated cylinder and recorded (V1). After baking, the empty volume above the cake in the pan was filled with rapeseed and that volume was also recorded (V2). Cake volume was calculated by taking the V1 and subtracting V2. Test was performed in triplicate.

$$\text{Cake Volume} = V1 - V2$$

COLOR

The Hunter L *a b* method was used to determine both crumb and top crust color. In the Hunter method, L=lightness or darkness, +a=redness, -a=greenness, +b=yellowness and –b=blueness (Nielsen 2003). HunterLab ColorFlex Colorimeter (Model 45/0, Reston, VA) was used to conduct determine L, *a*, *b*, values. Calibration was done according to manufacturer's instructions by using standard black and white color tiles (white standard tile L 93.13, *a* -0.87, *b* 0.33). This test was performed in triplicate.

WATER ACTIVITY

Water activity is a measurement of how efficiently the water contained in a food system can take part in a physical or chemical reaction. It is calculated by dividing the partial pressure of water above the food (p) by a pure solution under identical conditions (p_o). Water activity (Aw) was tested using the AquaLab Model Series 3TE (Decagon Devices, Inc, Pullman, WA). Disposable sample cups were filled half way with finished cake crumb sample and testing was conducted according to the manufacturer's instructions in triplicate.

$$Aw = \frac{p}{p_o}$$

MOISTURE CONTENT

Percentage moisture is calculated by subtracting the weight of the dry sample from the weight of the wet sample and then dividing this number by the weight of the wet sample (Nielson 2003). Finished cake crumb samples of 10 g each were tested in a vacuum oven (Model 1410, VWR scientific Products, West Chester, PA). This was done pursuant to AACC approved methods 44-40.01 (modified vacuum oven method) at 100°C under vacuum at 20 mm of Hg. This test was performed in triplicate.

$$\% \text{ Moisture} = \frac{\text{wt. wet sample - wt dry sample}}{\text{wt. wet sample}}$$

TEXTURE ANALYSIS

Texture of each cake sample was determined using a texture analyzer (TA.XT2, Texture Technologies, Scarsdale, NY). A 5cm x 5cm x 5cm sample was cut from each cake's center and a triple cycle test was conducted. A multi-needle puncture probe was used for testing with a test speed of 0.3 mm/s and puncture distance of 20 mm under a 5 kilogram load of force. Calibration was done with a 5 kilogram load cell according to the manufacturer's instructions.

DIFFERENTIAL SCANNING CALORIMETRY

Calorimetry involves the determination of the temperature and or the quality of heat absorbed or emitted when a material undergoes a specific chemical or physicochemical change such as baking. The thermal properties of the cake batters were analyzed using differential scanning calorimetry (model Q2000 differential scanning calorimeter, TA Instruments, New Castle, Delaware) to determine the effects of replacing sucrose with a mixture of rebaudioside-A and erythritol on starch gelatinization. Freeze-dried powders of the batter (4 mg) were placed in aluminum pans (T-Zero™ hermetic pans, TA Instruments, New Castle, Delaware) and distilled water (6 mg) was added. Samples were hermetically sealed and allowed to stand for one hour at room temperature before heating. The instrument was operated at a heating rate of 10°C per minute from 20 to 120°C. An empty sample container was used as the reference pan. Onset temperature (T_o), peak temperature (T_p) and enthalpy (ΔH) were automatically calculated.

CALORIE AND NUTRITIONAL ANALYSIS

Nutritional analysis of cake formulations was conducted using Genesis® R&D SQL software, version 8.0 (ESHA Research, Salem, OR). Calories, fat, cholesterol, sodium, carbohydrates and protein were automatically calculated for 100g samples. Nutrition facts panels and ingredient declarations listed in order of weight were generated using the software program.

SENSORY ANALYSIS

Sensory analysis of five chiffon cake formulations was performed in the Gifford Building food laboratories at Colorado State University in Fort Collins, CO. The Institutional Review Board administrator reviewed this study and declared it exempt from the requirements of FDA human subject protections regulations as described in CFR 46.101(b)(6). A copy of the exempt status letter can be found in Appendix III. Each participant received a letter explaining the research; its purpose, who was responsible for conducting and a list of allergens contained in the test products (see Appendix III). Their participation in the sensory study after receiving this constituted their implied consent.

Sensory testing was conducted at the Gifford Building at Colorado State University with a total of forty panelists comprised of students, faculty and staff. The purpose of this sensory test was to determine which level of sucrose replacement was preferred. Rebaudioside-A (Cargill Truvia) and erythritol (Cargill Zerose) were blended to substitute for sucrose at varying percentages (0, 25, 50, 75 and 100%). Each participant was given a tray which contained the five randomized samples of the chiffon cake, two unsalted crackers, a six ounce cup of water, pencil, napkin and the sensory score card (See Appendix). The chiffon cake samples were in cups with random three-digit codes and placed on the tray in randomized order.

The sensory score card in Appendix IV was used to collect information from the participant's evaluation. A nine-point hedonic scale was used to measure overall liking/acceptance of color of crumb, tenderness, sweetness, aftertaste and overall acceptability. Panelists were asked to rank preference of the five samples from one to five with one being the most preferred. Probing questions were asked for comments on both flavor and bitterness.

STATISTICAL ANALYSIS

Statistical analysis was done using SAS/STAT software, version 9.1.3 of the SAS System for Windows. The null hypothesis was that there were no differences among cakes prepared with or without rebaudioside-A and erythritol. A randomized complete block one factor design was used for analysis of specific gravity, cake volume, color, water activity, moisture and texture measurements. A percentage of sucrose was reduced in chiffon cakes by 0, 25, 50, 75 or 100%. The weight of the reduced amount of sucrose was replaced by a mixture of rebaudioside-A (Truvia™ rebaudioside-A; Cargill, Minneapolis, MN) and erythritol (Zerose™ erythritol; Cargill, Minneapolis, MN). Specific gravity, cake volume, crumb color, crust color, water activity, moisture and texture were measured with four major replications of each treatment (0, 25, 50, 75 and 100%). Results were examined to determine if significant differences existed. If so, differences among means were then examined using Tukey's honestly significant difference. The ANOVA table for specific gravity, cake volume, color, water activity, moisture analysis and texture measurements can be found in Table 3.3.

TABLE 3.3: ANOVA TABLE FOR SPECIFIC GRAVITY, CAKE VOLUME, COLOR, WATER ACTIVITY, MOISTURE ANALYSIS AND TEXTURE MEASUREMENTS OF CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

	df	*p*
5 Treatments (Control, 25, 50, 75, 100% reduced sucrose)	4	0.05
Replications (n = 4)	3	
Treatments x Replications	12	
Total	15	

Sensory data were collected using five chiffon cake formulations, each with a percentage of sucrose (0, 25, 50, 75 and 100%) replaced with a mixture of rebaudioside-A and erythritol. The experimental design was a randomized complete block one factor design with data blocked by panelist. Forty panelists were used in the sensory study to evaluate color of crumb, tenderness, sweetness, aftertaste and overall acceptability. Panelists also ranked cake samples for preference. Data were examined to determine if significant differences existed by ANOVA. If so, differences between means were then examined using Tukey's honestly significant difference. The ANOVA table for sensory analysis of baked chiffon cakes with varying amounts of sucrose replaced with rebaudioside-A and erythritol can be found in Table 3.4.

TABLE 3.4: ANOVA TABLE FOR SENSORY ANALYSIS OF CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

	df	*p*
5 Treatments (Control, 25, 50, 75, 100% reduced sucrose)	4	0.05
Replications (n = 40)	39	
Treatments x Replications	195	
Total	394	

CHAPTER IV

RESULTS AND DISCUSSION

Five chiffon cake formulations were prepared containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%). Analytical testing on specific gravity, volume, color, water activity, moisture, texture, differential scanning calorimetry and nutritional analysis was conducted on the five cake formulations as well as a consumer sensory test with forty panelists. A nine-point hedonic scale ranging from "like extremely" to "dislike extremely" was used to measure overall liking/acceptance of color of crumb, tenderness, sweetness, aftertaste and overall acceptability. Panelists were asked to rank preference of the five samples from one to five with one being the most preferred. Probing questions were asked for comments on both flavor and bitterness. Panelists were asked "what did you like or dislike about the flavor of the samples; please refer to the sample number." They also were asked if they found any of the samples to be bitter and if so to rank the bitter perception using a scale from one to five where one was "slightly bitter" and five was "very bitter."

