THE EFFECTS OF NON-NUTRITIVE SWEETENERS IN FLORENCE'S HOMESTYLE CHA-CHA

by

LESLIE BOONE

B.A., University of Arkansas at Pine Bluff, 1993
M.B.A, Fontebonne University, 2001

A REPORT

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2009

Approved by:

Major Professor
Dr. J. Scott Smith
Animal Sci and Ind

Abstract

Non-nutritive sweeteners are alternative sweeteners that provide the taste of sweetness without a caloric contribution. In this Florence's HomeStyle Cha-Cha study, aspartame, acesulfame K, sodium saccharin, and neotame were evaluated as a substitute for sucrose in the formulation. These sweeteners were used at their recommended equivalency levels to match the sweetness potency level to that of sucrose. Because they are more potent than sucrose, minimum amounts were needed to replace sucrose, and the remaining amounts were filled with maltodextrin. Using maltodextrin as bulk filler is common practice in the industry when replacing sucrose with a non-nutritive sweetener. This study evaluated the quality of the finished product in determining which non-nutritive sweetener had similar taste, aroma, and density qualities as the control, which was Cha-Cha sweetened with sucrose. The results of the evaluations indicated that Cha-Cha sweetened with acesulfame K was more similar to the control than the other non-nutritive sweeteners. Of the three taste characteristics evaluated for their degree of intensity, sweetness, saltiness, and bitterness, the acesulfame K sweetened treatment was closer to the control. In the GC/MS analysis in characterizing the volatile aroma compounds, acesulfame K contained all the aroma compounds with smaller differences in concentration than the other experimental treatments. In the density evaluation, the experimental treatments were similar to the control treatment. In conclusion, Florence's HomeStyle Cha-Cha sweetened with acesulfame K is similar in quality to the sucrose-sweetened control.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to extend thanks and grateful appreciation to the following people who assisted me in the planning, development and implementation of this project:

- The Management Committee of Florence's HomeStyle Cha-Cha for participating in the taste-testing panel and for their intense interest in this research.
- My daughter for being supportive, understanding…and for being a great teen. She maintained a 3.8—4.0 GPA during my graduate school years.
- Dr. J. Scott Smith…for his mentorship, understanding, and interest in this project.
- My Supportive Parents and brother for keeping me grounded and enabling me to manage my work, school and parenthood.
- My friends…for being supportive and for understanding the value of my time, especially during this last semester.
- Dr. Fadi Aramouni and Dr. Kelly J.K. Getty as Kansas State University committee members…for the mentorship and opportunity to present this research.

Dedication

I dedicate this research to Florence's HomeStyle Cha-Cha. This is a relatively new company which has tremendous potential for expanding into a productive, lucrative market. This research provides guidelines for the use of non-nutritive sweeteners in the company's product applications. Also, this research will create interest in further evaluating non-nutritive sweetener blends that will enable them to optimize and balance sweetness, and to mask the bitter taste characteristics in Cha-Cha. If Florence's HomeStyle Cha-Cha chooses to utilize and build on this research, they will access an excellent opportunity to expand their portfolio, as well as address the concerns of their sucrose-sensitive customers.

CHAPTER 1 - Introduction

Florence's HomeStyle Inc. is a family owned business that specializes in African American condiments. The company was established in 2000 with the intent of marketing Cha-Cha, a product that this family of seven sisters learned to make from their mother, Florence Gunnels. Florence's HomeStyle Cha-Cha is a green tomato-based food enhancer. It can be used as a condiment, an ingredient, a dipping sauce, and a marinade.

Cha-Cha is composed of green tomatoes, jalapeño peppers, red peppers, cabbage, onions, special spices, apple cider vinegar, high fructose corn syrup, and sucrose. The cabbage is shredded, and the other vegetables are diced. These ingredients are mixed with apple cider vinegar, high fructose corn syrup, sugar, and special spices, then thermal processed in a large kettle and packaged hot with a piston-filler in 13 oz. jars. The tamper evident button-pop lids are placed with a chuck capper machine. The end product is sweet and tangy in taste, slightly crunchy in texture, and greenish in appearance. There are two commercially available versions of Cha-Cha, "mild" and "hot." Both versions are sold in Missouri, Arkansas, and Ohio.

Marketing a new niche product, such as Cha-Cha has been challenging and rewarding. The most successful avenues used to market this product have been through local and national trade shows, store promotions, demonstrations, cooking shows, and press releases. Through continued participation in trade shows, the company has gained knowledge and information on ways the targeted consumers are using the product. These suggestions have been redirected back into the marketing campaign to educate consumers and increase consumer awareness. These marketing channels have helped identify a lucrative market.

Some people, however, have reservations on purchasing the product because of the high sugar (sucrose). Many of them indicated they would be interested in purchasing the product if it did not contain sucrose. This, of course, is a valid concern. The media and healthcare system constantly emphasizes the negative impact that sucrose has on people's health; and with the epidemic rise of diabetes and obesity in the United States, this is certainly understandable. Complications of diabetes can lead to blindness, heart disease, kidney failure, and/or lower extremity amputations. Complications from obesity can lead to diabetes, hypertension, and coronary heart disease. Excessive sucrose in the diet not only has an impact on medical conditions, but it also negatively impacts dental conditions. It causes tooth decay from the acid production it generates in the mouth (Kemp, 2006).

Due to these negative connotations of sucrose, it has become extremely important that sweetener alternatives be considered. The research conducted evaluated several non-nutritive sweeteners as a replacement for sucrose in the mild version of Florence's HomeStyle Cha-Cha. The research consisted of one trial for each study and a suggestive summary was reported. The sweeteners chosen for evaluation were aspartame, neotame, acesulfame K, and sodium saccharin. These non-nutritive sweeteners are unique in their composition, functionality, stability, and perception in taste. Therefore, the objective is to identify a non-nutritive sweetener that has a clean, quick onset of sweetness with little to no lingering aftertaste that mimics the taste of sucrose in Florence's HomeStyle Cha-Cha. The analyses conducted compared the control (Cha-Cha sweetened with sucrose) vs. the experimental treatments (Cha-Cha sweetened with non-nutritive sweeteners). A sensory analysis evaluating taste qualities, density evaluation, and volatile aroma profile by gas chromatography/mass spectrometry (GC/MS) were conducted.

Literature Review

The two widely accepted theories on the human perception of sweetness are the Three Point Attachment Theory and the Multi-Point Attachment Theory. Both of these theories suggest that sweet perception is based on a molecule that binds with a receptor site which triggers impulses to the brain to perceive the taste of sweetness. The degree of binding varies depending on the shape and structure of the molecule and how well it fits into the receptor (Purves, 2006). The molecules of the non-nutritive sweeteners (aspartame, neotame, acesulfame K, and sodium saccharin) have different affinities that help them bind to the receptor more snugly than sucrose which send more signals to the brain to perceive the taste of sweetness more than sucrose (Selim, 2005).

Three Point Attachment Theory

The three point attachment theory (AH-B-X) proposes that the structural element of all sweet tasting molecules have AH-B-X regions, and these regions bind with taste receptors to enable them to perceive the taste of sweetness. The AH-B part of this theory was proposed by Shallenberger and Acree (1967); then in 1972, Kier expanded the work and proposed the 3rd component, the "x". The A and B regions of a compound have electronegative atoms. The distance between the two regions must be greater than 2.4º Angstroms and less than 4.0º Angstroms for sweetness to be perceived. Outside of this range, the compound will be bitter. The A region possess an electronegative atom such as oxygen or nitrogen. This atom also has a proton attached to it by a single covalent bond, so AH can represent hydroxyl group, an imine or amine group or a methane group used for hydrogen bonding. The AH region is a hydrogen bond donor. The B region is the hydrogen bond acceptor. The B region is located 0.3 nm from the

AH proton it can be either an oxygen or nitrogen. The x region plays an important role in determining the intensity of sweetness (deMann, 1999). For instance, in sucrose, the AH and B regions are C2 and C3 hydroxyls of glucose respectively. The extended hydrophobic region x is the fructose ring that docks in a hydrophobic cleft of the receptor, facilitating optimal electrostatic interaction and sensory stimulation (Colonna et al., 2001).

The AH and B regions of sweetener's molecules must align suitably with the AH and B region of the receptor site to send signals to the brain to perceive the taste of sweetness. When a sweetener's molecule binds to the receptor site of the taste cell, the AH region hydrogen bonds to the B region of the receptor site, and the B region hydrogen bonds to the AH region of the receptor site. The x region of the molecule will attract similar lipophilic regions of the taste receptor, such as methylene (CH_2), methyl (CH_3), or phenyl (C_6H_5) groups. These interactions trigger a response by cells in the taste bud and the cells send electrical impulses to the brain to create the perception of sweetness (Lichtenthaler and Immel, 1993). Understanding these regions in non-nutritive sweeteners help us to understand why their intensities differ. In sucrose, there are hydrogens in the AH region that are available for hydrogen to bond with the complementary acceptor group in the receptor. Hydrogen is in the B region and is available for bonding. The x part is the area outside the fructose moiety (Lichtenthaler and Stefan, 1993). In aspartame, the AH region has a $-NH_3^+$ group in which hydrogen bonds to hydrogen. The B region has a $-COO^-$ group where it is the hydrogen bond acceptor; the x region is the $COOCH_3$ ester group (Lichtenthaler and Immel, 1993). These are just a couple examples that demonstrate the effects AH-B-and X regions of sweeteners.

Multi-Point Attachment Theory

The multi-point attachment theory proposed by Tinti and Nofre in 1996 suggests that there are eight receptor sites that will interact and bind with eight or less sweetener interaction sites. The receptor interaction site binds based on the structure of the binding ligands, not on the structure of the sweet receptor. The number of the binding sites determines the potency of the sweetener. The eight receptor sites are B, AH, XH, G1, G2, G3, G4 and D. Each of these receptor sites interact with eight of the sweetener interaction sites of the same names (B, AH, XH. G1, G2, G3, G4, and D). It is believed that the aromatic interactions with the D zone of the sweet receptor are responsible for the intense sweetness of peptide based ligands, such as aspartame and neotame (Nofre et al., 1996).

Sweet Taste Transduction

Sugar and high potency sweeteners bind to taste receptors and activate the sweet-responsive taste receptors called G protein coupled receptors (GPCR). Each of the G protein coupled receptors has two proteins that function as human sweet receptors, which are T1R2 and T1R3. The combination of T1R2 and T1R3 respond to many sweet ligands, including sugars, small molecule artificial sweeteners, and protein sweeteners. Both the T1R2 and T1R3 have a large NTD (amino terminal domain) with a cysteine-rich linker domain and a seven-transmembrane-spanning helical region (Lindemann, 2001). The T1R3 can perceive sweet taste alone if the concentrations of sweet compounds are high enough, and it has a well defined "pocket" where smaller molecules may enter and bind. Usually, the T1R3 form heteromers with T1R2 responds to sweet taste, such as artificial sweeteners (Lindemann, 2001). When binding occurs, sweet molecules bind to the NTD of the subunit of T1R2 and/or T1R3 and 7TMD of the

subunit T1R3. The NTD of the subunit of T1R2 and T1R3 is for small molecular weight compounds to bind. The 7TMD of the subunit of T1R3 is for high molecular weight compounds to bind. Therefore the binding sites for all sweet compounds are T1R2 NTD, T1R3 NTD, and T1R3 7TMD. The NTD of the T1R3 subunit binds sucrose more readily. The NTD of the T1R2 subunit binds neotame and aspartame more readily (Meyers and Brewer, 2008). Sucrose binds fairly well to the T1R3 that leads to a sweet sensation in the brain. Saccharin and aspartame binds to the T1R3 much stronger than sucrose, which send more signals to the brain, resulting in a longer lasting sweet taste sensation than sugar.

Sweet stimuli activate taste cells within the taste buds through two transduction pathways. Both of the pathways bind to G protein coupled receptors (GPCRs) that are in the membrane of the taste receptors cell that activates adenyl cyclase, form cyclic AMP (cAMP), and close K^+ channels that lead to deplorization of the cell (Reineccius, 2006). The first pathway is a GPCR-G_s-cAMP where sucrose and other saccharide sweeteners activate G_s by one or more coupled GPCRs. The receptor activated G_{as} activates adenyl cyclase to generate cAMP (cyclic adenosine monophosphate). The generated cAMP may act directly to cause influx of cations through cNMP gated channels or indirectly activate protein kinase to depolarize the taste cell by releasing Ca^{2+}. Depolarization triggers neurotransmitter release that send signals to the brainstem. The second pathway is GPCR-G_q/Gβγ-IP_3; this is the pathway for artificial sweeteners. Artificial sweeteners bind to and activate phospholipase (PLCβ2) to generate IP_3 and diacylglycerol (DAG). IP_3 and DAG draw the release of $Ca^{2+}$and cause cellular depolarization of the taste receptor cells and trigger neurotransmitter release to the brainstem (Reineccius, 2006).

In general, sucrose activates adenyl cyclase, and nonnutritive sweeteners activate IP_3 within the same cell. The intracellular levels of cAMP, cGMP, or IP_3 elevate producing a PKA-mediated phosphorylation of K^+ channels. The flow of K^+ is inhibited, resulting in cell depolarization. Calcium enters the cell through activated Ca^{2+} channels and electric current is produced sending signals to the brain to perceive the taste of sweetness (Reineccius, 2006).

Characteristics of Non-Nutritive Sweeteners

Aspartame, acesulfame K, neotame, and sodium saccharin are chemically derived non-nutritive sweeteners. These non-nutritive sweeteners are not metabolized by the body therefore no calories are being contributed to the diet (Lindsay, 2008). These non-nutritive sweeteners are not carbohydrates, so they do not function as carbohydrate sweeteners. They are unique in their physical and chemical properties, flavor, stability, and sweetness intensity. Because each of these sweeteners have specific characteristics, it is critical that formulators select the best one for their application (Pszczola, 2003). If one non-nutritive sweetener does not meet the quality specification of the product, it is not unusual for formulators to use more than one non-nutritive sweetener to achieve optimum taste and stability. The challenge for product applications that are "reduced calorie" or "sugar free" is being able to replace the sweet taste of sucrose that is clean with no lingering after taste with a non-nutritive sweetener. Generally, non-nutritive sweeteners tend to have a slower onset than sucrose and a lingering after taste that is normally bitter, metallic, astringent or drying (Salant, 1972).

Individual sweeteners have different temporal profiles, and these profiles are perceived differently from the onset of time sweetness is perceived until its duration. Sucrose takes 4.1 seconds to reach maximum sweetness and it lingers for 66.1 seconds. Most of the non-nutritive sweeteners last approximately 10 seconds longer than sucrose (Kuntz, 1995). When the temporal

characteristic of a sweetener system is in sync with sucrose, it is more likely that sweetener system would be acceptable to the palate. In some applications, having longer duration time is positive, such as chewing gums where long lasting sweetness and flavor is product quality (Corliss et al., 2002). However, having a long duration of sweetness in Cha-Cha will result in a negative or undesirable lingering aftertaste. To better understand how these non-nutritive sweeteners will impact the taste and aroma of Florence's HomeStyle Cha-Cha, an outline of their general characteristics are listed:

Aspartame

Aspartame's chemical name is alpha L-aspartyl-L-phenylalanine-1-methyl ester and molecular formula is $C_{14}H_{18}N_20_5$ (Figure 1.1) with a molecular weight of 294.3

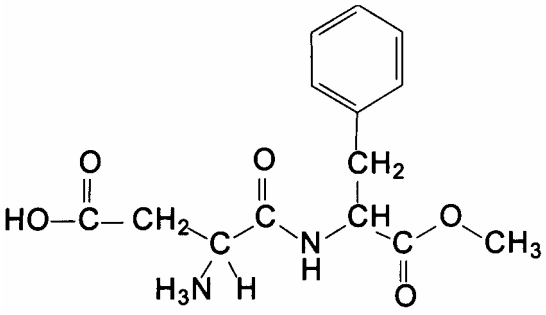

Figure 1.1 Aspartame Chemical Structure

It is a low calorie nonnutritive sweetener that contains 4 kcal/g, but because of its minimal usage level, it contributes no calories to the diet (Kuntz, 1993). It is 180 times sweeter than sucrose and provides no initial bitter aftertaste. Its sweetness has a slower onset and a longer duration than sucrose (Burg, 1998). It is sold under the brand names NutraSweet and Equal (Theodore, 2006). It is broken down in the body into amino acids, aspartic acid, phenylalanine, and small amounts of methanol (Lindsay, 2008). Because of its phenylalanine component, aspartame carries a health risk for people with phenylketonuria. Products containing

aspartame must indicate on the label that the product contains phenylalanine. Because aspartame has two chiral carbons, it has four different configurations when it is synthesized. Only one of the four configurations fits into the receptor site which contributes to the sweet sensation. Two of the other configurations are tasteless, and the other is bitter (Reineccius, 2006). .