SPECIFIC GRAVITY

Mean specific gravity results for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) are reported in Table 4.1. No differences were found among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). Specific gravity of cake batter means ranged from 0.42 to 0.44 g/ml. The cake batters containing 0, 25, 50, 75 or 100% reduction of sucrose with rebaudioside-A and erythritol had similar abilities to retain air.

TABLE 4.1: MEANS* BY TREATMENT OF SPECIFIC GRAVITY FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	Mean (g/ml)
Control (0% Reduced Sucrose)	0.44 ± 0.01
RS-025 (25% Reduced Sucrose)	0.43 ± 0.01
RS-050 (50% Reduced Sucrose)	0.45 ± 0.01
RS-075 (75% Reduced Sucrose)	0.42 ± 0.01
RS-100 (100% Reduced Sucrose)	0.42 ± 0.01

*Results are presented as mean of 12 values ± standard deviation. Means are NOT significantly different ($p > 0.05$).

CAKE VOLUME

Mean cake volume results for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) are reported in Table 4.2. No differences were found among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). Cake volume means ranged from 1668.75 to 1745.00 ml. Pictures of whole cakes, cross sections and crumb can be found in Appendix V. The chiffon cake formulas were not adjusted for altitude. After baking, the researchers observed the center of each control cake had less springiness and a shorter height than the other samples. Baeva and others (2000) reported similar results with their sponge cakes prepared with 100% sucrose compared to ones with reduced levels. Starch gelatinization plays an important role in giving springiness to sponge-type cakes and sucrose has a retarding effect on this process (Baeva et al. 2000). In our study, as the sucrose replacement level increased, the more uniform height was observed. During baking, gas expansion is restricted by starch gelatinization (Baeva et al. 2000). It is possible that the amount of sucrose in the control formulation was at a level that retarded gelatinization to a point of allowing for some gas to release from the food system prior to firming of the internal structure.

TABLE 4.2: MEANS* BY TREATMENT OF CAKE VOLUME FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	Mean (ml)
Control (0% Reduced Sucrose)	1670.83 ± 20.80
RS-025 (25% Reduced Sucrose)	1745.00 ± 20.80
RS-050 (50% Reduced Sucrose)	1690.00 ± 20.80
RS-075 (75% Reduced Sucrose)	1688.75 ± 20.80
RS-100 (100% Reduced Sucrose)	1668.75 ± 20.80

*Results are presented as mean of 12 values ± standard deviation. Means are NOT significantly different ($p > 0.05$).

COLOR

Color was determined for both the crust and crumb of each treatment and reported using the L-a-b value system. The L-value is a measurement of lightness/darkness of a given sample where 100 corresponds to white and 0 to black. The a-value represents redness (positive) and greenness (negative). The b-value assesses yellowness (positive) and blueness (negative) in the sample.

Mean crumb color results for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) are reported in Table 4.3. No differences in crumb L-values were found among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). L-values ranged from 69.4 to 78.49. There was a trend from cakes without sucrose to be slightly lighter in color (100% reduced = 78.49). This corresponds with a visually detectible variation in lightness of crumb as the amount of sucrose in formulation was reduced. The mixture of rebaudioside-A and erythritol is white in color and non-reducing. This may explain the lighter colored crumb as concentration in formulation was increased. No differences in crumb a-values were found among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). Crumb a-values ranged from 1.18 to 1.42. The control cake and 25% reduced sucrose crumb b-values were significantly more yellow in color than the 100% reduced sucrose sample ($p < 0.05$). The results ranged from 27.42 to 32.64 with the control value at 32.64. This visually corresponds with an observed more pronounced yellow crumb of the control cake.

TABLE 4.3: MEANS* BY TREATMENT OF CRUMB COLOR L-, a- AND b-VALUES FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	L-value Mean	a-value Mean	b-value Mean
Control (0% Reduced Sucrose)	69.40 ± 3.56 *a*	1.59 ± 0.35 *a*	32.64 ± 1.69 *a*
RS-025 (25% Reduced Sucrose)	73.56 ± 3.56 *a*	1.18 ± 0.35 *a*	31.71 ± 1.69 *a*
RS-050 (50% Reduced Sucrose)	70.08 ± 3.56 *a*	1.33 ± 0.35 *a*	29.35 ± 1.69 *ab*
RS-075 (75% Reduced Sucrose)	73.99 ± 3.56 *a*	1.42 ± 0.35 *a*	29.53 ± 1.69 *ab*
RS-100 (100% Reduced Sucrose)	78.49 ± 3.56 *a*	1.18 ± 0.35 *a*	27.42 ± 1.69 *b*

*Results are presented as mean of 12 values ± standard deviation. Means with the same letter are NOT significantly different ($p > 0.05$).

Mean crust color results for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) are reported in Table 4.4. No differences in crust L-values were found among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). Crust L-values ranged from 39.16 to 56.98. There was a trend for cakes without sucrose to have a lighter color crust. This corresponds with a visually detectible variation in lightness of crust as the amount of sucrose in formulation was reduced. The visual lightening of samples as more sucrose was replaced with rebaudioside-A and erythritol may be attributed to the higher erythritol content. Erythritol contains polyhydroxyl compounds with no reducing group. Thus, the substance does not caramelize or participate in carbonyl-amine browning reactions (Lin et al. 2010).

No differences in crust a-values were found among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). Crust a-values ranged from 11.15 to 12.63 and no differences were visually observed. No differences in crust b-values were found among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). Crust b-values ranged from 29.57 to 32.16 and no differences were visually observed.

TABLE 4.4: MEANS* BY TREATMENT OF CRUST COLOR L-, a- AND b-VALUES FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	L-value Mean	a-value Mean	b-value Mean
Control (0% Reduced Sucrose)	40.69 ± 4.28	12.63 ± 0.53	29.57 ± 1.60
RS-025 (25% Reduced Sucrose)	39.16 ± 4.28	12.49 ± 0.53	28.34 ± 1.60
RS-050 (50% Reduced Sucrose)	41.74 ± 4.28	11.15 ± 0.53	27.46 ± 1.60
RS-075 (75% Reduced Sucrose)	56.98 ± 4.28	11.41 ± 0.53	32.34 ± 1.60
RS-100 (100% Reduced Sucrose)	56.15 ± 4.28	11.48 ± 0.53	32.16 ± 1.60

*Results are presented as mean of 12 values ± standard deviation. Means are NOT significantly different ($p > 0.05$).

WATER ACTIVITY

Mean water activity for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) are reported in Table 4.5. Water activity of the control cake prepared with 100% sucrose with a value of 0.91 was higher than the 50 or 75% reduced sucrose chiffon cakes ($p < 0.05$). However, the 25 and 100% reduced sucrose cakes were statistically the same as the control ($p > 0.05$). The mean water activity of cakes prepared with 25% reduced sucrose was significantly higher than the 75% reduced sucrose cakes ($p < 0.05$). The use of alternative sweeteners in baked products may impact water activity of final product. Zoulias and others (2000) reported a decrease in water activity of cookies prepared with acesulfame-K as replacement for sucrose. In doughnuts prepared with Truvia™ as replacement for sucrose researchers reported reduced water activity when compared to those prepared with sucrose (Wetzel 2010). Kim and others (2003) reported water activity decreasing as concentration of polyols increased when replacing sucrose in formulation. If shelf stability is desired, the formulas would need to be adjusted to reduce water activity. However, the values are acceptable for cakes prepared for same or next-day consumption in a commercial foodservice facility.

TABLE 4.5: MEANS* BY TREATMENT OF WATER ACTIVITY FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	Mean (Aw)
Control (0% Reduced Sucrose)	0.91 ± 0.01 *a*
RS-025 (25% Reduced Sucrose)	0.90 ± 0.01 *ab*
RS-050 (50% Reduced Sucrose)	0.88 ± 0.01 *bc*
RS-075 (75% Reduced Sucrose)	0.86 ± 0.01 *c*
RS-100 (100% Reduced Sucrose)	0.89 ± 0.01 *ac*

*Results are presented as mean of 12 values ± standard deviation. Means with the same letter are NOT significantly different ($p > 0.05$).

MOISTURE

Mean moisture results for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) are reported in Table 4.6. No differences were found among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). Percent moisture of cake batter means ranged from 32.08 to 33.33% and there were no visual differences among cake samples.

TABLE 4.6: MEANS* BY TREATMENT OF PERCENT MOISTURE FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	Mean (% Moisture)
Control (0% Reduced Sucrose)	32.33 ± 0.39
RS-025 (25% Reduced Sucrose)	32.08 ± 0.39
RS-050 (50% Reduced Sucrose)	33.25 ± 0.39
RS-075 (75% Reduced Sucrose)	33.33 ± 0.39
RS-100 (100% Reduced Sucrose)	33.25 ± 0.39

*Results are presented as mean of 12 values ± standard deviation. Means are NOT significantly different ($p > 0.05$).

TEXTURE

Mean texture analysis results for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) are reported in Table 4.7. Both the 25 and 50% reduced sucrose chiffon cakes were more tender than the control prepared with 100% reduced sucrose ($p < 0.05$). Texture analysis means ranged from 178.01 to 389.25 grams of force. The cake prepared with 25% reduced sucrose had the softest texture with an average value of 178.01 grams of force; the hardest texture was that of the 100% reduced sucrose cake with a value of 389.25 grams of force. The control samples required an mean of 263.15 grams of force to penetrate. There were no differences between control value and other treatments ($p > 0.05$).

Visually, there was a difference between the integrity of air cells in the baked control samples compared to other treatments. Pictures of the whole cakes as well as cross-sections can be found in Appendix V. There was a slight "white" and slightly grainy-textured layer close to the crust of the 75 and 100% reduced sucrose samples after baking, possibly due to the higher erythritol content. According to Lin and others (2010) erythritol does not attract water and tends to crystallize during baking. Their research using erythritol in Danish cookies found erythritol crystallization in their cookie using 100% erythritol as a replacement for sucrose.

TABLE 4.7: MEANS* BY TREATMENT OF TEXTURE ANALYSIS FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	**Mean (g - force)**
Control (0% Reduced Sucrose)	263.15 ± *32.17 ab*
RS-025 (25% Reduced Sucrose)	178.01 ± *32.17 b*
RS-050 (50% Reduced Sucrose)	210.51 ± *32.17 b*
RS-075 (75% Reduced Sucrose)	308.92 ± *32.17 ab*
RS-100 (100% Reduced Sucrose)	389.25 ± *32.17 a*

*Results are presented as mean of 12 values ± standard deviation. Means with the same letter are NOT significantly different ($p > 0.05$).

DIFFERENTIAL SCANNING CALORIMETRY

Differential scanning calorimetry results by treatment for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) can be found in Table 4.8. Onset temperature (To), peak temperature (Tp) and enthalpy change were automatically calculated using Universal Analysis software published by TA Instruments, New Castle, Delaware. As the amount of sucrose in formulation was decreased, both the onset and peak temperatures decreased. Control cakes had the highest onset and peak temperature; 72.64 °C and 74.44 °C, respectively. The treatment with 100% sucrose replacement showed the lowest onset (68.01 °C) and peak (69.81 °C) temperatures. Figure 4.1 illustrates the linear onset and peak temperature change among treatments. These results correspond with differential scanning calorimetry testing conducted by Lim and others (1992) on wheat starch, sucrose and water interactions. The researchers reported the increase of onset temperature as sucrose concentrations were increased (Lim et al. 1992).

TABLE 4.8: THERMAL PROPERTIES OF CAKE BATTER BY TREATMENT FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	**T_O (°C)**	**T_P (°C)**	**ΔH (J/g)**
Control (0% Reduced Sucrose)	72.64	74.44	*0.887*
RS-025 (25% Reduced Sucrose)	71.88	74.34	*0.880*
RS-050 (50% Reduced Sucrose)	70.88	72.66	*0.877*
RS-075 (75% Reduced Sucrose)	68.87	70.97	*0.907*
RS-100 (100% Reduced Sucrose)	68.01	69.81	*1.101*

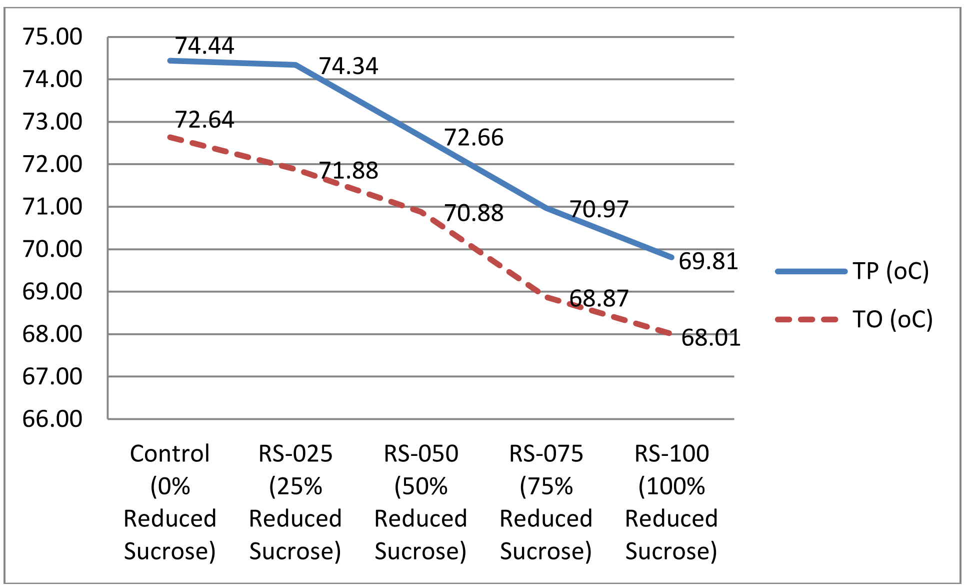

FIGURE 4.1: DIFFERENTIAL SCANNING CALORIMETRY ONSET AND PEAK TEMPERATURE CHANGE BY TREATMENT FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Differences in enthalpy change among treatments were observed; however, they were small and would likely not be detectable by consumers. These results correspond with differential scanning calorimetry testing for change in enthalpy reported by Lin and Lee (2005) on chiffon cakes prepared with indigestible dextrin and sucralose as replacement for sucrose. Figure 4.2 illustrates enthalpy change in joules per gram. The lowest reported value was for the control cake, 0.887 J/g. The highest enthalpy change was observed in the 100% reduced sucrose treatment with a value of 1.101 J/g. Minimal change in enthalpy between the control, 25 and 50% reduced sucrose treatments with reported values of 0.887, 0.880, 0.877 J/g, respectively. Change in enthalpy from the control treatment to the 75% reduced sucrose cake was 0.02 J/g. The greatest change was observed from the control to 100% reduced sucrose with a 0.214 J/g difference.