Aspartame has a melting point between 246-247 ºC and will decompose at temperatures above 280 ºC. It has a solubility of 1.0 g/100 mL at pH 3.0. Its solubility is dependent upon pH and temperature; the solubility is ideal at pH ranges 3.0 to 5.0 (Lee, 2006). It is sparingly soluble in alcohol and insoluble in fats and oils (Kemp, 2006). Aspartame lowest isoelectric point is at pH 5.2. When aspartame is in solution, its ester bond may be hydrolyzed resulting in a reduction in sweetness. In an application below 8% moisture at ambient temperatures, aspartame is stable for several years. Its maximum stability in an aqueous solution is pH 4.3. Products containing aspartame with pH ranging below 3.0 and above 5.0 should compensate for decomposition during shelf life and increase the inclusion of aspartame in the formulation (Lee, 2006).

Aspartame has a free amino group that reacts with carbonyl-containing food ingredients. Aspartame has a peptide that causes it to be susceptible to hydrolysis causing its taste in sweetness to gradually degrade (Kroger et al., 2006). Being an amine it can react with aldehydes. Aspartame can withstand high-temperatures at a short-time and ultra high temperature processing, such as pasteurization and asceptic processing (Kemp, 2006). At elevated temperature over time, it becomes unstable due to the hydrolysis of either the methyl ester on phenylalanine or the peptide bond between the two amino acids. Aspartame undergoes an intramolecular condensation, especially at elevated temperatures to yield diketopiperazine (5-benzyl-3,6-dioxo-2-piperazine acetic acid). This reaction normally occurs at neutral and alkaline

pH because non-protonated amine groups on the molecules are more available for reaction under these conditions (Lee, 2006).

Under acidic and alkaline pH, aspartame is unstable and rapidly degrades to the rate where sweetness will gradual be lost (Lindsay, 2008). For this reason aspartame is not ideal for cooking and baking. The shelf life of aspartame can be prolonged between nine months to a year when used in combination with other non-nutritive sweeteners, such as acesulfame K, a more stable sweetener (Tragash and Tomiyama, 2005). Aspartame can be encased with fats or maltodextrin to improve shelf-life. An encapsulated version of aspartame is available for use in baked products to enhance its stability properties (Kroger et al., 2006).

Aspartame enhances fruit flavors in food and beverage products. It used in many fruit flavored syrups. Aspartame under specific conditions can generate brown and slight roasted notes by its amine group, initiating Maillard reaction conditions. These conditions can be favorable for some applications and not others (Kroger et al., 2006). Therefore, some formulators use aspartame instead of other non-nutritive sweeteners to generate desirable notes for applications requiring roasted/savory flavors (Kuntz, 1995).

The United States Food and Drug Administration (USFDA) approved aspartame in 1981 for regulatory use in dry foods. In 1983, it was approved for use in carbonated beverages, and in 1996 it was approved as a general purpose sweetener for use as a food additive. This allowed aspartame to be used in all categories of foods and beverages. Its acceptable daily intake (ADI) is 50.0 mg/kg of body weight per day. This is the estimated amount a person can safely consume on average per day over a lifetime without a risk (Kroger et al., 2006).

Acesulfame K

Acesulfame K is the potassium salt of 6-methyl-1,2,3-oxathiazin-4(3H)-one dioxide (Figure 1.2). Its molecular formula is $C_4H_4KNO_4S$ and has a molecular weight of 201.2.

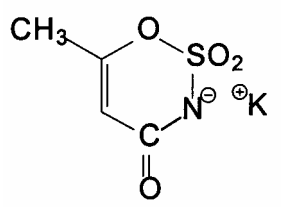

Figure 1.2 Acesulfame K Chemical Structure

It is a non-caloric sweetener that is 200 times sweeter than sucrose. Its brand names are Sunett, Sweet One, and Sweet n' Safe. It has a chemical structure and taste profile that is similar to saccharin (Lee, 2006). Its temporal profile is rapid and carries its flavor well (Burg, 1998). It has a melting point of 225 ºC, and a solubility of 27.0 g/100mL of water (Linden and Lorient, 1999). Acesulfame K is not metabolized by the body, but it is excreted unchanged, and its potassium does not have an influence of the potassium that is intake by the body (Kroger et al., 2006). Acesulfame K is sweet, but presents a bitter aftertaste when used as a single sweetener at high concentration levels (Lindsay, 2008). Acesulfame K blended with other non-nutritive sweeteners mask bitterness providing an acceptable product (Lindley, 2004). Despite the bitterness, acesulfame K has good stability properties with no deterioration in sweetness over time. It is heat stable at various temperatures with wide pH ranges (Meyer and Riha, 2002). Its stability properties are good where it can be added to products before and during heating, cooking, extrusion, and spray drying processing (Kemp, 2006). When acesulfame K is blended with sweeteners with poor stability issues, such as aspartame; acesulfame K minimizes the stability problems, making the product more stable for storage (Tragash and Tomiyama, 2005). In addition to blending acesulfame K with other sweeteners to mask bitterness, some blends with acesulfame K may offer mouthfeel and perceived to be thick, full of texture, and flavor (Meyer

and Rhia, 2002). Acesulfame K is more beneficial to be used in combination with other sweeteners than to be used alone (Rorie, 2006). Acesulfame K can be found in pharmaceuticals, oral hygiene products, and foods (Pszczola, 2003). It is considered to be a general purpose sweetener that is used in combination with other sweeteners because of its good stability properties (Kroger et al., 2006).

The United States Food and Drug Administration (USFDA) approved acesulfame K in 1988 for regulatory use in a variety of dry food products. At that time, the Joint Expert Committee on Food Additives (JECFA) approved its acceptable daily intake (ADI) for 9.0 mg/kg per day; by 1991 it increased to 15.0 mg/kg per day. Subsequently, it was approved as a sweetener for other types of foods and alcoholic beverages. In 1998, the USFDA approved acesulfame K for use in nonalcoholic beverages (carbonated and non-carbonated). In 2000, the European Union's Scientific Committee on Food (SCF) reaffirmed it is a safe sweetener for use. In 2003, the USFDA approved its use as a general purpose sweetener, so it can be used as a food additive in all categories of foods and beverages (Kroger et al., 2006).

Neotame

Neotame is a non-caloric sweetener that is 6000 times sweeter than sugar. It is the sweetest, no calorie non-nutritive sweetener available on the market. Its chemical name is N-[N-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1 –methyl ester and molecular formula is $C_{20}H_{30}N_2O_5$ (Figure 1.3) with a molecular weight of 378.5 (Corliss et al., 2002).

Neotame is a derivative of a dipeptide made up of aspartic acid and phenylalanine, but the potential release of phenylalanine is so limited that a PKU warning label is not needed (Pszczola, 2003). Neotame delivers a clean, sweet taste similar to that of sucrose with no

aftertaste. It has a slower onset time and a longer linger time than sucrose. Its temporal profile is closer to that of aspartame.

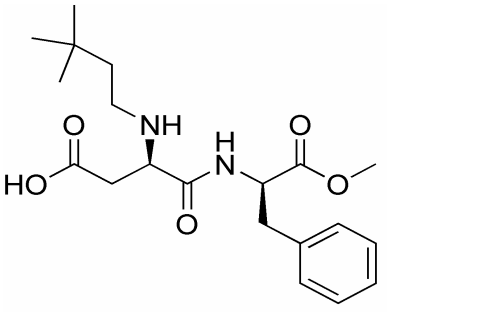

Figure 1.3 Neotame Chemical Structure

Neotame has a structure that is similar to aspartame in that two of the terminal nitrogen of aspartame is substituted with hydrophobic groups, 3,3-dimethylbutyl group. It has better stability properties than aspartame due to the N-substitution which prevents formation of the diketopiperazine derivative making it more stable in baking (Kemp, 2006). Although neotame is similar to aspartame, it is 40 times sweeter than aspartame, but once its methyl ester is hydrolyzed, its sweet taste is lost (Lee, 2006).

Neotame has a carboxylic acid and a secondary amino group with pKa values of 3.03 and 8.08 respectively. Its solubility in water is 1.3 g/ 100mL @ 25 ºC and is more soluble in solvents than aspartame. Neotame is stable at elevated temperatures, and it is ideal for cooking, baking, and canning. The major degradation product formed from thermal process is the de-esterified neotame by hydrolysis of the methyl ester group and it is not sweet in taste (Corliss et al., 2002).

Neotame has functional properties that enhance the flavors of mint, vanilla, berry, and citrus flavors, and it has been known to complement the flavor of root beer soda (Gallo-Torres, 2005). Neotame provides a mouthfeel which enhances the sweetness palatability of fruit juices. It also masks undesirable notes, such as bitterness, astringency, and cooling sensations that are

often found in products containing vitamins, nutraceuticals, pharmaceuticals, and soy ingredients. Neotame reduces the bitter taste of potassium chloride in salt substitutes and eliminates beany flavor notes in soy products. It is compatible with reducing sugars, aldehydes, and ketone flavoring agents (Corliss et al., 2002).

The United States Food and Drug Administration (USFDA) approved neotame in 2002 as a general purpose sweetener. In 2004, the JECFA established an ADI of 2.0 mg/kg per day. The ADI in the United States is 18.0 mg/kg per day. It is approved for use on all food products (Kroger et al., 2006).

Saccharin

Saccharin is the least expensive and the first artificial sweetener discovered. Chemically, it is known as 2,3 dihydro-3-oxobenzisosulfonazole.

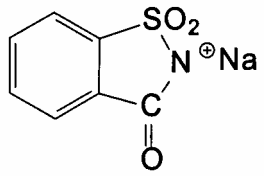

Figure 1.4 Sodium Saccharin Chemical Structure

It is 300 times sweeter than sucrose and is available in granular and liquid forms (Kroger et al., 2006). Its brand names are Sweet N Low, Sweet Twin, and Necta Sweet (Artificial Sweeteners: No Calories ….Sweet, 2006). Like many of the other non-nutritive sweeteners, it goes through the body without being digested or calories exchanged. Saccharin presents a pleasant onset taste, but it is followed by a lingering, bitter aftertaste with a thin mouthfeel. Sensitivity to bitterness can vary from person to person, but at high concentrations of saccharin, most people can detect the unpleasant bitter aftertaste (Lee, 2006).

Saccharin is a sulfimide, and it is normally sold as acid saccharin, and sodium or calcium salt. The chemical name for sodium saccharin is 1,1 dioxo-1,2-benzothialzol-2-id-3-one (Figure 1.4). Its molecular formula is $C_7H_4NNaO_3S$ with a molecular weight of 205.2. The sodium salt is commonly sold on the market, but people with sodium restrictions normally use the calcium salt form of saccharin. The acid form of saccharin is also sold in the food industry. The salt forms of saccharin are more stable in water than the acidic forms; the salt form of saccharin solubility is 12.8g/ 100 mL of water. It has a melting point between 228-229 ºC and will boil at 300 ºC; whereas, a sweetener like aspartame will decompose at these temperatures. Saccharin has a moisture range of 4.5-5.8% (Salant, 1972). It has good stability properties over elevated temperatures and wide pH ranges; therefore ideal for cooking, baking and canning and does not chemically react with other ingredients (Lee, 2006). Saccharin delivers a sweet taste at diluted concentrations; however, at high concentrations it delivers a bitter taste due to its sulfur bond (Linden and Lorient, 1999). Compounds, such as tartaric acid, dipeptides, and gluconates can help reduce the bitter after taste of saccharin, as well as other non-nutritive or nutritive sweeteners (Kuntz, 1995). Saccharin is used as a tabletop sweetener in pharmaceuticals, mouthwash, baked goods, soft drinks, jams, and chewing gums. Because of its stability properties, saccharin is used widely in diet fountain sodas (Kemp, 2006).

Saccharin is the oldest non-nutritive sweetener. It was discovered in 1878 prior to the regulations by the USFDA. Saccharin was classified as GRAS, “generally recognized as safe” on the basis of scientific testing and the history use of the food. In 1972, the USFDA removed it from the GRAS list due to studies that showed saccharin caused bladder cancer in rats. By 1977, USFDA proposed warning labels to be placed on packages with foods containing saccharin. From 1977 to 2000 many studies took place on its safety. In December 2000, Congress passed

legislation to remove the warning labels. Its current regulatory use is permitted for use under an interim regulation that specifies the amount saccharin permitted in beverages, processed foods, and sugar substitutes. The label must state saccharin in the ingredient declaration and indicate the amount used.

Each of these non-nutritive sweeteners deliver a sweet taste, but they can be differentiated by their sweetness intensity, temporal onset, and lingering effects. The non-nutritive sweetener in each treatment (Cha- Cha with a sweetener) replaced the sucrose in Cha-Cha at the sucrose equivalency level to mimic the taste of sweetness in the control (Cha-Cha sweetened with sucrose). Based on the characteristics of the non-nutritive sweeteners, all the sweeteners are sweet with at least a slightly clean taste, but their temporal profile is different and degree of lingering aftertaste differs. Therefore, the ideal sweetener should have a rapid temporal profile, one that is closer to that of sucrose and have good stability properties. The characteristics of some of these non-nutritive sweeteners are not stable, so the ideal must be stable in a low pH system and under pasteurization temperatures, since Cha-Cha has a pH of 3.5 and is thermal processed at a pasteurization temperature.

Sucrose

Sucrose is a carbohydrate, nutritive sweetener that is metabolized by the body yielding energy of approximately 4 kcal/g. Sucrose has a molecular formula of $C_{12}H_{22}O_{11}$ with a molecular weight of 342.3 (Figure 1.5).

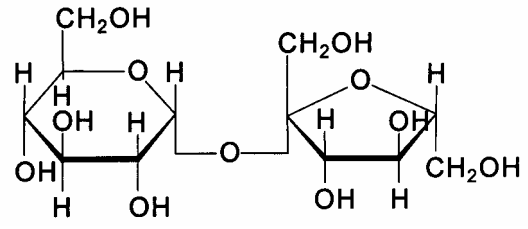

Figure 1.5 Sucrose Chemical Structure

Sucrose is commonly known as table sugar. Throughout this research, "sugar" will be referred to as sucrose. Sucrose is a disaccharide composed of α –D-glucopyranosyl unit and a β-D-fructofuranosyl unit that are linked head-to-head (reducing end to reducing end linkage) and not head-to-tail linkage. Because it has no reducing end, it is not a reducing sugar, so it does not take part in Maillard type reactions (Lindsay, 2008). However, when sucrose is hydrolyzed, an equimolar of glucose and fructose are released allowing these reducing sugars to take part of Maillard type reactions. Inversion, hydrolysis of sucrose, can occur with an enzyme or by a low pH system at elevated boiling temperatures. The hydrolysis increases the sugar solids and yields an invert sugar (Kemp, 2006). The invert sugar is sweeter and yields moisture and lowers freezing point in products (Linden and Lorient, 1999). However, after the inversion process, many reactions can occur depending on the pH and temperature conditions. In an acidic medium, 5-hydroxymethyl furfural is formed which rapidly decomposes into dark-colored compounds with off flavors. In an alkaline medium, lactic acid is formed and glucose is rearranged to a mixture of mannose and fructose. In a medium with α-amino acids, the invert sugar participates in Maillard reactions in which dark color compounds and flavors are generated (Colonna et al., 2001).

A molecule of sucrose has eight hydroxyl groups, three hydrophilic oxygen atoms, and fourteen hydrogen atoms which contribute to the formation of hydrogen bonds with water molecules making it readily soluble in water. Its solubility is at 20 ºC is 67g/ 100 mL of water (Linden and Lorient, 1999). When sucrose is soluble in water, the interaction of sucrose with water molecules influence the partition behavior of some aroma compounds and it reduces the vapor pressure of hydrophobic aroma compounds. The interaction of sucrose with water makes the system more hydrophobic and result in more solubility to lipophilic odorants. If sucrose is

greater than 40% in solution, there may be a "salting out" effect. This effect decreases the amount of free water in the food matrix which increases the concentration of flavor compounds available in free water that affects the equilibrium of the volatile compounds in the gas phase (Delarue and Giampaoli, 2006). The "salting out" increases lipophilic aroma components that leads to imbalance aroma compounds that effect the flavor character being perceived (Reineccius, 2006).

Sucrose melts and decomposes at 186 ºC to form caramel (Colonna et al., 2001). When sucrose is heated in the dry state at or above melting point temperatures, it turns pale yellow, amber, orange brown, red brown, then to a very dark brown before foaming and carbonization that yields a black residue. These thermal conditions not only have an impact on the color of sucrose, but it also affects its taste. Sucrose that is heated under thermal conditions to a amber and medium brown color will have a bitter and sweet taste. Burnt sucrose produces carbon dioxide and water. The main volatile compound of caramel from sucrose product is 5 – (hydroxymethyl)-2-furfuraldehyde (Davis, 1995).

The functionality of sucrose has an impact on the appearance, color, texture, and shelf life of foods. It adds body to finished products that enhances the flavor to be more pronounced and rounded (Knehr, 2005). The body it adds contributes to the richness and fullness of finished products. A lack of thickness may cause problems during processing and functionality of the product. Sucrose contributes to the perceived texture of foods in thickness, crunchiness, and crispness. It contributes to perceived appearance of food in color and surface appearance, such as a shiny or glaze appearance (Kemp, 2006). Sucrose aids in browning, increases viscosity, adds bulk, provides mouthfeel, and enhances sweetness (Davis, 1995). The sweetness it provides is quick and clean followed by a sharp cut-off without a lingering after taste. Since sucrose is the

standard in which non-nutritive sweeteners are compared to, their molecular weights and complexity of their chemical structure must be comparable to sucrose in order to achieve a quick onset in sweet taste as sucrose. Molecular size and complexity of a sweetener's chemical structure impacts the time it takes to the maximum intensity of sweetness. The molecular mass of sucrose is 342.2, and its onset in perceiving sweetness is quick (Frank, 2006). Sucrose sweetness threshold in solution is 0.2-0.5% and its highest sweetness intensity ranges between 32-38 ºC (Colonna et al., 2001).

CHAPTER 2 - Processing Cha-Cha with Sweeteners

Materials and Ingredients for Processing Cha-Cha

Florence's HomeStyle Cha-Cha was produced in five beakers; one beaker was the control that contained sucrose as the sweetener, and the other four beakers contained maltodextrin and either aspartame, acesulfame K, sodium saccharin, or neotame as its sweetener system. Only one replication of these five sweetener treatments was produced. The ingredients were added to the beakers and the mixture was thermal processed, homogenized, and packaged. All the non-nutritive sweeteners were supplied by Univar (Redmond, WA.). The maltodextrin was supplied by Grain Processing Corporation (Muscatine, IA.).

The ingredient composition consisted of green tomatoes, jalapeño peppers, serrano peppers, and onions that were individually diced, then weighed in their proper proportions and added to each beaker. The cabbage was shredded and weighed in its proper proportion to the beaker. The dicing and shredding of the vegetables were processed with a Hamilton Beach food processor. All the vegetables were purchased from Schnucks, a local grocery store in St. Louis, MO. The liquid ingredients (apple cider vinegar, water, and 40 dextrose equivalent - high fructose corn syrup) were weighed to their respective proportions and added to the beaker. The apple cider vinegar was supplied by Fleischmann's Vinegar (Santa Fe, CA.). Isoclear 42 high fructose corn syrup was supplied by Cargill (Eddyville, IA.). The liquid ingredients were mixed in the beaker with the vegetables. The dry ingredients (salt, red pepper, and special spices) were weighed to their respective proportions and added to the beaker with the vegetables and the liquid ingredients. Salt was purchased from Schnucks, a local grocery store in St. Louis, MO., and the special spices were provided by Florence's HomeStyle, Inc (St. Louis, MO.).

The maltodextrin and sweetener system are added last. The control contains no maltodextrin, so sucrose is added to this mixture prior to thermal processing. The maltodextrin and non-nutritive sweeteners are added last to the batch of the experimental treatments prior to thermal processing.

Being that these sweeteners are of high potency, their inclusion levels are less than sucrose, but the levels used in this experiment were the suppliers recommended sweetness equivalency level to that of sucrose. The sucrose equivalency is the amount of sweetener used to be equivalent in sweetness to a 10% solution of sucrose. For instance, it takes 1/200 of sucrose in weight to obtain the same sweetness intensity as sucrose (Meyer and Rhia, 2002). The inclusion level of sucrose used in the Florence's HomeStyle Cha-Cha is 7.7% (see Table 2.1). However, each of the non-nutritive sweeteners inclusion levels are less than 7.7%, so maltodextrin-M100 (Grain Processing Corporation, Muscatine, IA) was used as a filler to increase the remaining quantity to 7.7%. Maltodextrin-M100 has a DE range of 9-12; it was chosen for this application because of its inability to contribute to sweetness, ability to build solids, and it is commonly used as a filler in the industry when a non-nutritive sweetener is replaced with sucrose.

The appropriate amount of non-nutritive sweetener needed to match the sweetness equivalency to that of sucrose was determined by dividing 7.7% sucrose by the concentration level of the non-nutritive sweetener (7.7/200 concentration of aspartame). The appropriate amount of sweetener was added to the mixture of Cha-Cha last, after the maltodextrin addition and prior to thermal processing. The aspartame sweetened treatment consisted of 0.038% aspartame (aspartame is 200X sweeter than sucrose based on a 10% solution) and 7.64% maltodextrin. The acesulfame K sweetened treatment consisted of 0.038% acesulfame K (acesulfame K is 200X sweeter than sucrose) and 7.64% maltodextrin . The sodium saccharin sweetened treatment consisted of 0.025% sodium saccharin (sodium saccharin is 300X sweeter

than sucrose) and 7.65% maltodextrin. The neotame sweetened treatment consisted of .001% neotame (neotame is 6000X sweeter than sucrose) and 7.66% maltodextrin (See Table 2.1 - Cha-Cha formulations). The control consisted of 7.7% sucrose and no maltodextrin.

Table 2.1 Cha-Cha Formulations with Sucrose vs Non-Nutritive Sweeteners

Sweetener Systems	Sucrose	Aspartame	Acesulfame K	Sodium Saccharin	Neotame
Sucrose	7.7%				
Aspartame		0.038%			
Acesulfame K			0.038%		
Sodium Saccharin				0.025%	
Neotame					0.001%
Maltodextrin		7.64%	7.64%	7.65%	7.66%

Each experimental and control batches were allocated the same thermal process conditions using digital computerized hot plates (Dataplate PMC 720 series; Barnstead International; Dubuque, IA.) to maintain heating and cooling consistencies. After thermal processing, the products were cooled to 160°F and analyzed for density. The treatments were then homogenized with a high sheer mixer, Silverson L4R (Silverson Machines; East Long Meadows, MA.) into a homogenous treatment and packaged in labeled glass vials for gas chromatography/mass spectrometry (GC/MS) and sensory analyses. The pH of each treatment was measured after thermal processing and cooling at 160 ºF with a Denver Instrument Ultra Basic – pH probe model UB-5 (Denver Instruments; Denver, CO.). The pH was measured on each treatment (aspartame, acesulfame K, neotame, sodium saccharin, and sucrose – sweetened treatments). Since this is a low pH product, the expected shelf-life is approximately 6 months at room temperature. This is based on color change that is observed on the finished product and not

an accelerated storage test. Below are pictures of Cha-Cha being produced during the heat treatment phase to homogenization with the Silverson (Figure 2.1 – 2.3).

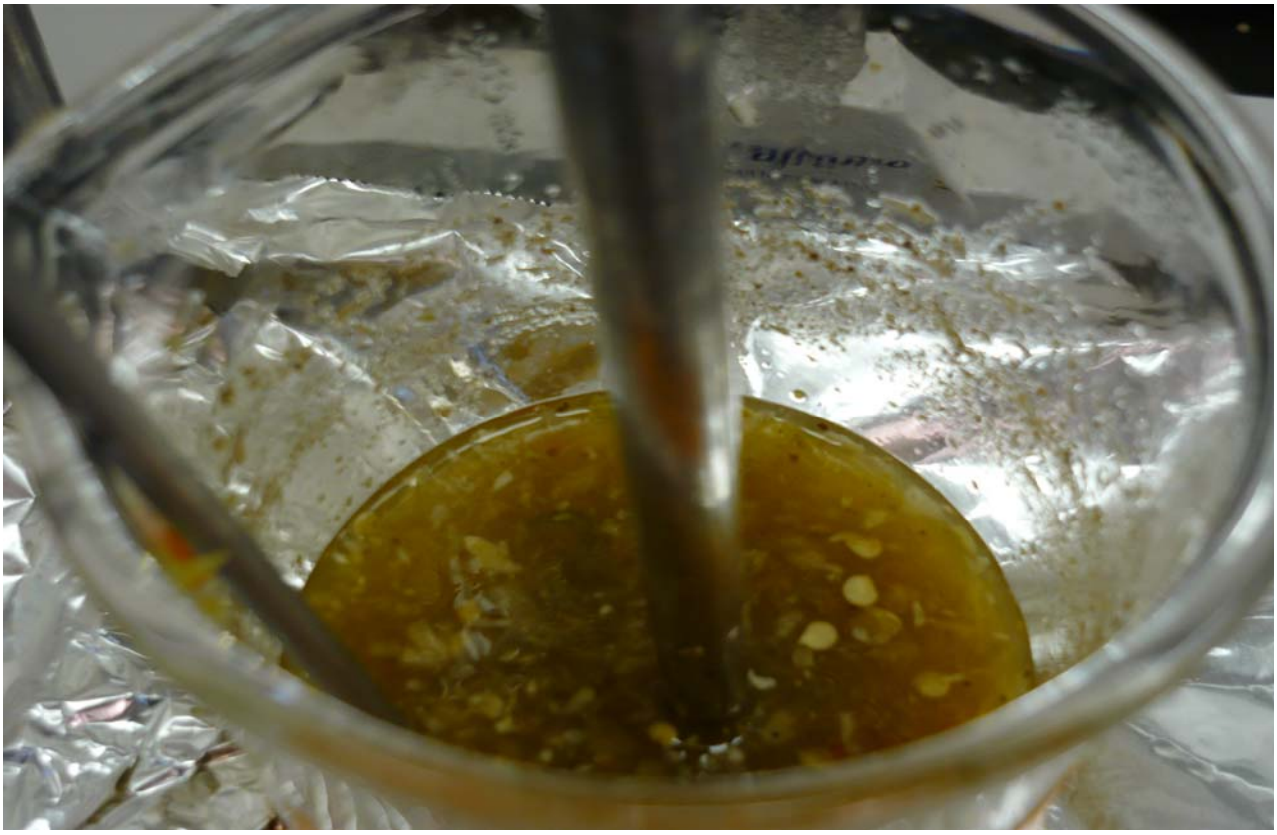

Figure 2.1 Cha-Cha with Aspartame Prior to Thermal Processing.

Figure 2.2 Cha-Cha with Aspartame after Thermal Processing (Finished Product)

Figure 2.3 Finished Product of Cha-Cha Homogenized for Gas Chromatography/Mass Spectrometry and Sensory Analysis

Quality Assurance of Incoming Raw Materials

Incoming control points for raw materials are important for product quality, safety, and consistency. All incoming raw materials are checked to assure they meet their product specifications. In addition to the raw material quality checks, the packaging is checked for damages and the freight is checked for temperature, pests, filth, and odors.

The incoming raw vegetables for Cha-Cha are individual quick frozen (IQF). Upon arrival, they are checked to assure the temperature is < -20ºC. They are ordered a couple days prior to production and are stored in a freezer until processed. All the raw are checked for sensory analysis in color, flavor, and particle size. For instance, the specifications for acceptable incoming raw material for green tomatoes would be green in color with no more than 2% tomatoes orange, a dice size of ¼", and flavor of a tart tomato taste. A shipment of green tomatoes that would be reject based on the specifications would consist of 2% or more of green tomatoes that are orange or red or a dice size larger than ¼", and a tomato with an off odor or flavor. Another basis for rejecting the tomatoes would be inadequate temperature of product due to freight temperature which may have induced microbial growth.

Jalapeno and serrano peppers also have incoming sensory specification that includes color, heat, flavor, and particle size. For the shipment to be accepted, they both have particle specification of a dice size of 3/8". The color of the jalapeno should be medium to dark green and the serrano should be light green. The Scoville heat units for jalapeno should be 2,500 to 3,500 and the serrano should be 5,000 to 6,000. The jalapeno should have a fresh jalepeno flavor and the serrano should have a fresh serrano flavor.

Dry ingredients, such as salt is packaged in 50lb. bags with polyethylene liners in kraft boxes. The incoming lot is checked for tears in the liners and damage to the boxes. A certificate of analysis with percent of sodium chloride and calcium are listed. An acceptable lot would have sodium chloride level of 99.0% to 99.9% and a calcium level of 257.0 ppm. Any calcium level above 300.0 ppm would be rejected.

Lastly, apple cider vinegar has chemical and physical properties for acceptance on incoming raw materials. An acceptable lot of the product would have an acidity (acetic acid) of 50 grain and the color should range from light to medium amber.

Methods for Sensory and Analytical Analysis

Sensory Analysis

The experimental treatments and the Cha-Cha control were placed in coded vials. The treatments were served a week later after they were stored at room temperature after processing. They were served at room temperature and in numerical order based on the codes listed on the vials. Each of the eight panelists received napkins, a pencil, bottled water, pipette, spit cup, and test ballots. Several days prior to testing, the panelists were trained on evaluating the optimal level of sucrose in Cha-Cha. The panelists were offered 4 samples with different levels of sucrose, one sample was the control (containing 7.7% sucrose). The panelists were able to distinguish between the four samples, the Cha-Cha with the correct level of sucrose. Water was used to help clean palates between evaluations in the training and in this study. Serving size was 50 g; each panelist sampled 10.0 g with a 10 mL pipette.

Density Measurement

The density of each treatment was evaluated using a 10 mL graduated cylinder that was tarred on a scale. Each treatment was filled to the graduated cylinder's 10 mL mark. The weight of the treatment was recorded in grams. Density was calculated as weight (mass) divided by volume g/mL.

Solid Phase Micro Extraction and Gas Chromatography/Mass Spectrometry of Volatile Aroma Analysis

A Varian Star 3600 Gas Chromatography – Mass Spectrometer (Walnut Creek, CA.) was used for the analyses of these treatments. It has an attached CombiPal autosampler with Solid

Phase Micro Extraction (SPME) option, and a heat block for treatment conditioning and extraction at different temperatures.

Five 10 mL headspace vials from VWR International (West Chester, PA.) were labeled with the experimental treatment number and the control. An amount of 2.0 g of each treatment were weighed in the 10 mL vial, capped, before loading onto the autosampler tray. Treatments were conditioned in a heat block set at 53 ºC for ten minutes, followed by extraction of the volatile components onto the SPME fiber 2 cm -50/30 µm Divinylbenzene/Carboxen Polydimethylsiloxane (DVB/CAR/PDMS) from Sigma-Aldrich (St. Louis, MO.) for forty minutes. The volatiles were desorbed into the GC injector maintained at 250 ºC for six minutes.