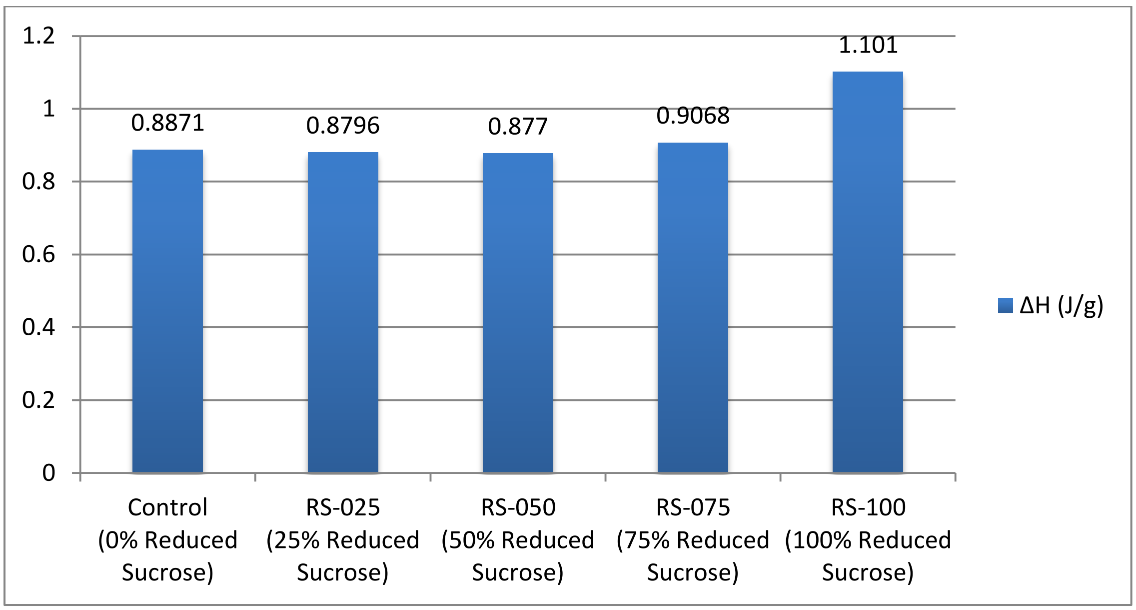

FIGURE 4.2: DIFFERENTIAL SCANNING CALORIMETRY CHANGE IN ENTHALPY BY TREATMENT FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

CALORIC AND NUTRITIONAL ANALYSIS

Caloric results by treatment for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels (0, 25, 50, 75 and 100%) can be found in Table 4.9. Complete nutrition facts panels for each treatment are in Appendix VII. Results per 100 gram sample were calculated using Genesis® R&D SQL software, version 8.0 (ESHA Research, Salem, OR). As the replacement level of rebaudioside-A and erythritol for sucrose increased, the caloric level of treatments decreased. The control was the highest with 300 calories per 100 grams of cake and the 100% reduced sucrose sample had the lowest with 200 calories per 100 grams of cake. According to Food and Drug Administration regulations, the term "reduced calorie," "reduced in calories," "lower in calories," "fewer calories," "calorie reduced" or "lower calorie" can be used on a food label provided that the product contains at least 25 percent fewer calories from the reference food (FDA 2012). Therefore both the 75 and 100% reduced sucrose cakes qualify for such a label claim. Additionally, according to CFR 101.13 (j) (2), a product can contain a "relative claim" where "the identity of the reference food and the percentage (or fraction) of the amount of the nutrient in the reference food by which the nutrient in the labeled food differs" (FDA 2012). Therefore, as long as the percentage of calories reduced for both the 25 and 50% reduced sucrose samples compared to the control are disclosed, those percentages can be advertised on the food packaging. The 50% reduced sucrose sample allows for the greatest reduction in calories (20%) compared to the control without compromising quality. Other than a consistent reduction in grams/serving of sugar in cakes as the replacement level of rebaudioside-A and erythritol for sucrose increased, no other changes in nutrients were observed (Appendix VII).

TABLE 4.9: CALORIES PER 100 GRAMS FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	Calories Per 100 Grams	Percent Reduced From Control
Control (0% Reduced Sucrose)	300	
RS-025 (25% Reduced Sucrose)	280	7.14
RS-050 (50% Reduced Sucrose)	250	20.00
RS-075 (75% Reduced Sucrose)	230	30.44
RS-100 (100% Reduced Sucrose)	200	50.00

SENSORY ANALYSIS

Sensory data were collected from forty panelists using five chiffon cake formulations, each with a percentage of sucrose (0, 25, 50, 75 and 100%) replaced with a mixture of rebaudioside-A and erythritol. A nine-point hedonic scale ranging from "like extremely" (9.0) to "dislike extremely" (1.0) was used to measure overall liking of color of crumb, tenderness, sweetness, aftertaste and overall acceptability. Panelists were asked to rank preference of the five samples from one to five with one being the most preferred. Probing questions were asked for comments on flavor and bitterness. Panelists were asked "what did you like or dislike about the flavor of the samples; please refer to the sample number." In respect to bitterness they were asked if they found any of the samples to be bitter and if so to rank the bitter perception using a scale from one to five where one was "slightly bitter" and five was "very bitter."

TABLE 4.10: HEDONIC* SENSORY MEAN** VALUES FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Treatment	**Color of Crumb**	**Tenderness**	**Sweetness**	**Aftertaste**	**Overall Acceptability**
Control (0% Reduced Sucrose)	7.0 ± 0.2 *a*	6.4 ± 0.3 *b*	7.0 ± 0.2 *bc*	6.3 ± 0.3 *bc*	6.7 ± 0.2 *dc*
RS-025 (25% Reduced Sucrose)	7.2 ± 0.2 *a*	7.6 ± 0.3 *a*	7.7 ± 0.2 *a*	7.3 ± 0.3 *a*	7.6 ± 0.2 *ba*
RS-050 (50% Reduced Sucrose)	7.5 ± 0.2 *a*	7.6 ± 0.3 *a*	7.6 ± 0.2 *ba*	7.4 ± 0.3 *a*	7.7 ± 0.2 *a*
RS-075 (75% Reduced Sucrose)	7.2 ± 0.2 *a*	7.0 ± 0.3 *ba*	7.4 ± 0.2 *ba*	6.7 ± 0.3 *ba*	7.0 ± 0.2 *bc*
RS-100 (100% Reduced Sucrose)	7.0 ± 0.2 *a*	6.4 ± 0.3 *b*	6.4 ± 0.2 *c*	5.7 ± 0.3 *c*	6.1 ± 0.2 *d*

*Hedonic ranking scale ranges from 9.0—like extremely to 1.0—dislike extremely

**Results are presented as mean of 40 replications ± standard deviation. Means with the same letter are NOT significantly different ($p > 0.05$)

Mean results of crumb color “liking” for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels are reported in Table 4.10. No differences were found for “liking” of crumb color among cake samples containing 0, 25, 50, 75 or 100% reduction of sucrose ($p > 0.05$). Panelist score means ranged from 7.0 to 7.5. This corresponds with instrumental crumb color analysis results showing no difference in L or a-values.

Mean results of tenderness “liking” for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels are reported in Table 4.10. Tenderness of both the 25 and 50% reduced sucrose chiffon cakes were preferred over other treatments ($p < 0.05$). Comparing to texture analysis results, both the 25 and 50% reduced sucrose chiffon cakes were more tender than other treatments ($p < 0.05$).

Mean results of sweetness “liking” for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels are reported in Table 4.10. Sweetness of 25, 50 and 75% reduced sucrose chiffon cakes were statistically more preferred over other treatments ($p < 0.05$). The sample with 100% reduction in sucrose scored the lowest on the 9-point hedonic scale for sweetness with a score of 6.4. However, the value is not different from the control score of 7.0 ($p > 0.05$). Since steviosides are considered the most bitter of the most common non-nutritive high-intensity sweeteners, especially at higher concentrations (Schiffman et al. 1995), panelists were asked to indicate if any sample was bitter and if so, which ones. While the majority of panelists (90%) found no bitterness in any samples, four of the forty panelists (10%) reported bitterness. When asked to report which samples they found to be bitter, two indicated the 100% reduced sucrose cake, one the 75% reduced sucrose and one responded the control.