Treatments were analyzed on the DB-5 column (60 m x 0.32 mm x I.D. 0.5 µm film thickness) from J & W Scientific Inc. (Folsom, Calif.). The temperature programming conditions were as follows. Initial temperature of the column was set at 40 ºC and held for two minutes. The column was then heated to 80 ºC at a rate of 2 ºC per minute, and then held 80 ºC for two minutes before heating up to 250 ºC at a rate of 5 ºC per minute. At this temperature, the column was held for ten minutes before the end of the run.

Chromatograms obtained from the analysis were analyzed using the Varian Star Software (version 6.42). The peaks shown on the chromatogram were later identified using the NIST (National Institute of Standards and Technology Mass Spectral Library Version 2005) and AMDIS (Automated Mass Spectral Deconvolution and Identification System Version 6.2) Libraries (Agilent Technologies, Inc., Santa Clara, Calif.). A difference analysis was conducted to identify differences in volatile compounds between the control and the experimental treatments.

Sensory Analysis Procedures

Eight trained panelists who were trained only on Cha-Cha were selected to evaluate the intensity of sweetness, saltiness, and bitterness in the experimental treatments. The panelists were very familiar with the flavor and aroma characteristics of Cha-Cha and have over 20+ years experience in making this product in their kitchens. The panelists were a mixture of males and females whose ages range from 39-71. Four of the panelists were female siblings ages 63-70. All of the panelists have consumed products containing non-nutritive sweeteners, such as diet drinks, ice cream, and candy bars.

The panelists were instructed not to eat or drink one hour before testing, expectorate the treatment after tasting, and rinse their mouths with water to clean palate between treatment evaluations. The panelists compared the experimental treatments to the control and evaluated the intensity of sweetness, saltiness, and bitterness, using an interval scale of 1.0-9.0. The interval scale value of a 5.0 indicated the treatment is the same as the control, 6.0-9.0 indicated that the treatment was more intense than the control, 1.0-4.0 indicated that the treatment was less intense than the control, and 0 indicated there was no taste attribute perceived. The panelists recorded their results on a score card ballot.

The panelists tasted the experimental treatment, rinse their mouths, then tasted the control treatment, and finally, determined the degree of intensity of taste characteristics between the two treatments. In the first evaluation, the panelists evaluated the experimental treatment and the control to determine if the intensity of sweetness was the same or different than the control. The second analysis evaluated the intensity of saltiness. The panelists determined if the intensity of saltiness was the same or different than the control. The third analysis evaluated the intensity of bitterness. All analyses were conducted once, and no repetitive testing was conducted for confirmation.

Sensory Analysis Results: Intensity of Sweetness, Saltiness, and Bitterness

The sensory evaluation measured the intensity of sweetness, saltiness, and bitterness in the experimental treatments versus the control. The intensity is measured on an interval scale ranging from 1.0 – 9.0. The control treatment (Cha-Cha sweetened with sucrose) on this scale is 5.0. This indicates any experimental treatments close to 5 is closer in the taste attributes evaluated to the control. In the first evaluation, the perception of sweetness showed that Cha-Cha sweetened with acesulfame K was more similar in sweetness to the control (sucrose sweetened Cha-Cha) than the other treatments evaluated. The acesulfame K interval mean value was 5.6, which indicates this treatment is similar in sweetness to the control. An interval value of a 5.0 indicates that the treatment is the same as the control and any value above or below the 5.0 value is different based on how far that interval value is from 5.0. In this evaluation, acesulfame K was the only experimental treatment that had an interval value close to 5.0. This data set range was 3.0, indicating the interval value chosen by the panelists was from 4.0-7.0, but the mode for this data set was 5.0, which indicates the panelists chose this interval value the most. Three out of the eight panelists rated this treatment to be similar to be the control. Literature indicates acesulfame K is often used with other non-nutritive or nutritive sweeteners to improve taste characteristics and stability, and provide sweetness synergy (Meyer and Rhia, 2002). It can be suggested there is synergistic sweetness potency and improved temporal flavor with acesulfame K and high fructose corn syrup (ingredient in Cha-Cha) that creates a taste similar to that of sucrose.

The neotame sweetened treatment was the sweetest and noticeably different in taste than the control and the other treatments. All of the panelists indicated this treatment had a strong lingering aftertaste and the taste was displeasing. The treatment mean of the interval values for this data set was 9.0, and the range was 0, which indicates 100% of the panelists chose this

product to be much sweeter than the control. Unlike other sweeteners, neotame can reach its maximum sweetness intensity (plateau) of 15.1% sweetness equivalency in water; whereas aspartame, acesulfame K, and sodium saccharin attain their maximum sweetness intensity in water at 16.0%, 11.6%, and 9.0% sweetness equivalency. This was established by an outside panelist in a separate study that measured the concentration-response curve for neotame (Corliss et al., 2002). Due to the sweetness potency of neotame, it is blended with acesulfame K, maltodextrin, or maltitol or a combination of the three to balance the sweetness for applications. Univar Inc. markets a blend of neotame, acesulfame K, and maltitol named SucraSweet HIS 600 (Gallo-Torres, 2005).

The other two treatments sweetened with aspartame and sodium saccharin had an interval value of 6.5 and 6.3, respectively. Both of these treatments were shown to be sweeter than the control, but not as sweet as the neotame sweetened treatment. However, the aspartame treatment range was much smaller, in that the interval range was from 5-7. See Figure 2.5 and Table 2.2 of the perception of sweetness of the four treatments evaluated versus sucrose sweetened treatment.

Table 2.2 Sensory Evaluation – Intensity of the Perception of Sweetness

N = 8	Aspartame	Acesulfame K	Sodium Saccharin	Neotame
Mean	6.5	5.6	6.4	9.0
Standard Deviation	0.76	1.1	1.5	0
Mode	7.0	5.0	5.0	9.0
Range	2.0	3.0	4.0	0

* sucrose control = 5.0

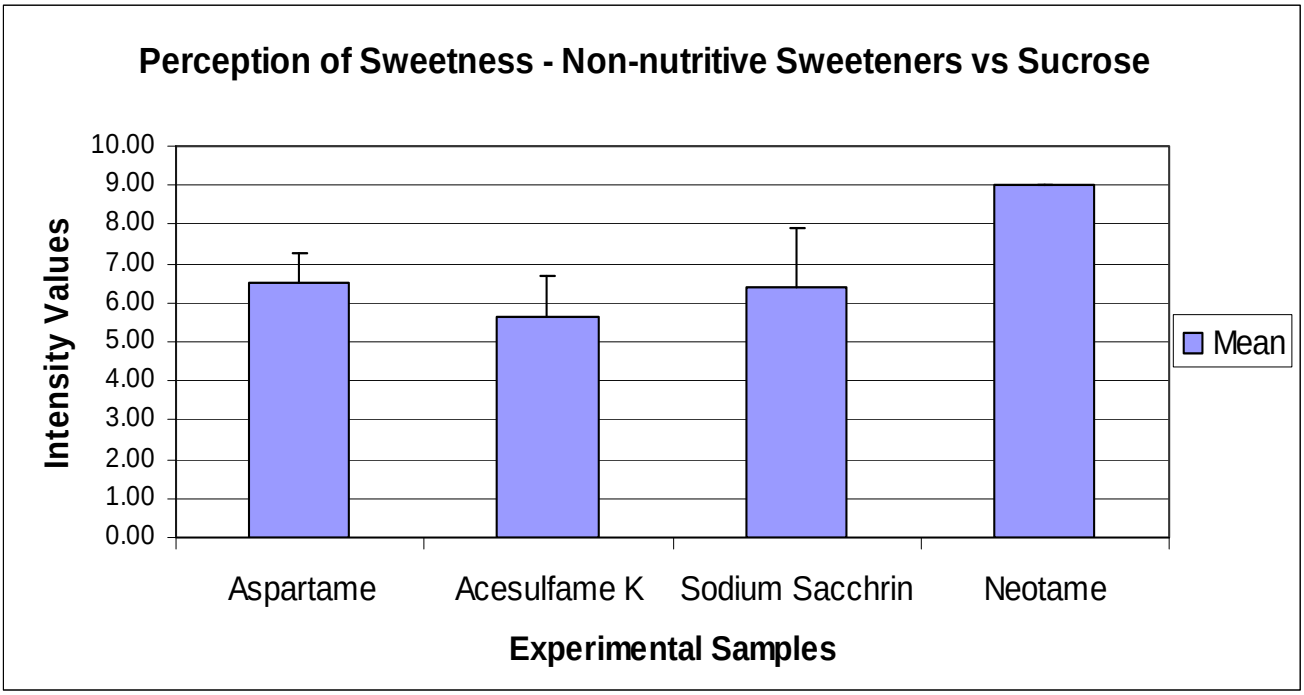

Figure 2.4 Sensory Evaluation – Intensity of the Perception of Sweetness
Control = intensity value of 5.0

The sensory evaluation for saltiness showed that the aspartame sweetened treatment was more similar in saltiness to the control than the other treatments. The treatment mean of the interval value for this data was 5.48. It had a lower standard deviation than the other treatments (0.74). The treatment range for this data set was 2.0, indicating that the panelists selected interval values between 4.0- 6.0. The mode for this data set is 6.0, which indicates that the interval value of 6.0 was chosen the most. The treatment perceived to be the saltiest was the neotame sweetened treatment. However, its range was 4.0, indicating that panelists chose interval values between 5.0-9.0, but the mode was 9.0 indicating the panelist chose 9.0 the most. With acesulfame K, sodium saccharin, and neotame having an interval value greater than 5.0, they were perceived to be saltier than the sucrose-sweetened control; see Figure 2.6 and Table 2.3.

Table 2.3 Sensory Evaluation – Intensity of the Perception of Saltiness

N = 8	Aspartame	Acesulfame K	Sodium Saccharin	Neotame
Mean	5.4	6.1	6.0	7.2
Std. Dev.	0.74	1.2	1.2	1.7
Mode	6.0	5.0	5.0	9.0
Range	2.0	2.0	2.0	4.0

* control = 5.0

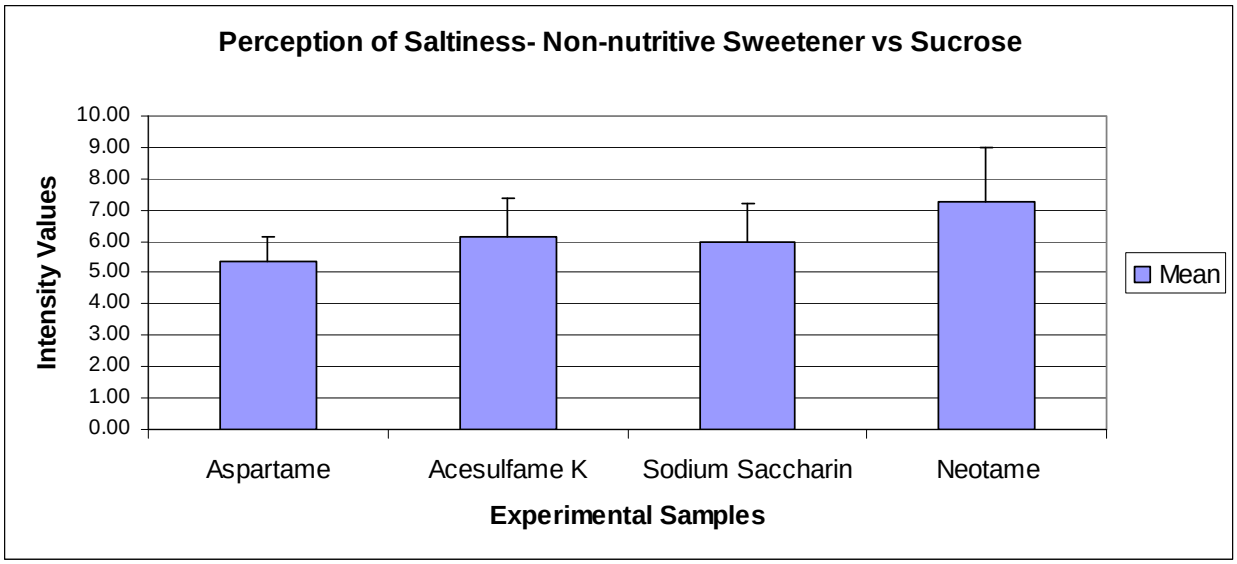

Figure 2.5 Sensory Evaluation – Intensity of the Perception of Saltiness
Control = intensity value of 5.0

The sensory evaluation for bitterness showed 100% of the non-nutritive sweetener treatments were bitter than the control. The acesulfame K treatment was perceived to be more similar to the control than the other treatments. The neotame treatment was perceived to more bitter than the control and the other non-nutritive sweeteners. The acesulfame K treatment resulted in an interval mean of 5.7, and a range of 2.0 indicating that the panelist chose interval values between 5.0- 7.0. The neotame sweetened treatment had a data mean of 7.6, which indicated that the treatment was intensely bitter than the control. The data range for the neotame treatment was 4.0, which indicates that the panelists chose interval values between 5.0 -9.0. The

aspartame, acesulfame K, and sodium saccharin sweetened treatments, all had a mode of 5.0 indicating more panelists selected this value the most. Although the results showed differences, there was no difference in pH between the treatments (Cha-Cha with sweeteners) that could contribute to these results; pH 3.5 was measured on each treatment. Based on the literature, bitterness is a taste characteristic that is normally associated with non-nutritive sweeteners. Non-nutritive sweeteners tend to not have a clean taste similar to sucrose. Due to this issue, non-nutritive sweeteners are normally used in combination with other nutritive and non-nutritive sweeteners to mask the taste perception of bitterness (Lindsay, 2008) (Figure 2.7 and Table 2.4).