Mean results of aftertaste "liking" for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels are reported in Table 4.10. Aftertaste of 25, 50 and 75% reduced sucrose chiffon cakes were statistically more preferred over other treatments ($p < 0.05$). Some comments regarding the negative attributes of the 100% reduced sucrose cake is that it had a "grainy aftertaste and texture," "did not like the cool, tangy aftertaste" and some panelists simply responded that they just disliked the samples. Lin and others (2010) reported a similar "cooling" sensation when replacing sucrose with erythritol in Danish cookies. The researchers reported that the "cooling effect may be due to the negative heat of dissolution of erythritol" (Lin et al. 2010).

Mean results of overall acceptability for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels are reported in Table 4.10. Results indicate panelist overall "liking" of the chiffon cake samples with the lowest mean score of 6.1 indicating "like slightly" and the highest of 7.7 indicating a score between "like moderately" to "like very much." There were significant preferences for either the 25 and 50% reduced sucrose chiffon cakes with mean scores of 7.6 and 7.7 respectively ($p < 0.05$). Panelists found the 100% reduced sucrose and the control samples to be the preferred with the lowest mean scores of 6.1 and 6.7 respectively ($p < 0.05$). From written comments, panelists preferred the flavor, sweetness and tenderness of both the 25 and 50% reduced sucrose cakes. There were multiple comments that the control cake was too dense which corresponds with texture analysis findings. More grams of force were exerted to penetrate the control cake as opposed to 25 and 50% reduced sucrose. They also disliked the "grittiness" or "dryness" of the 100% reduced sucrose sample. The "grittiness" and "dryness" may be attributed to the higher concentration of erythritol. According to Lin and others (2010) "Erythritol does not attract water, which can cause baked goods made with the sugar alcohol to dry out more quickly. It also has a tendency to crystallize."

Panelists were asked to rank the five samples in order of preference. A score of one assigned to "most preferred" and five assigned to "least preferred." Mean results for ranking preference for chiffon cake formulations containing rebaudioside-A and erythritol as replacement for sucrose at varying levels are reported in Table 4.11. These results are reported in order from most preferred to least preferred. There was preference for either the 25 or 50% reduced sucrose chiffon cakes ($p < 0.05$). Panelists also found the control as well as the 100% reduced sucrose to be the least preferred ($p < 0.05$). Written comments about the two most preferred samples included "I liked the flavor and texture," "light and fluffy texture and good tasting," "617 (25% reduced sucrose sample) was by far the best in every category," "103 (50% reduced sucrose sample) tasted the most like a cake I would make." As for the two least preferred, overall comments were regarding the sweetness or lack thereof, dislike in texture and dislike of aftertaste.

TABLE 4.11: MEAN* SENSORY RANKING** BY TREATMENT IN ORDER FROM MOST PREFERRED TO LEAST FOR CHIFFON CAKE FORMULATIONS CONTAINING REBAUDIOSIDE-A AND ERYTHRITOL AS REPLACEMENT FOR SUCROSE AT VARYING LEVELS

Rank of Preference	**Treatment**	**Mean**
1	RS-050 (50% Reduced Sucrose)	2.2 ± 0.2 *c*
2	RS-025 (25% Reduced Sucrose)	2.3 ± 0.2 *c*
3	RS-075 (75% Reduced Sucrose)	2.9 ± 0.2 *b*
4	Control (0% Reduced Sucrose)	3.6 ± 0.2 *a*
5	RS-100 (100% Reduced Sucrose)	4.0 ± 0.2 *a*

*Results are presented as mean of 40 replications ± standard deviation. Means with the same letter are NOT significantly different ($p > 0.05$).
**Rank of 1 = most preferred; 5 = least preferred.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Research was conducted to determine the physicochemical and sensory effects of replacing a mixture of rebaudioside-A and erythritol for sucrose at varying levels (0, 25, 50, 75 and 100%) in chiffon cake. Analytical testing on specific gravity, texture, volume, water activity, moisture, color and differential scanning calorimetry was conducted on five cake formulations as well as nutritional analysis and a consumer sensory analysis. Acceptable replacement level from a physicochemical and sensory perspective was determined.

Instrumental testing results for specific gravity, cake volume and moisture percentage found no differences among the five chiffon cake formulations ($p > 0.05$). Therefore replacing sucrose with a mixture of rebaudioside-A and erythritol showed no effect on the amount of air held within the batter, the baked chiffon cake volume or the final moisture content. Color testing determined there were no differences in color of crust or crumb among any of the replacement levels with the exception that the crumb color of both the control and 25% reduced sucrose samples were more yellow than the 100% reduced sucrose cake ($p < 0.05$). Since the mixture of rebaudioside-A and erythritol is white in color and does not participate in Maillard browning explains the lighter yellow hue of crumb as replacement levels increased.

The replacement level of rebaudioside-A and erythritol for sucrose had an effect on the amount of bound water within the food system. Water activity of the control cake was significantly higher than both the 50 and 75% reduced sucrose samples ($p < 0.05$). Additionally, the water activity of 25% reduced sucrose sample was significantly higher than the 75% reduced sample ($p < 0.05$). These results suggest a mixture of rebaudioside-A and erythritol as replacement for sucrose in a chiffon cake may impact water activity.

Cake texture was affected as the replacement level of rebaudioside-A and erythritol for sucrose increased to 100%. Texture analysis showed the 25 and 50% reduced sucrose cakes were more tender than the 100% reduced sucrose cake ($p < 0.05$). There was a slight "white" and slightly grainy-textured layer close to the crust of the 75 and 100% reduced sucrose samples after baking. Erythritol does not attract water during baking and crystallizes at higher concentrations.

Differential scanning calorimetry results showed the higher the replacement level of sucrose for a mixture of rebaudioside-A and erythritol, both the onset and peak temperatures decrease. Enthalpy stayed around constant for the control, 25 and 50% reduced sucrose samples and then increased for both the 75 and 100% samples; however, differences were small and would likely not be detectable by consumers. In home or commercial baking, as sucrose is replaced with a mixture of rebaudioside-A and erythritol, baking time or temperature may require adjustment. Calorie and nutrition analysis showed a decrease in both calories and sugars as the sucrose replacement level increased. All other nutrient levels remained constant among treatments.

Consumer sensory evaluation with forty panelists showed an overall preference for the 25 and 50% reduced sucrose samples ($p < 0.05$) and an overall dislike of samples prepared with a 75 and 100% reduction based from "overall acceptability" and "ranking" questions. Four of forty panelists reported bitterness; two referring to the 100% reduced sucrose sample, one to the 75% sucrose sample and one to the control. Additionally panelists found the 25 and 50% reduced sucrose samples to be the most tender ($p < 0.05$). This result corresponds with instrumental texture analysis results. For sweetness and aftertaste, they preferred the 25, 50 and 75% reduced sucrose cakes ($p < 0.05$). These findings suggest a chiffon cake formulated with 50% sucrose and 50% rebaudioside-A and erythritol results in a product with high overall consumer acceptability and 20% fewer calories than one formulated with 100% sucrose.

Future research may be aimed at optimizing the ratio of sucrose to rebaudioside-A and erythritol possibly with 55, 60 or 65% replacement. This mixture may prove to be a suitable all-natural competitor to other “baking” blends currently on the market such as Splenda Sugar Blend for Baking™ (McNeil Nutritionals LLC 2012). Our research also suggests there may be a relationship with the effect of replacing sucrose with rebaudioside-A and erythritol on baking time and temperature. Once an optimum replacement level is determined, future research should focus on defining suitable adjustments in baking time and/or temperature. Possible formulation adjustment for high altitude baking may be explored. However, a mixture of rebaudioside-A and erythritol at a 25% or higher replacement level for sucrose does not appear to need altitude adjustment as visually observed in pictures found in Appendix V. Investigating the blend’s use in other types of baked goods such as quick breads, shortened cakes, muffins or scones could be investigated.

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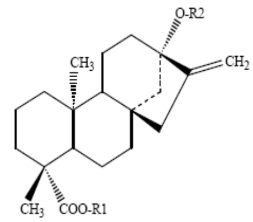
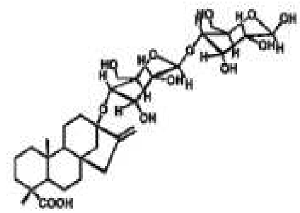
APPENDIX I

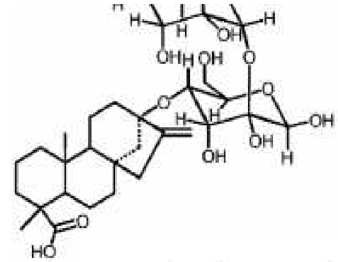
DIAGRAMS OF STEVIOL GLYCOSIDES



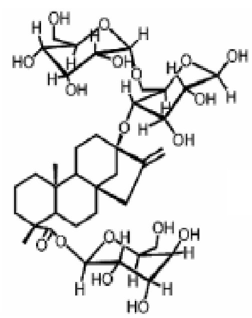
Steviol



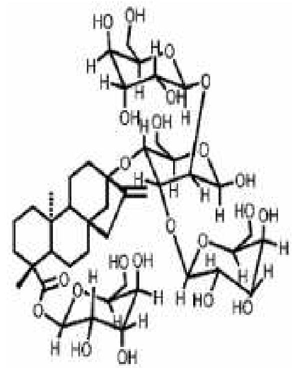
Steviolbioside



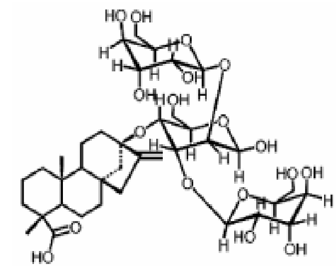
Dulcoside A



Stevioside



Rebaudioside-A



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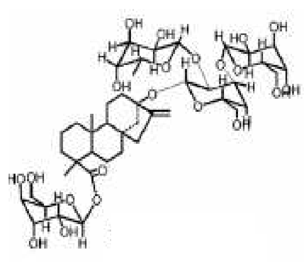

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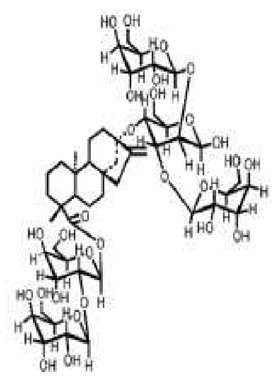

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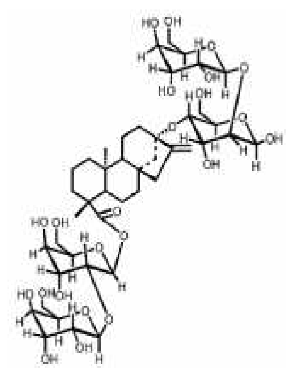


Rebaudioside E

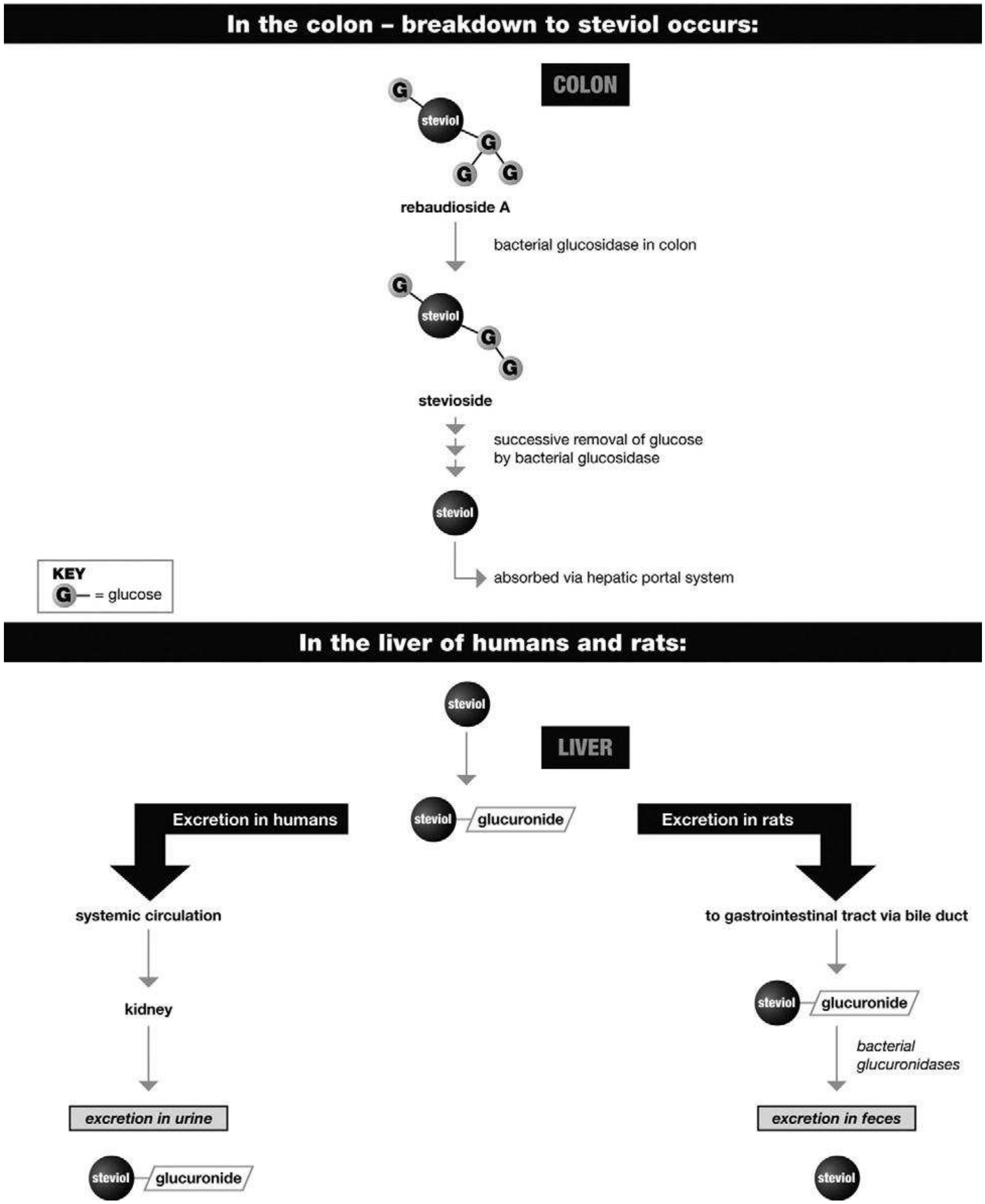
STEVIOL GLYCOSIDES

(Carakotas et al. 2008)

APPENDIX II

DIAGRAM OF ABSORPTION, METABOLISM AND EXCRETION OF

REBAUDIOSIDE-A



ABSORPTION, METBOLISM AND EXCRETION OF REBAUDIOSIDE-A

(Carakostas et al. 2008)

APPENDIX III

INSTITUTIONAL REVIEW BOARD EXEMPT STATUS APPROVAL
AND
LETTER TO SENSORY PANELISTS

Colorado State University

Research Integrity & Compliance Review Office
Office of Vice President for Research
Fort Collins, CO 80523-2011
(970) 491-1553
FAX (970) 491-2293

DATE: April 27, 2011

TO: Martha Stone, FSHN
Robert Lothrop, FSHN

Janell Barker

FROM: Janell Barker, IRB Administrator
Research Integrity & Compliance Review Office

TITLE: Sensory Quality of Chiffon Cake Prepared with Erythritol and Rebaudioside-A as Replacement for Sucrose

IRB ID: 052-12H **Review Date:** April 27, 2011

The Institutional Review Board (IRB) Administrator has reviewed this project and has declared the study exempt from the requirements of the human subject protections regulations as described in 45 CFR 46.101(b)(6): Taste and food quality evaluation and consumer acceptance studies, a) if wholesome foods without additives are consumed or b) if a food is consumed that contains a food ingredient at or below the level and for use found to be safe by the FDA or approved by the EPA or the Food Safety and Inspections Service of the USDA.