Table 2.4 Sensory Evaluation – Intensity of the Perception of Bitterness

N = 8	Aspartame	Acesulfame K	Sodium Saccharin	Neotame
Mean	5.9	5.7	5.9	7.6
Std. Dev.	1.4	1.0	1.0	1.7
Mode	5.0	5.0	5.0	9.0
Range	4.0	2.0	2.0	4.0

* control = 5.0

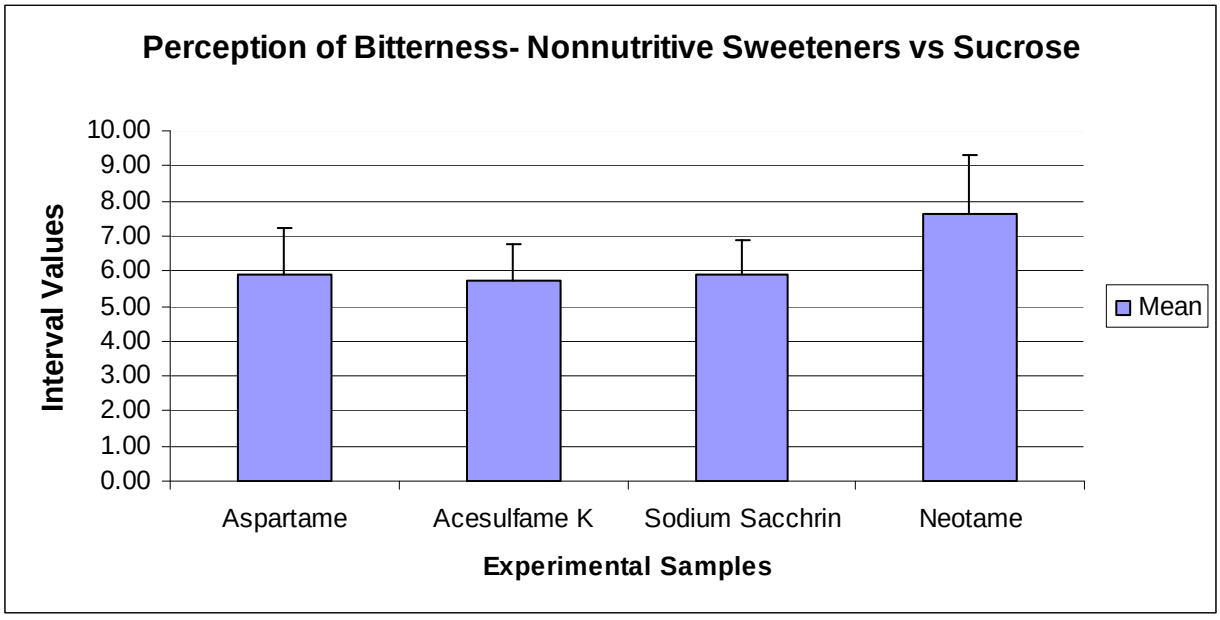

Figure 2.6 Sensory Evaluation – Intensity of the Perception of Bitterness
Control = intensity value of 5.0

Based on the sensory analysis evaluation on taste characteristics, Cha-Cha sweetened with acesulfame K was more similar to the control than Cha-Cha sweetened with the other non-nutritive sweeteners. This analysis has not been confirmed with a second round of testing. This sweetener may be good for this application because of its stability properties in not losing its sweetness quality in high temperature processing and storage. Acesulfame K taste quality was similar to that of sucrose, and the panelist did not perceive the aftertaste to be overwhelming and lingering. The taste may have been perceived to have an onset similar to that of sucrose with combination of high fructose corn syrup and acesulfame K, which can complement each other in elevating taste and sweetness to that of sucrose. Although high fructose corn syrup was used in all treatments, it can be suggested that acesulfame K had more synergism with improved sweetness potency and temporal profile than the other sweeteners evaluated. Lastly, acesulfame K is stable in a medium at pH 3, whereas some of the other sweeteners tend to break down and lose their sweetness in products at low pH (Kemp, 2006). The pH of Cha-Cha is 3.5, and based on the results from the panelist, the acesulfame K treatment delivered a sweet, clean, and quick onset that suggests its stability properties are good in this low pH application indicating its performance was at its optimal level. In a different study with soft drinks at low pH, a blend of saccharin and acesulfame K with 2-3% fructose was shown to improve the taste quality of soft drinks. Not all blends are synergistic, for instance a blend of saccharin and acesulfame K was shown by panelists to be bitter suggesting they activate a common bitter receptor resulting in no improvement in taste quality (Walters D.E., 2006).

Of the two theories of sweet taste proposed, the AH-B-X theory may explain why the sense of sweetness may have a lingering effect like the taste the panelist experience with neotame. The AH-B-X proposed by Shallenberger and Kier indicates that all sweet compounds

have AH-B glucophore that interacts with a similar AH-B unit that exist at the taste bud receptor site through the formation of hydrogen bonds, and the strong nature of these bonds is why the sense of sweetness could have a lingering sensation, where the X region determines sweetness (deMann, 1999). Based on this theory, it can be suggested the AH-B unit of neotame react at the receptor sites at the proper alignment to create a lingering sensation that was not pleasing to the panelist.

Density

Density is the measurement of mass (g)/volume (mL). Evaluation of the density of the experimental treatments and control treatments would determine if there is a packaging volume difference. The results indicate the experimental treatments and the control were similar. The experimental treatments and the control yielded a density value of 12.1g/10mL. This measurement was based one experimental treatment weighed for each treatment. The results suggest there may not be a difference in the amount of product that could be packaged into a jar of Cha-Cha if one of the non-nutritive sweeteners was selected for the formulation. However, a difference in density would be expected if maltodextrin was not used as filler; the density values of the experimental treatments would differ from the control.

CHAPTER 3 - Volatile Aroma Profile Analysis

The GC/MS was used to identify differences in volatile compounds between the sucrose sweetened treatment (control) and the non-nutritive sweetened treatments. The peaks were analyzed first by NIST then confirmed by AMDIS spectral libraries. The peaks where evaluated at three different points, both sides and the middle for a spectrum match for identification. Since the compounds were not a 100% match, but had high probability of 70-98% as a match, the retention time and peaks of the compounds were tentatively confirmed with the AMDIS library. Once the volatile aroma compound was identified, the area percent of each compound's peak was identified to show the differences between the experimental treatments and the control. The experimental treatment with the least differences than the control is the treatment identified as being similar to the control. Table 3.4 lists the volatile aroma compound and their area of percent identified in each treatment by GC/MS. Within this table, the volatile compounds with an area percent greater than 0.5% are shown. Compounds with an area percent greater than 0.5% were considered prominent in the treatment. However, this is not an indication that compounds with an area percent less than 0.5% are not contributing to the aroma profile of the finished product.

Table 3.1 Area Percent of Volatile Aroma Compounds Identified by GC/MS on the Influence of High Potency Non- Nutritive Sweeteners in Cha-Cha

		Area Percent of Volatile Compounds				
Compounds	Retention Time	Sucrose	Aspartame	Acesulfame K	Sodium Saccharin	Neotame
Dimethyl Sulfide	4.23	0.154	0.091	0.083	0.042	0.03
3 Methylbutanal	8.14	0.743	0.056	0.396	0.389	0.205
Hexanal	13.33	2.007	0.162	0.139	0.382	0.165
Furfural	15.75	0.109	0.181	0.559	0.158	0.207
Camphene	23.97	1.087	0.362	0.501	0.244	1.04
Isoxazole, 5 methyl	24.57	0.492	0	0.394	0	0.35
Trans - 2-Heptenal	24.58	0.207	0.104	0.394	0.244	0.35
Pinene	27.00	0.392	0.687	0.402	0.742	0.578
Linalool	33.11	1.157	0.987	0.425	0.969	0.122
Disulfide Diproply	33.63	0.088	0.456	0.405	2.415	0.727
Terpinyl Acetate	37.27	0.386	0.69	0.299	0.548	0.432
2-Hydroxymethylbenzoate	37.49	0.386	0.526	0.226	0.764	0.068
1- Phenoxypropan-2-ol	39.27	0	0.159	0.127	0.036	0.054
Ethyl Decanoate	43.54	1.673	0	0.521	0	0.034
Phenol, 2 Methoxy – 4 (1 propenyl)	44.50	0.001	0.042	0.117	0.03	0.156
6-allyl-4-methoxy-1,3 benzodioxide	47.64	0.023	0.174	0.217	0.163	0.167

The prominent compounds identified in Cha-Cha sweetened with aspartame were pinene, linalool, terpinyl acetate, and 2- hydroxymethylbenzoate. The prominent compounds identified in Cha-Cha sweetened with acesulfame K were furfural, camphene, and ethyl decanoate. The prominent compounds identified in Cha-Cha sweetened with sodium saccharin were pinene, linalool, disulfide diproply, terpinyl acetate, and 2-hydroxymethylbenzoate. The prominent compounds identified in Cha-Cha sweetened with neotame were camphene, pinene, and disulfide diproply. In the control treatment, the most prominent compounds identified were 3-methylbutanal, hexanal, camphene, linalool, and ethyl decanoate. Figure 3.4 shows the graphical view of the volatile aroma compounds and their area of percent in the treatments identified by GC/MS.

Volatile compounds contribute to the taste and aroma of food products. Aromas are the key to flavor perception, and they are perceived in humans by the nasal olfactory epithelium. The human threshold for perception of a volatile molecule is 0.007 μg L^{-1}. Combinations of volatiles and their components determine the aroma properties of foods (Lewinsohn, et al., 2001).

Green tomatoes are the main ingredient in Cha-Cha. They are not fully ripe and are harvested while they are green. Unlike red or ripe tomatoes, they will not receive ethylene ripening treatment. Tomatoes are made up of the 70.0% pulp with 0.3% seeds, 20.0% fluid, 10.0% skin. These different parts of the tomatoes contain volatiles at different levels (Buttery et al., 1988). Over 400 volatile compounds have been identified in tomatoes, however only several are considered to be important for flavor based on their odor threshold. These compounds are acetaldehyde, acetone, methanol, ethanol, 1-penten-3-one, hexanal, cis-3-hexenal, trans-2-hexenal, trans-2-heptenal, 6-methyl-5-hepten-2-one, cis-3-hexenol, geranylacetone, 2-isobutythiazole and β-ionone (Abegaz, et al., 2004). In addition to these key volatiles, research by Buttery et al., (1987) showed the volatile concentrations of green tomatoes are lower than those found in ripe tomatoes. As they lose their green color due to chloroplast degradation, they release membrane lipids which are rich in aroma precursors (Reineccius, 2006). Of the volatile aroma compound identified in the Cha-Cha treatments, linalool was at prominent levels (area percent >0.5%) in the control, aspartame, and sodium saccharin. Linalool is a monoterpene alcohol that influences the flavor quality of tomatoes. It is present in fresh tomatoes at 1.0 and 20.0 μg g^{-1}(Lewinsohn et al., 2001). Buttery Buttery et al. (1986) showed that linalool occurred at different levels in certain parts of the tomatoes. It occurred in the pulp at 3.0 ppb, fluid at 4.0 ppb, skin at 4.0 ppb, and none was found in the seed of the tomato. Also, it is found in high

concentrations in fresh heated tomatoes than those at cold temperatures (Buttery et al., 1971). Linalool is an aromatic alcohol that tends to be weak, but has pleasant odor characters (Reineccius, 2006). It is described as floral, woody, sweet with a green, spicy, topical nuance. It occurs in products such as, apples, citrus peel oil, berries, celery, peas, potato, tomato, cloves, ginger, herbs, flowers, and wood. In tomatoes, it is formed from geranyl diphosphate by the isopernoid pathway (Lewinsohn et al., 2001). Linalool can occur in either its optical active forms (*d*- and *l-)* or optically inactive form. Its optically inactive form normally occurs in oils from herbs, leaves, flowers, and wood. It has an aroma threshold value at 4.0 to 10.0 ppb. It has a taste threshold value at 30 ppm. Linalool has a molecular formula of $C_{10}H_{18}O$ with a molecular weight of 154.2 (Burdock, 2005). It can be suggested linalool was identified in Cha-Cha by GC/MS from the usage of tomatoes and spices in the product.

Ethyl decanoate is an ester, and esters are major contributors to both flavor and aroma. Ethyl decanoate is typically found in products that have undergone a fermentation process with yeast such as grape wine, apple juice, and apple wines. Wang, L. et al. (2004) conducted a study that showed apple juice from Fuji apples contained 0.010 mg/L of ethyl decanoate, and wine made from Fuji apples contained 1.50 mg/L of ethyl decanoate. Cha-Cha contains apple cider vinegar which is a fermentation process from apple cider. However, ethyl decanoate was only identified in the control, acesulfame K, and the neotame treatments, but it was at prominent levels in the control and acesulfame K treatments. Ethyl decanoate is known to have a fatty, brandy, and fruit odor like cognac. It natural occurs in cognac and wines. It has an aroma threshold at 8.0 – 12.0 ppb, and it is approved to be used in beverages at 2.0 ppm and food at 10.0 ppm. Ethyl decanoate has a molecular formula of $C_{12}H_{24}O_2$ with a molecular weight of 200.3 (Burdock, 2005). In relations to Cha-Cha, it can be suggested that apple cider vinegar is

the main contributor of this compound being identified by GC/MS. However, another perspective of the explanation of this compound being identified is its capability of being formed during the thermal processing steps, since it was identified in three of the five treatments.

Camphene and pinene are terpenoid hydrocarbons. They act as diluents and carriers for oxygenated flavoring constituents. They add a sense of freshness to the odor and the flavor profile. They are normally found in essential oil, and when the oils are deterpenated they lack the attribute of freshness and become flat (Reineccius, 2006). Camphene was identified at prominent levels in the control, acesulfame K, and neotame treatments. This volatile compound is known to have a terpene and camphoraceous taste. It occurs in products such as, spices, carrots, orange peel oil, lemon oil, and turmeric. It is used in baked goods, gelatins, pudding, nonalcoholic beverages, and soft candies. Its usual level in nonalcoholic beverages is 17.93 ppm and soft candy is 81.79 ppm. Camphene has a molecular formula of $C_{10}H_{16}$ with a molecular weight of 136.2 (Burdock, 2005). It can be insinuated the spices used in the treatments are the major contributors to this compound being identified by GC/MS.

Pinene is another volatile compound that occurs in spices, and it was identified in Cha-Cha mostly likely from the spices and tomatoes that were used in the product. It is described as intense woody, piney, and terpy with camphoraceous, and turpentine note. At certain concentrations, pinene notes can appear herbal, spicy, and topical. Pinene naturally occurs in products such as apples, tomatoes, spices, bell peppers, kiwifruit, hops oil, strawberry, and celery. It has an aroma threshold at 2.5 to 62.0 ppb and taste threshold values at 5.0 to 100.0 ppm. Pinene has a molecular formula of $C_{10}C_{18}$ with a molecular weight of 136.2 (Burdock, 2005).

Terpinyl acetate is an ester, and esters have a wide spectrum of odor and flavoring effect (Reineccius, 2006). Terpinyl acetate is described to be sweet spicy and herbaceous. In Cha-Cha, it occurred at prominent levels in the aspartame and sodium saccharin treatments. It is used in products such as alcoholic and nonalcoholic beverages, condiments, relishes, meat products, and gravies. It has an aroma threshold value at 2.5 ppm. It naturally occurs in the essential oils of cypress, Malabar cardamom, and pine. It can be found in the grapefruit juice, spices, lemon balm, eucalyptus oil, and cocoa. It is approved for use in beverage at 5.0 ppm and food 40.0 ppm. Terpinyl acetate has a molecular formula of $C_{12}H_{20}O_2$ with a molecular weight of 196.2 (Burdock, 2005). In relations to Cha-Cha, terpinyl acetate was identified by GC/MS from the spices used in the product.

Hexanal is a six carbon aldehyde that occurs in vine ripe tomatoes at 2.0-3.7 ppm and green tomatoes at 0.3 ppm (Buttery et al., 1987). It also occurs in different parts of the tomatoes at different levels. It occurs in the pulp at 8100 ppb, fluid at 3500 ppb, seed at 36 ppb, and at 9700 ppb in the skin (Buttery et al., 1988). Hexanal occurred at prominent levels in the control, but not in the other treatments. It is described as fatty, green, grassy, strong, penetrating characteristic fruity odor and taste (Burdock, 2005). Other aldehydes C8-C10 (octanol, nonanal, and decanal, respectively) are described as floral and optimal at low concentrations in which a floral nuance is required. Hexenal occurs in products such as tomato, cabbage, apple, melon, berries, peaches, and onion. It has an aroma threshold value at 4.1 to 22.8 ppb and aroma threshold at 4.5 ppb (Buttery et al., 1987). It is approved for use in beverages at 3.0 ppm and food at 10.0 ppm. Hexanal has a molecular formula of $C_6H_{12}O$ with a molecular weight of 100.1 (Burdock, 2005). Hexanal and the formation of other short-chain aldehydes and alcohols in tomatoes takes place through the action of lipases, hydoperoxide lysases, and cleavage enzymes

on lipid components followed by alcohol dehydrogenases (Lewinsohn et al., 2006). It can be suggested that hexanal was identified by GC/MS from the tomatoes, cabbage, and onions in the product.

Dimethyl sulfide and *trans*-2-heptenal were not identified at prominent levels in the treatments, but they are compounds that occur in tomatoes which is the main ingredient in Cha-Cha. Dimethyl sulfide is an aliphatic sulfur compound that occurs in cooked tomatoes. It is the major contributor to the aroma of heated tomato products at 0.3 ppb. It is an important component in canned tomato juice formed by the heat during processing (Buttery et al., 1971). Dimethyl sulfide also occurs in cabbage and onion, which are other ingredients in Cha-Cha. Dimethyl sulfide is described as intense, cabbage, and a vegetable green note at 0.1 to 3.0 ppm. It has an aroma threshold value at 0.3 – 10.0 ppb. A few other products dimethyl sulfide occurs in are celery, carrots, peas, potato, milk, wines, tea, hop oil, sweet corn, and krill. It is approved for use in beverages at 1.0 ppm and food 5.0 ppm. Dimethyl sulfide has a molecular formula of C_2H_6S with a molecular weight of 62.1 (Burdock, 2005). When dimethyl sulfide is heated, it presents an off-odor characteristic in the product (Yoo et al., 2003). Trans-2-Heptenal is described as intense green, sweet, fresh fruity apple-skin note at 4.0 ppm. It is found in vine ripe tomatoes at 0.05 ppm and green tomatoes at <0.005 ppm. In sliced vine ripe tomatoes it has an odor threshold of 13.0 ppb (Buttery et al., 1987). Other than tomatoes, it occurs in other products such as, in peas, butter, asparagus, strawberry, and malt. It has an aroma threshold value at 13.0 to 51.0 ppb. It is approved to be used at 5.0 ppm in foods. Tras-2-Heptenal has a molecular formula of $C_7H_{12}O$ with a molecular weight of 112.1 (Burdock, 2005).