The IRB determination of exemption means that:

- **You do not need to submit an application for annual continuing review.**
- **You must carry out the research as proposed in the Exempt application**, including obtaining and documenting (signed) informed consent if stated in your application or if required by the IRB.
- **Any modification of this research should be submitted to the IRB through an email to the IRB Administrator, prior to implementing any changes**, to determine if the project still meets the Federal criteria for exemption. If it is determined that exemption is no longer warranted, then an IRB proposal will need to be submitted and approved before proceeding with data collection.
- **Please notify the IRB if any problems or complaints of the research occur.**

Please note that you must submit all research involving human participants for review by the IRB. **Only the IRB may make the determination of exemption**, even if you conduct a similar study in the future.

Colorado State University
Knowledge to Go Places

Department of Food Science and Human Nutrition
College of Applied Human Sciences
234 Gifford Building
502 West Lake Street
1571 Campus Delivery
Fort Collins, Colorado 80523-1571
Office: (970) 491-3663 FOOD
FAX: (970) 491-3875 or (970) 491-7252
website: www.cahs.colostate.edu/fshn

Dear Sensory Panelist,

Thank you for volunteering to participate in our sensory test. Today, we will have you taste five samples of chiffon cake made with Truvia™ brand sweetener, complete a score card and answer questions about the cakes that you tasted. The purpose of this study is to determine if Truvia™ is a suitable replacement for sugar in a chiffon cake and if so, at what level. The study will take place in the food laboratories of the Gifford Building at Colorado State University and will take approximately 15-20 minutes to complete.

This study is completely anonymous and all sensory score cards will be kept in a locked file cabinet. There is no compensation for participating in the study; however, there will be soft drinks and bottled water available for you after completion. This research is being conducted by Robert Lothrop, PhD candidate in the Department of Food Science and Human Nutrition under the guidance of Dr. Martha Stone.

IF YOU ARE ALLERGIC TO ANY OF THE FOLLOWING FOODS, DO NOT PARTICIPATE IN THIS SENSORY TEST AS THESE ARE PRESENT IN THE CAKES: **SOY, WHEAT, EGGS, DAIRY.** All of the products used in the production of the cakes are GRAS (Generally Recognized as Safe) by the FDA. If you have any questions regarding the study, please contact Robert Lothrop at 720-427-5397 or rslothrop@comcast.net.

Thank you for your participation.

______________________________	______________________________
Martha Stone, PhD	Robert Lothrop, MBA
Primary Investigator	Co-Investigator

APPENDIX IV

SENSORY SCORE CARD

SENSORY SCORE CARD

COLOR OF CRUMB					
SAMPLE NUMBER→→	505	617	103	702	352
Like Extremely					
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like or Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					
Dislike Extremely					

AFTERTASTE					
SAMPLE NUMBER→→	505	617	103	702	352
Like Extremely					
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like or Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					
Dislike Extremely					

TENDERNESS					
SAMPLE NUMBER→→	505	617	103	702	352
Like Extremely					
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like or Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					
Dislike Extremely					

OVERALL ACCEPTABILITY					
SAMPLE NUMBER→→	505	617	103	702	352
Like Extremely					
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like or Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					
Dislike Extremely					

SWEETNESS					
SAMPLE NUMBER→→	505	617	103	702	352
Like Extremely					
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like or Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					
Dislike Extremely					

What did you like or dislike about the flavor of the samples; please refer to the sample numbers

____

____

Did you find any of the samples to be bitter? YES NO ***(circle one)***

If you answered YES, please indicate the sample(s) you found to be bitter and the level 1-5 (1= slightly bitter; 5= very bitter).

____

____

Please rank the samples in order of preference (write in sample number)
(1=FAVORITE; 5=LEAST FAVORITE)

____	____	____	____	____
1	2	3	4	5

APPENDIX V

PHOTOGRAPHS OF CAKES AND CRUMB

CONTROL

Outside Edge Height: 1 11/16 inch
Outside Middle Height: 1 6/8 inch

Center Edge Height: 1 6/8 inch
Center Middle Height: 1 5/16 inch

RS-025

Outside Edge Height: 1 6/8 inch
Outside Middle Height: 1 13/16 inch

Center Edge Height: 1 13/16 inch
Center Middle Height: 1 13/16 inch

RS-050

Outside Edge Height: 1 13/16 inch
Outside Middle Height: 1 7/8 inch

Center Edge Height: 1 7/8 inch
Center Middle Height: 1 7/8 inch

RS-075

Outside Edge Height: 1 6/8 inch
Outside Middle Height: 1 6/8 inch

Center Edge Height: 1 11/16 inch
Center Middle Height: 1 9/16 inch

RS-100

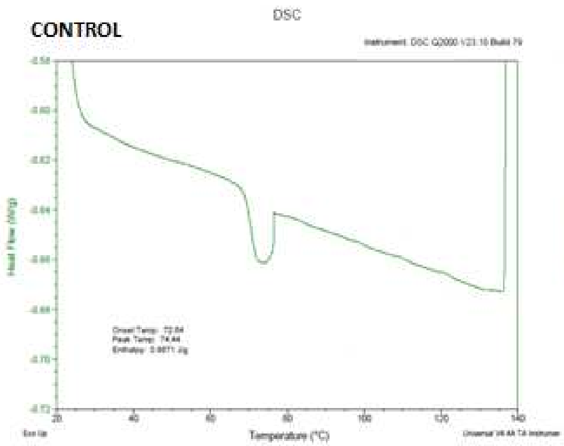
Outside Edge Height: 1 1/2 inch
Outside Middle Height: 1 1/2 inch

Center Edge Height: 1 1/2 inch
Center Middle Height: 1 1/2 inch

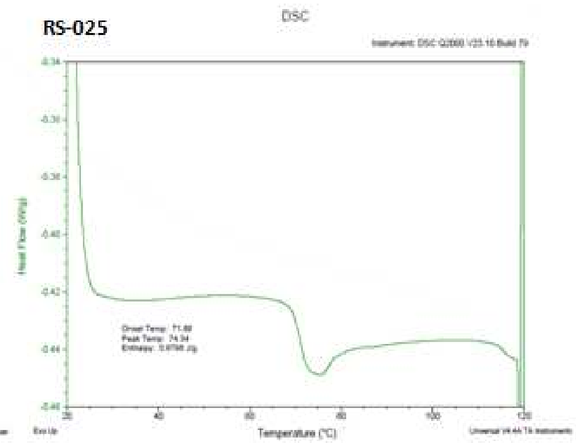
APPENDIX VI

DIFFERENTIAL SCANNING CALORIMETRY THERMAL GRAPHS

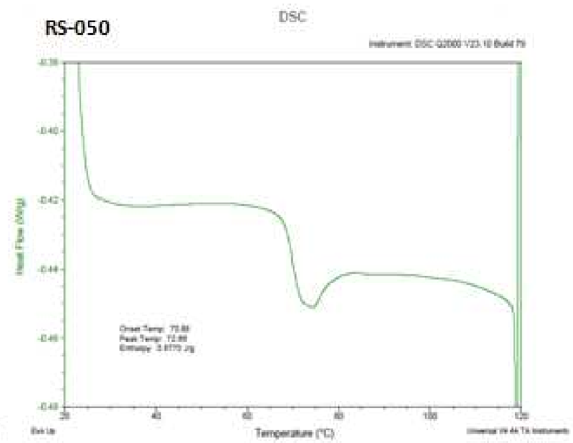
CONTROL



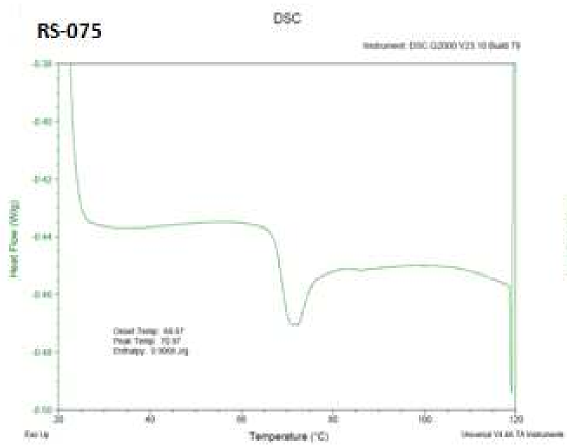
RS-025



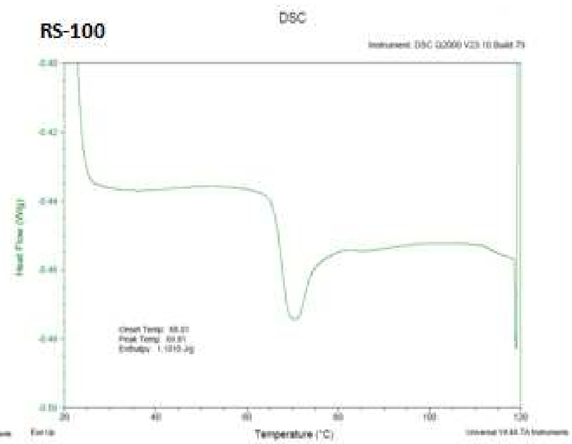
RS-050



RS-075



RS-100



APPENDIX VII

NUTRITION FACTS PANELS AND INGREDIENT DECLARATIONS

	Control **0% Reduced Sucrose**	**RS-025** **25% Reduced Sucrose**	**RS-050** **50% Reduced Sucrose**	**RS-075** **75% Reduced Sucrose**	**RS-100** **100% Reduced Sucrose**
	Nutrition Facts	**Nutrition Facts**	**Nutrition Facts**	**Nutrition Facts**	**Nutrition Facts**
	Serving Size (100g) Servings Per Container	Serving Size (100g) Servings Per Container	Serving Size (100g) Servings Per Container	Serving Size (100g) Servings Per Container	Serving Size (100g) Servings Per Container
	Amount Per Serving	**Amount Per Serving**	**Amount Per Serving**	**Amount Per Serving**	**Amount Per Serving**
	Calories 300 Calories from Fat 110	**Calories** 280 Calories from Fat 110	**Calories** 250 Calories from Fat 110	**Calories** 230 Calories from Fat 110	**Calories** 200 Calories from Fat 110
	% Daily Value*	**% Daily Value***	**% Daily Value***	**% Daily Value***	**% Daily Value***
	Total Fat 12g **18%**	**Total Fat** 12g **18%**	**Total Fat** 12g **18%**	**Total Fat** 12g **18%**	**Total Fat** 12g **18%**
	Saturated Fat 2.5g **13%**	Saturated Fat 2.5g **13%**	Saturated Fat 2.5g **13%**	Saturated Fat 2.5g **13%**	Saturated Fat 2.5g **13%**
	Trans Fat 0g	Trans Fat 0g	Trans Fat 0g	Trans Fat 0g	Trans Fat 0g
	Cholesterol 105mg **35%**	**Cholesterol** 105mg **35%**	**Cholesterol** 105mg **35%**	**Cholesterol** 105mg **35%**	**Cholesterol** 105mg **35%**
	Sodium 280mg **12%**	**Sodium** 280mg **12%**	**Sodium** 280mg **12%**	**Sodium** 280mg **12%**	**Sodium** 280mg **12%**
	Total Carbohydrate 43g **14%**	**Total Carbohydrate** 43g **14%**	**Total Carbohydrate** 43g **14%**	**Total Carbohydrate** 43g **14%**	**Total Carbohydrate** 43g **14%**
	Dietary Fiber 0g **0%**	Dietary Fiber 0g **0%**	Dietary Fiber 0g **0%**	Dietary Fiber 0g **0%**	Dietary Fiber 0g **0%**
	Sugars 27g	Sugars 21g	Sugars 14g	Sugars 8g	Sugars 1g
	Protein 6g	**Protein** 6g	**Protein** 6g	**Protein** 6g	**Protein** 6g
	Vitamin A 4% • Vitamin C 0%	Vitamin A 4% • Vitamin C 0%	Vitamin A 4% • Vitamin C 0%	Vitamin A 4% • Vitamin C 0%	Vitamin A 4% • Vitamin C 0%
	Calcium 6% • Iron 10%	Calcium 6% • Iron 10%	Calcium 6% • Iron 10%	Calcium 6% • Iron 10%	Calcium 6% • Iron 10%
	*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs: Calories: 2,000 2,500 Total Fat Less than 65g 80g Saturated Fat Less than 20g 25g Cholesterol Less than 300mg 300 mg Sodium Less than 2,400mg 2,400mg Total Carbohydrate 300g 375g Dietary Fiber 25g 30g	*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs: Calories: 2,000 2,500 Total Fat Less than 65g 80g Saturated Fat Less than 20g 25g Cholesterol Less than 300mg 300 mg Sodium Less than 2,400mg 2,400mg Total Carbohydrate 300g 375g Dietary Fiber 25g 30g	*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs: Calories: 2,000 2,500 Total Fat Less than 65g 80g Saturated Fat Less than 20g 25g Cholesterol Less than 300mg 300 mg Sodium Less than 2,400mg 2,400mg Total Carbohydrate 300g 375g Dietary Fiber 25g 30g	*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs: Calories: 2,000 2,500 Total Fat Less than 65g 80g Saturated Fat Less than 20g 25g Cholesterol Less than 300mg 300 mg Sodium Less than 2,400mg 2,400mg Total Carbohydrate 300g 375g Dietary Fiber 25g 30g	*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs: Calories: 2,000 2,500 Total Fat Less than 65g 80g Saturated Fat Less than 20g 25g Cholesterol Less than 300mg 300 mg Sodium Less than 2,400mg 2,400mg Total Carbohydrate 300g 375g Dietary Fiber 25g 30g
	Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4	Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4	Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4	Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4	Calories per gram: Fat 9 • Carbohydrate 4 • Protein 4
	INGREDIENTS: Sugar, Cake Flour (Bleached Wheat Flour, Thiamin Mononitrate, Riboflavin, Folic Acid), Egg Whites, Egg Yolks, Soybean Oil, Non-Fat Dried Milk, Baking Powder, Salt, Cream of Tartar	INGREDIENTS: Sugar, Cake Flour (Bleached Wheat Flour, Thiamin Mononitrate, Riboflavin, Folic Acid), Egg Whites, Egg Yolks, Soybean Oil, Erythritol, Non-Fat Dried Milk, Baking Powder, Salt, Cream of Tartar, Rebaudioside-A	INGREDIENTS: Sugar, Cake Flour (Bleached Wheat Flour, Thiamin Mononitrate, Riboflavin, Folic Acid), Erythritol, Egg Whites, Egg Yolks, Soybean Oil, Non-Fat Dried Milk, Baking Powder, Salt, Cream of Tartar, Rebaudioside-A	INGREDIENTS: Erythritol, Cake Flour (Bleached Wheat Flour, Thiamin Mononitrate, Riboflavin, Folic Acid), Egg Whites, Egg Yolks, Soybean Oil, Sugar, Non-Fat Dried Milk, Baking Powder, Salt, Cream of Tartar, Rebaudioside-A	INGREDIENTS: Erythritol, Cake Flour (Bleached Wheat Flour, Thiamin Mononitrate, Riboflavin, Folic Acid), Egg Whites, Egg Yolks, Soybean Oil, Non-Fat Dried Milk, Baking Powder, Salt, Cream of Tartar, Rebaudioside-A