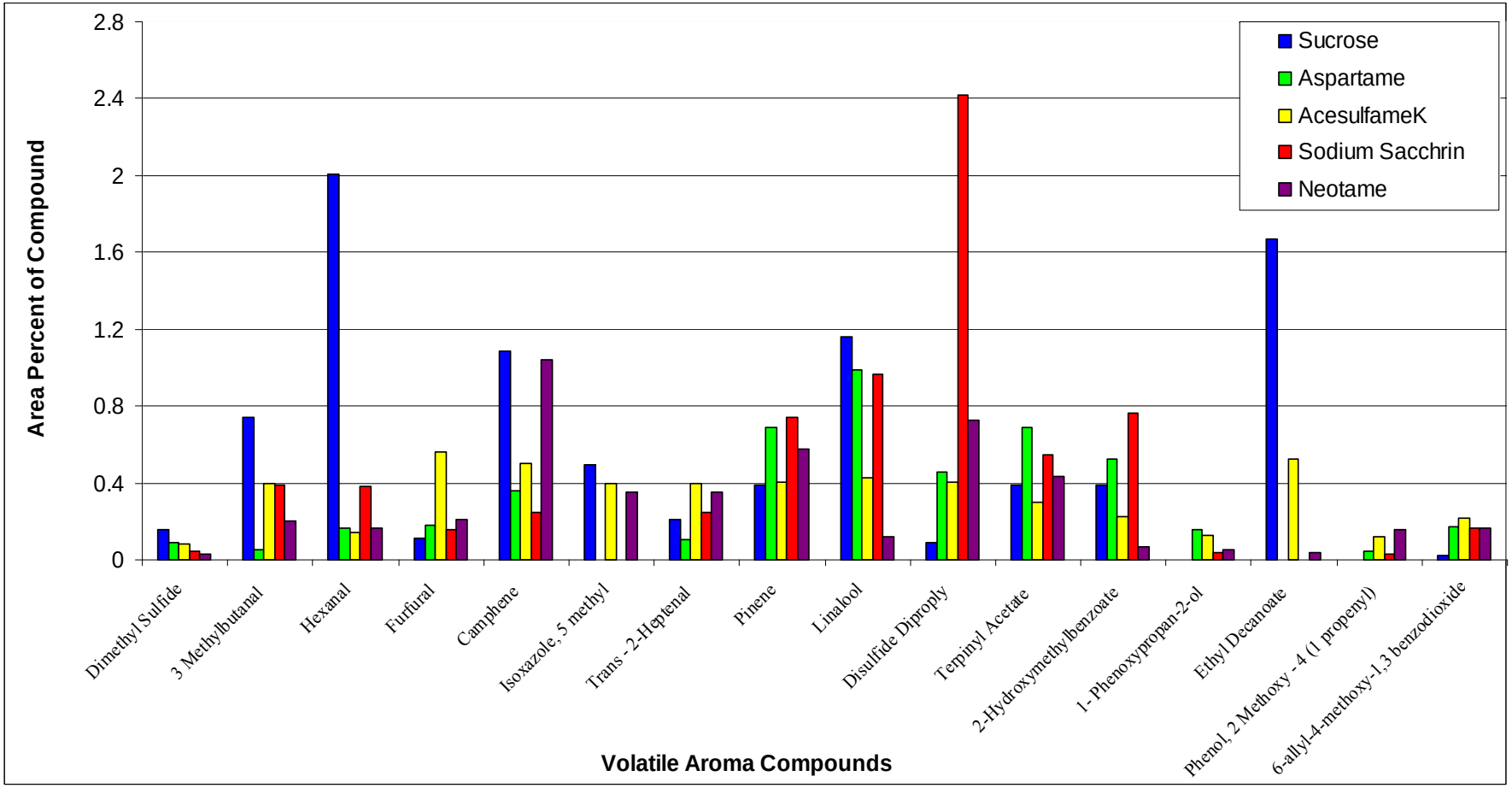

Figure 3.1 Graphical View of the Area Percent of Volatile Aroma Compounds Identified by GC/MS on the Influence of High Potency Non-Nutritive Sweeteners in Cha-Cha

In comparison to the control, some of the experimental treatments had volatile compounds camphene, linaool, and ethyl decanoate present at prominent levels, but they were all present at prominent levels in the control (sucrose sweetened Cha-Cha). The retention times of these compounds resolved from the GC column were 23.97 minutes for camphene, 33.11 minutes for linaool, and 43.55 minutes for ethyl decanoate.

Linalool is a volatile compound that was shown to be prominent in Cha-Cha sweetened with aspartame, sodium saccharin, and sucrose (control). The aspartame and sodium saccharin treatments had linalool present at similar areas of percent than that of the control (sucrose sweetened treatment), that indicates the concentration amount of linalool in these treatments are similar. The area percent of linalool in Cha-Cha sweetened with aspartame was 0.987, and the control was 1.157, indicating a 1.17:1 difference. The area percent of linalool in Cha-Cha sweetened with sodium saccharin was 0.969, and the control was 1.157 indicating a 1.2:1 difference. Although linalool was prominent in the acesulfame K sweetened treatment, its concentration was 2:1 less than the control. The area percent of linalool in Cha-Cha sweetened with acesulfame K was 0.425 and the control was 1.157, this indicated the control had twice as much linalool.

Camphene is another prominent compound identified in Cha-Cha. This compound was also identified in the acesulfame K and neotame sweetened treatments. Camphene's area percent in the control (sucrose sweetened) was 1.087 and neotame was 1.04. In acesulfame K sweetened treatment, the area percent was 0.501. The difference of camphene in between the acesulfame K vs control was 2.2:1, and the neotame sweetened treatment had a difference of 1.0:1. These results indicate that the neotame sweetened treatment and the control has similar concentration of camphene, but the acesulfame K treatment has 2X times less than the control. Camphene was

also identified in the other experimental treatments, but their area of percent was less than 0.5%, which is not considered to be prominent in this report.

Ethyl decanoate is another volatile compound identified at prominent levels (area percent > 0.5%) in the control and Cha-Cha sweetened with acesulfame K. The sucrose sweetened control seemed to contain more ethyl decanoate than the acesulfame K treatment. This compound was not detected in the aspartame nor sodium saccharin sweetened treatments. The ethyl decanoate area percent in acesulfame K was 0.521, while the ethyl decanoate area percent in the control was 1.673. This indicates that the concentration is 3.2:1 times more in the control than in the acesulfame K sweetened treatment.

The GC/MS chromatograms of cha-cha sweetened with each sweetener follow: (Figures 3.5-3.14). A full and split GC/MS chromatograms are illustrated for each cha-cha treatment. In the tables (Table 3.2 3.7) are a list of the volatile compounds and their retention times identified by GC/MS. The asterisks listed by the volatile compounds are the prominent volatile compounds indicated in each treatment.

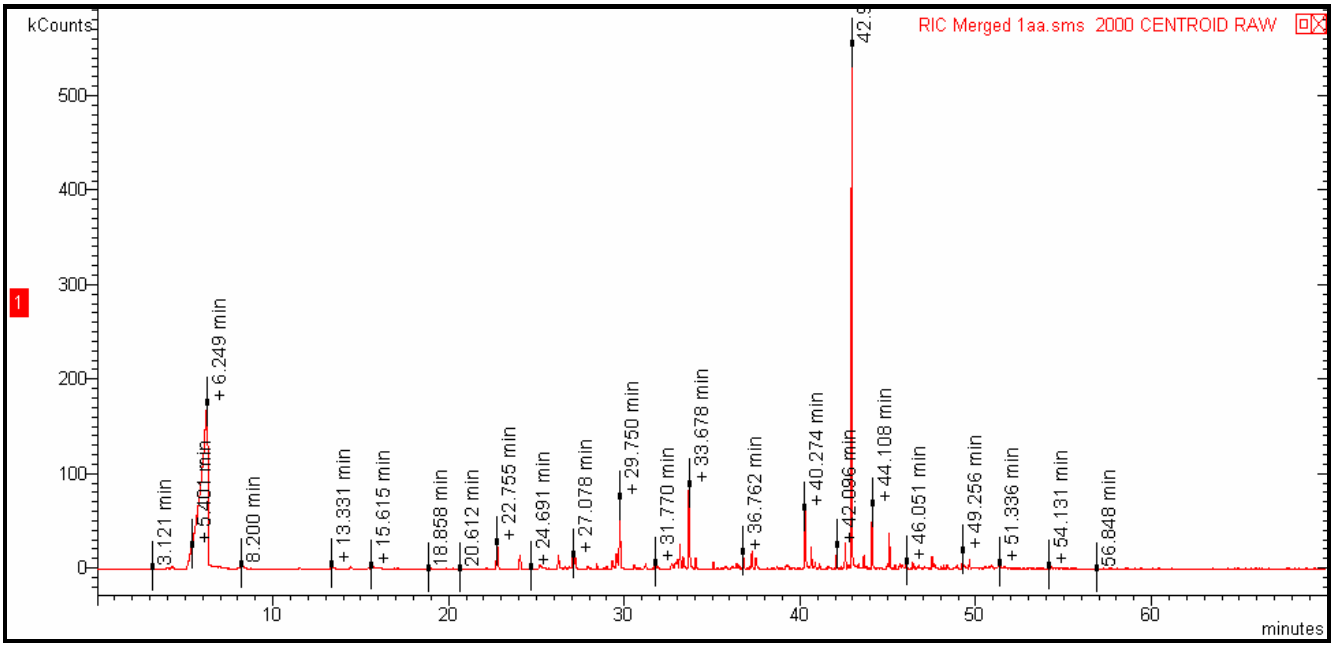

Figure 3.2 Full GC/MS Chromatogram of Cha-Cha Sweetened with Aspartame

Table 3.2 Volatile Compounds Identified in Cha-Cha Sweetened with Aspartame

Volatile Compounds	Retention Time
Dimethyl Sulfide	4.23
3-Methylbutanal	8.14
Hexanal	13.33
Furfural	15.75
Camphene	23.97
Trans – 2-Heptenal	24.58
* Pinene	27.00
* Linalool	33.11
Disulfide Diproply	33.63
* Terpinyl Acetate	37.27
* 2-Hydroxymethylbenzoate	37.49
1- Phenoxypropan-2-ol	39.27
Phenol, 2 Methoxy – 4 (1 propenyl)	44.50
6-allyl-4-methoxy-1,3 benzodioxide	47.64

* Prominent Volatile Compounds

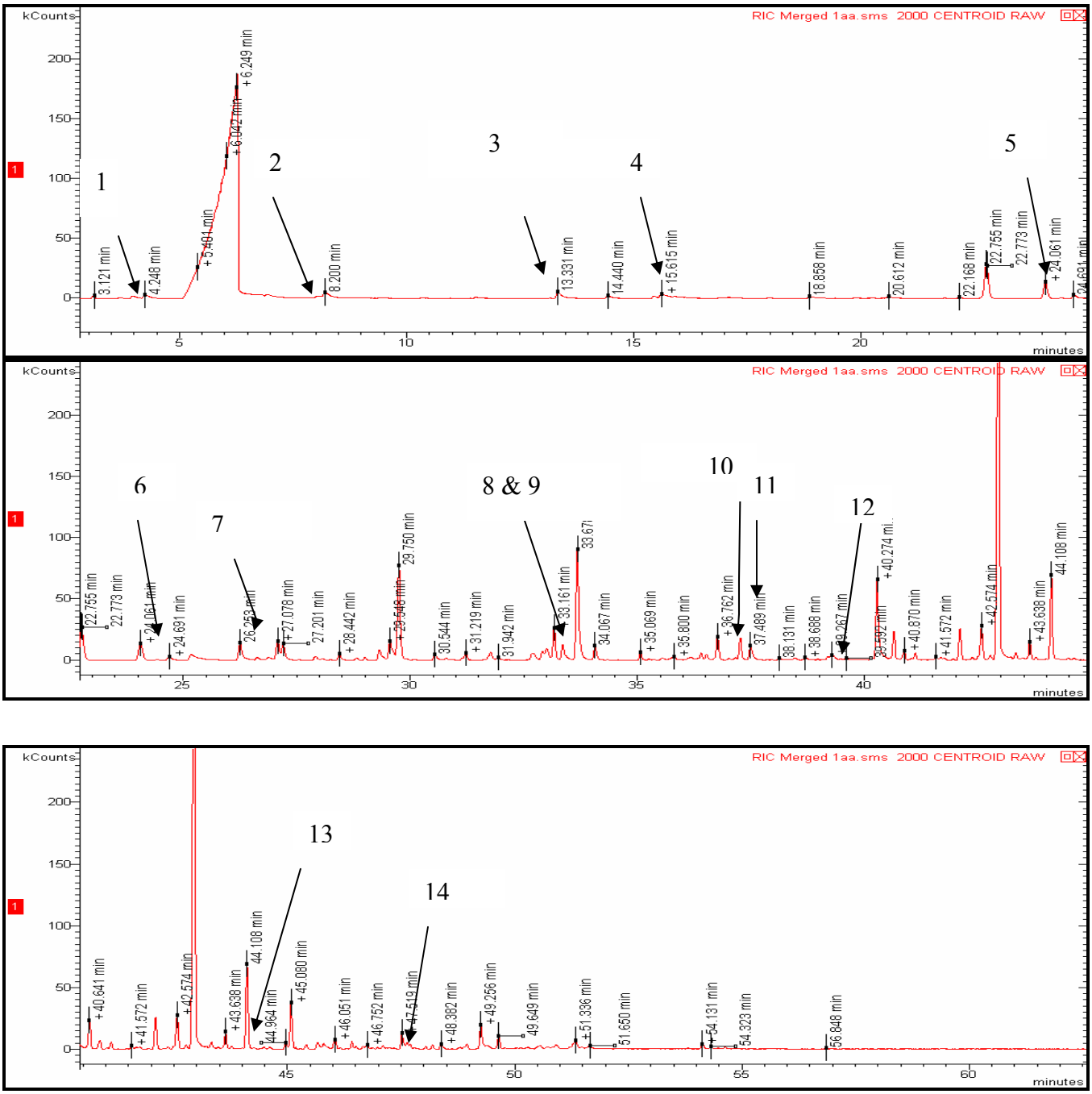

Figure 3.3 Split GC/MS Chromatogram of Cha-Cha Sweetened with Aspartame

1- Dimethyl Sulfide
2- 3-Methylbutanal
3- Hexanal
4- Furfural
5- Camphene
6- *Trans*-2-Heptenal
7- Pinene
8- Linalool
9-Disulfide Diproply
10- Terpinyl Acetate
11- 2-Hydroxymethylbenzoate
12- 1-Phenoxypropan-2-ol
13- Penol, 2 Methoxy-4(1-propenyl)
14- 6-ally-4-methoxy-1,3 benzodioxide

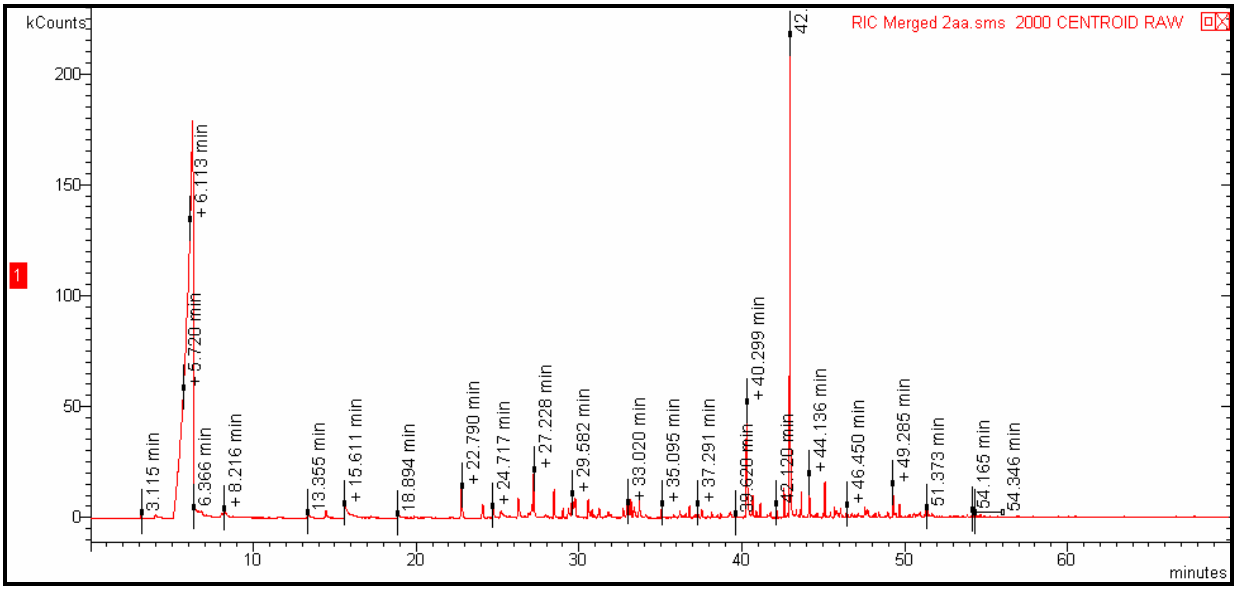

Figure 3.4 Full GC/MS Chromatogram of Cha-Cha Sweetened with Acesulfame K

Table 3.3 Volatile Compounds Identified in Cha-Cha Sweetened with Acesulfame K

Volatile Compounds	Retention Time
Dimethyl Sulfide	4.23
3-Methylbutanal	8.14
Hexanal	13.33
* Furfural	15.75
* Camphene	23.97
Isoxazole, 5 methyl	24.57
Trans – 2-Heptenal	24.58
Pinene	27.00
Linalool	33.11
Disulfide Diproply	33.63
Terpinyl Acetate	37.27
2-Hydroxymethylbenzoate	37.49
1- Phenoxypropan-2-ol	39.27
* Ethyl Decanoate	43.54
Phenol, 2 Methoxy – 4 (1 propenyl)	44.50
6-allyl-4-methoxy-1,3 benzodioxide	47.64

*Prominent Volatile Compounds

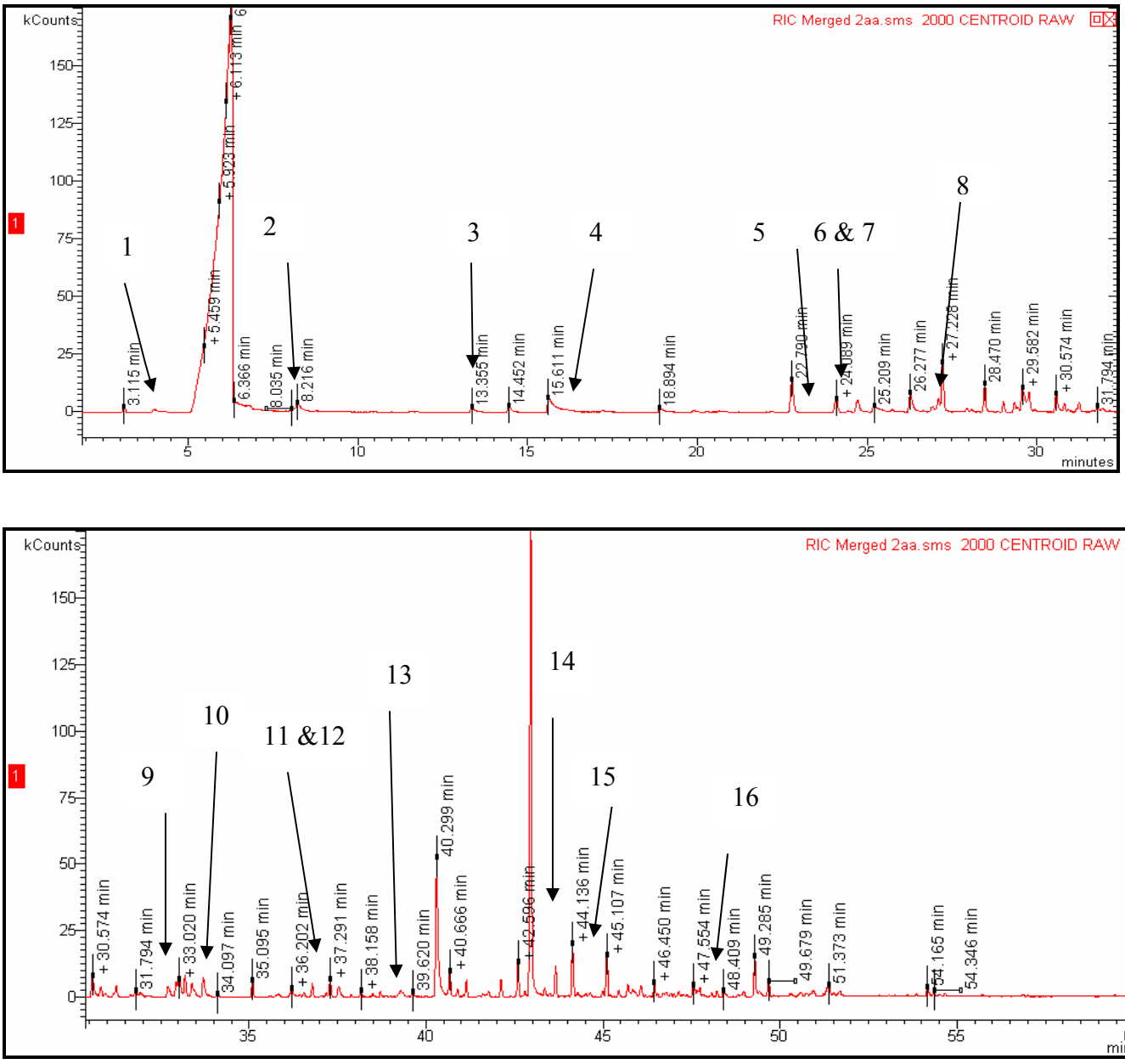

Figure 3.5 Split GC/MS Chromatogram of Cha-Cha Sweetened with Acesulfame K

1- Dimethyl Sulfide
2- 3-Methylbutanal
3- Hexanal
4- Furfual
5- Camphene
6- Isoxazole, 5 Methyl
7- *Trans*-2-Heptenal
8- Pinene
9- Linalool
10- Disulfide Diproply
11- Terpinyl Acetate
12- 2-Hydroxymethylbenzoate
13- 1-Phenoxypropan-2-ol
14- Ethyl Decanoate
15- Phenol, 2 Methoxy-4(1 propenxyl)
16- 6-ally-4-methoxy-1,3 benzodioxide

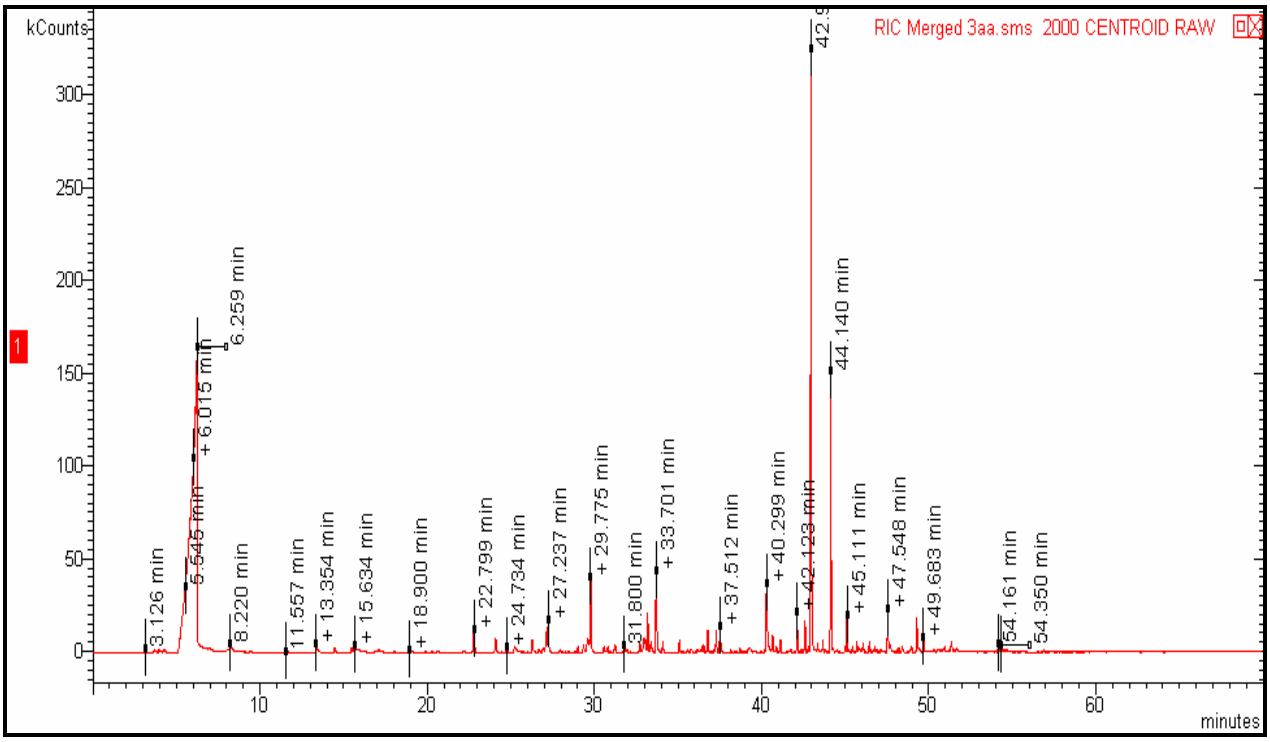

Figure 3.6 Full GC/MS Chromatogram of Cha-Cha Sweetened with Sodium Saccharin

Table 3.4 Volatile Compounds Identified in Cha-Cha Sweetened with Sodium Saccharin

Volatile Compounds	Retention Time
Dimethyl Sulfide	4.23
3-Methylbutanal	8.14
Hexanal	13.33
Furfural	15.75
Camphene	23.97
Trans – 2-Heptenal	24.58
* Pinene	27.00
*Linalool	33.11
*Disulfide Diproply	33.63
*Terpinyl Acetate	37.27
*2-Hydroxymethylbenzoate	37.49
1- Phenoxypropan-2-ol	39.27
Phenol, 2 Methoxy – 4 (1 propenyl)	44.50
6-allyl-4-methoxy-1,3 benzodioxide	47.64

*Prominent Volatile Compounds

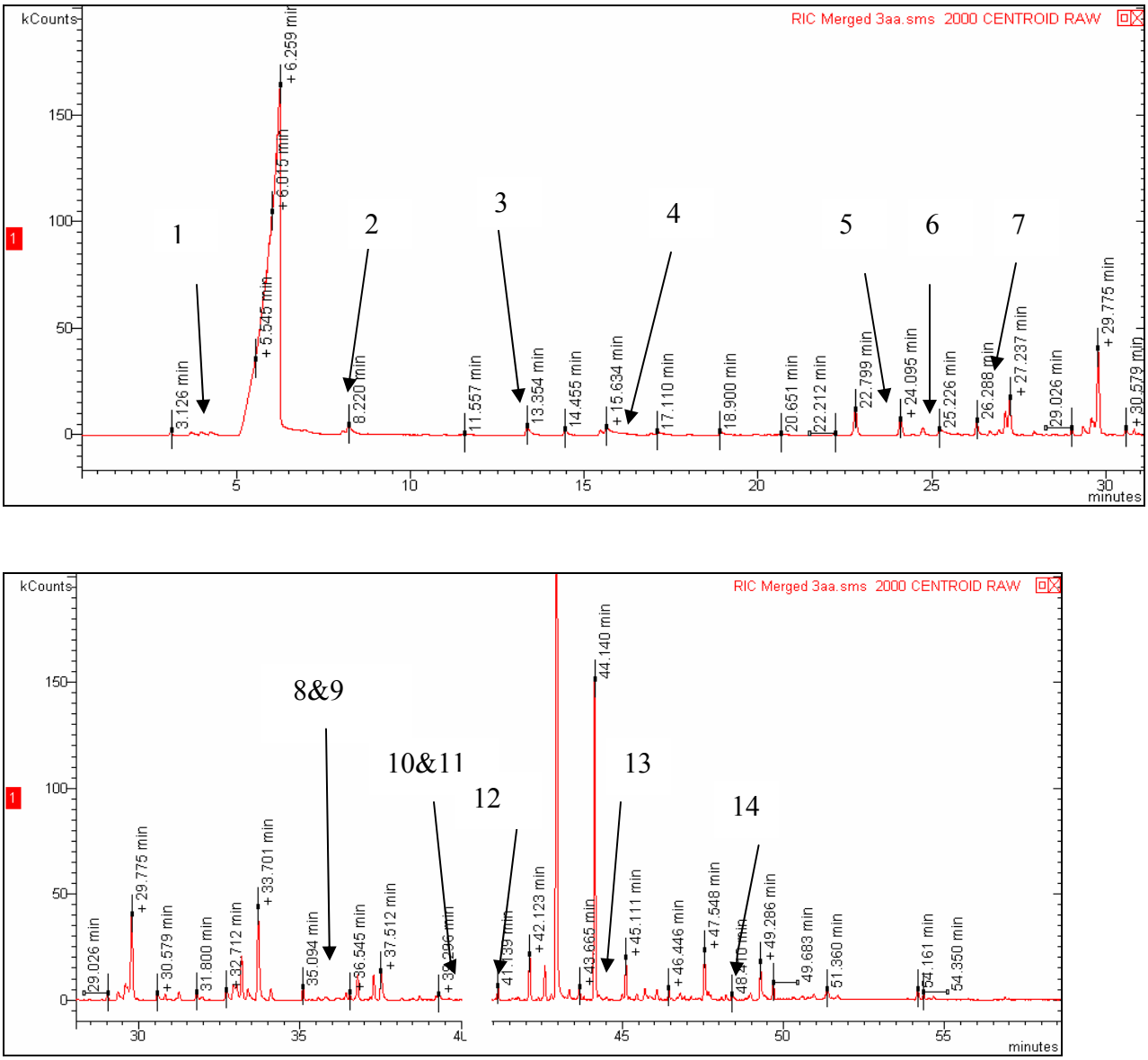

Figure 3.7 Split Chromatogram of Cha-Cha Sweetened with Sodium Saccharin

1- Dimethyl Sulfide
2- 3-Methylbutanal
3- Hexanal
4- Furfual
5- Camphene
6- *Trans*-2-Heptenal
7- Pinene
8- Linalool
9-Disulfide Diproply2
10- Terpinyl Acetate
11- 2-Hydroxymethylbenzoate
12- 1-Phenoxypropan-2-ol
13- Phenol, 2 Methoxy – 4 (1 propenyl)
14- 6 ally-4-methoxy-1,3 benzodioxide

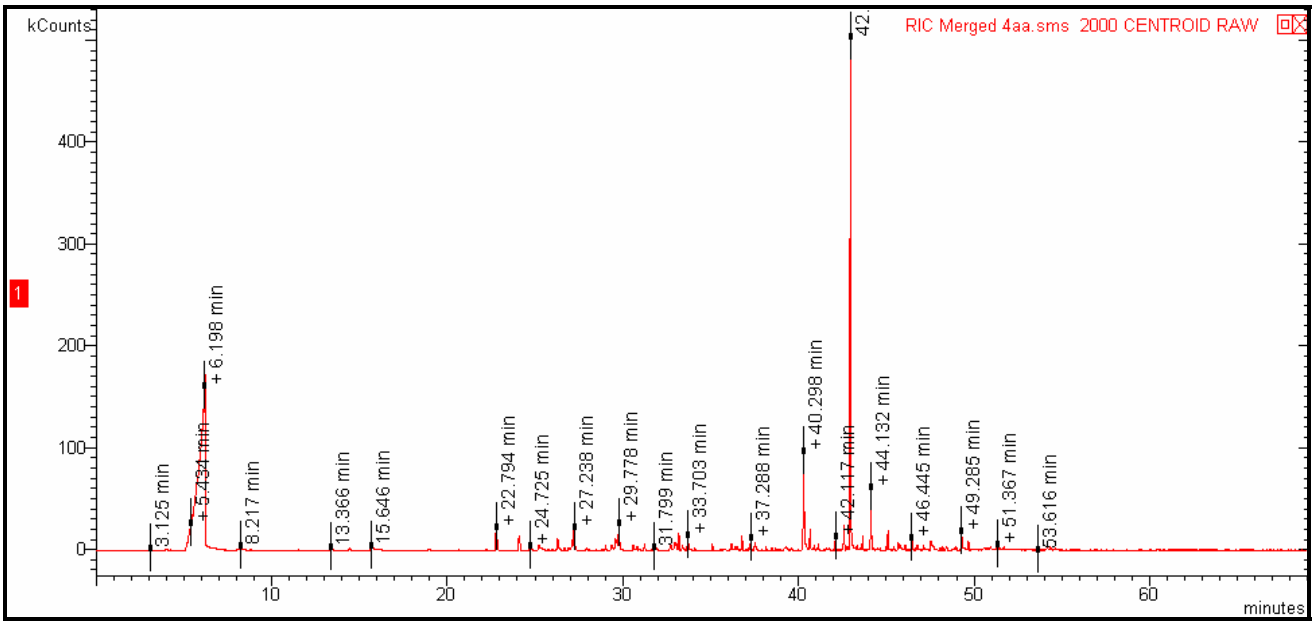

Figure 3.8 Full GC/MS Chromatogram of Cha-Cha Sweetened with Neotame

Table 3.5 Volatile Compounds Identified in Cha-Cha Sweetened with Neotame

Volatile Compounds	Retention Time
Dimethyl Sulfide	4.23
3-Methylbutanal	8.14
Hexanal	13.33
Furfural	15.75
*Camphene	23.97
Isoxazole, 5 methyl	24.57
Trans – 2-Heptenal	24.58
*Pinene	27.00
Linalool	33.11
*Disulfide Diproply	33.63
Terpinyl Acetate	37.27
2-Hydroxymethylbenzoate	37.49
1- Phenoxypropan-2-ol	39.27
Ethyl Decanoate	43.54
Phenol, 2 Methoxy – 4 (1 propenyl)	44.50
6-allyl-4-methoxy-1,3 benzodioxide	47.64

* Prominent Volatile Compounds

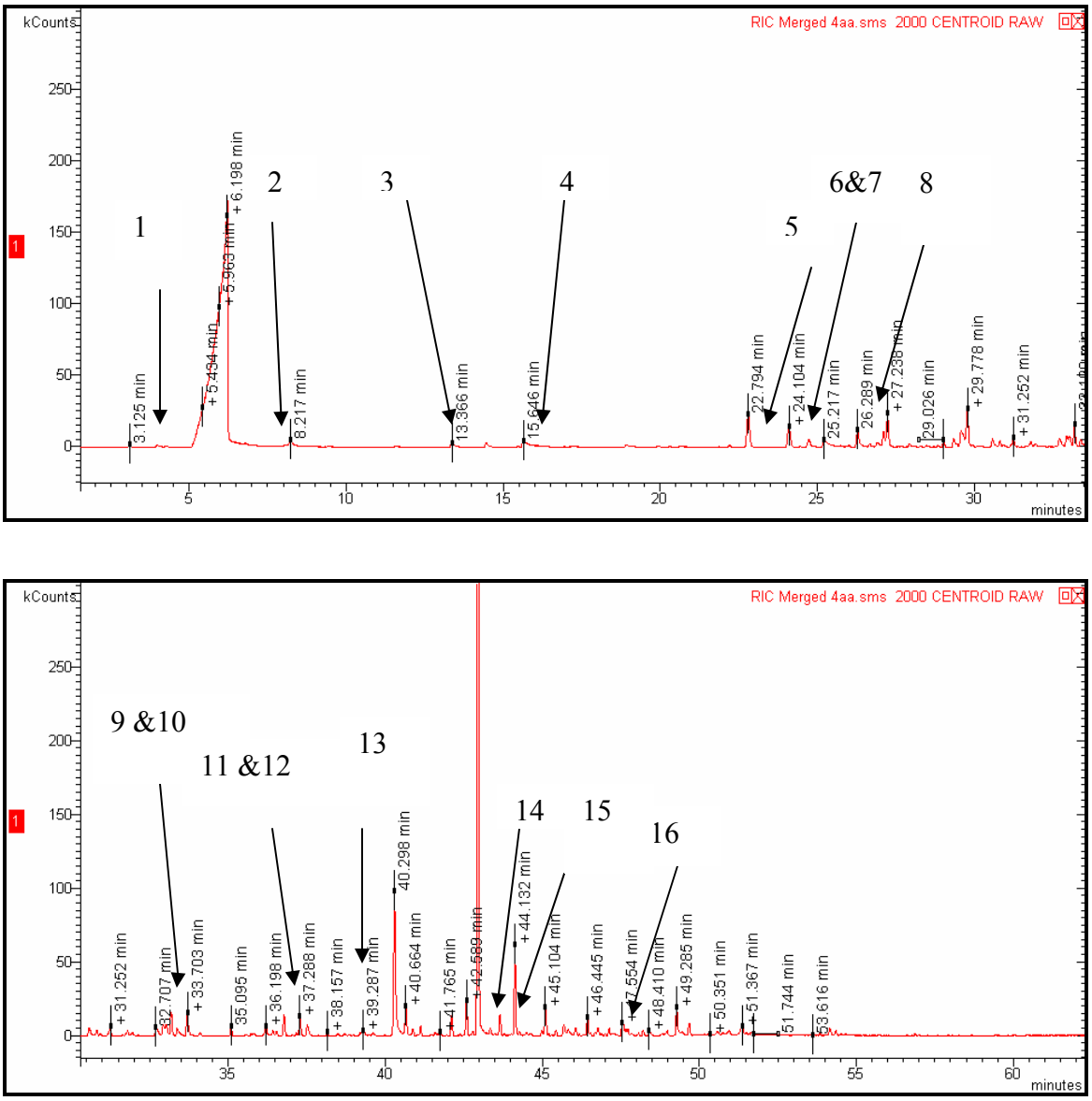

Figure 3.9 Split GC/MS Chromatogram of Cha-Cha Sweetened with Neotame

1- Dimethyl Sulfide
2- 3-Methylbutanal
3- Hexanal
4- Furfual
5- Camphene
6- Isoxazole, 5 Methyl
7- *Trans*-2-Heptenal
8- Pinene
9- Linalool
10- Disulfide Diproply
11- Terpinyl Acetate
12- 2-Hydroxymethylbenzoate
13- 1-Phenoxypropan-2-ol
14- Ethyl Decanoate
15- Phenol, 2 Methoxy-4(1 propenxyl)
16- 6-ally-4-methoxy-1,3 benzodioxide

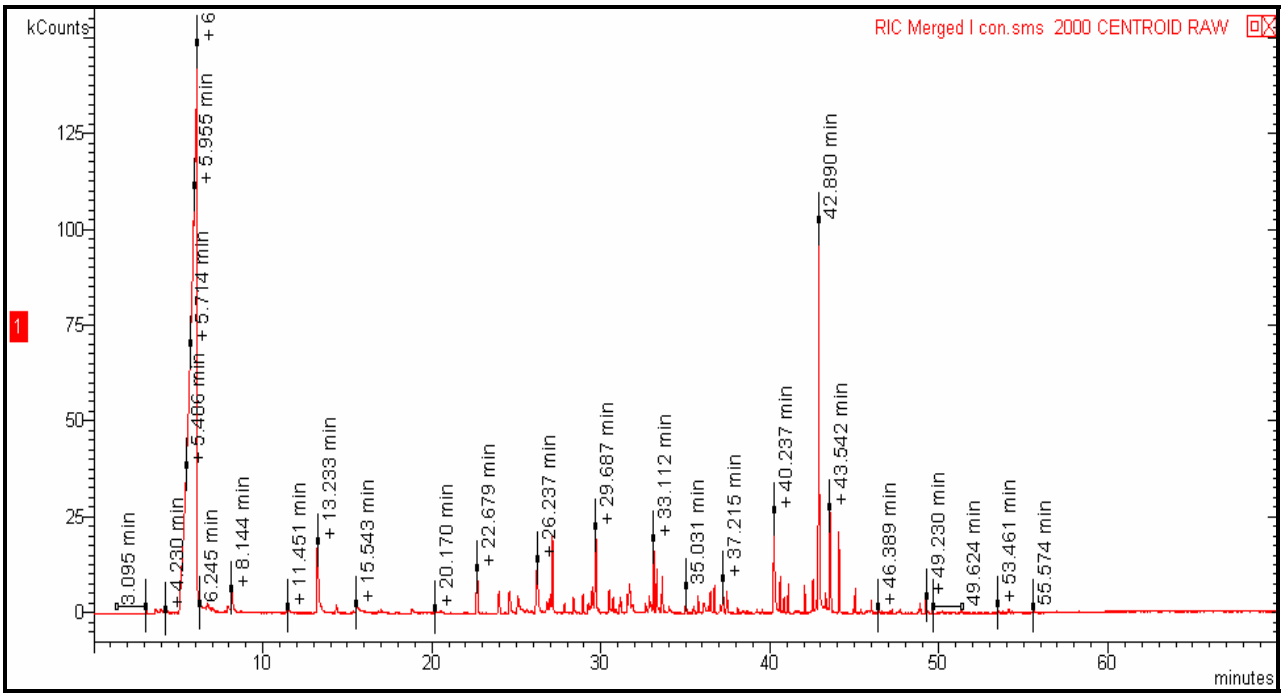

Figure 3.10 Full GC/MS Chromatogram of Cha-Cha Sweetened with Sucrose

Table 3.6 Volatile Compounds Identified in Cha-Cha Sweetened with Sucrose

Volatile Compounds	Retention Time
Dimethyl Sulfide	4.2
*3-Methylbutanal	8.14
*Hexanal	13.33
Furfural	15.75
*Camphene	23.97
Isoxazole, 5 methyl	24.57
Trans - 2-Heptenal	24.58
Pinene	27.00
*Linalool	33.11
Disulfide Diproply	33.63
Terpinyl Acetate	37.27
2-Hydroxymethylbenzoate	37.49
1- Phenoxypropan-2-ol	39.27
*Ethyl Decanoate	43.54
Phenol, 2 Methoxy – 4 (1 propenyl)	44.50
6-allyl-4-methoxy-1,3 benzodioxide	47.64

*Prominent Volatile Compounds

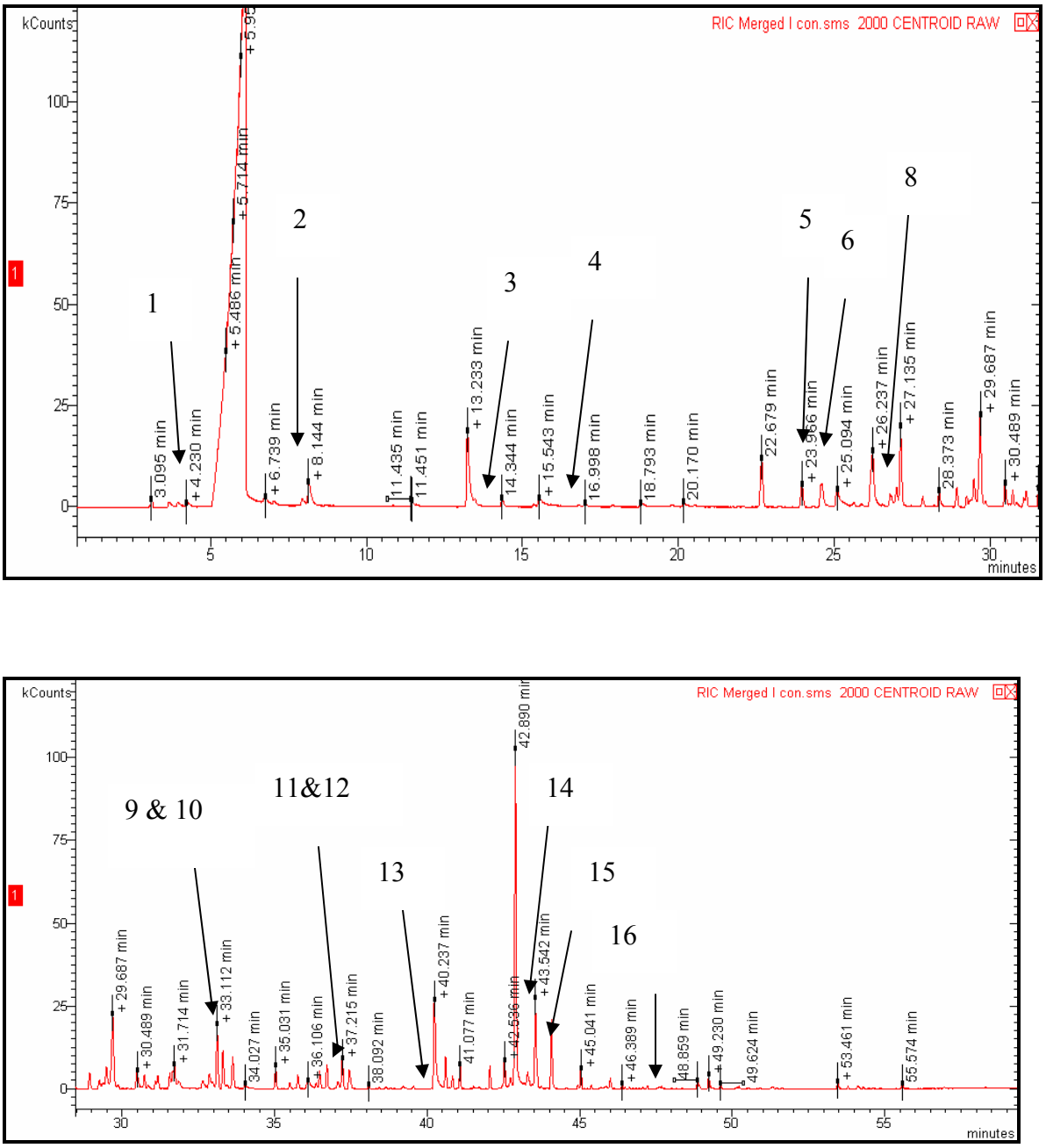

Figure 3.11 Split GC/MS Chromatogram of Cha-Cha Sweetened with Sucrose

1- Dimethyl Sulfide
2- 3-Methylbutanal
3- Hexanal
4- Furfural
5- Camphene
6- Isoxazole, 5 Methyl
7- *Trans*-2-Heptenal
8- Pinene
9- Linalool
10- Disulfide Diproply
11- Terpinyl Acetate
12- 2-Hydroxymethylbenzoate
13- 1-Phenoxypropan-2-ol
14- Ethyl Decanoate
15- Phenol, 2 Methoxy-4(1 propenxyl)
16- 6-ally-4-methoxy-1,3 benzodioxide

Based on the percent comparison of the volatile aroma compounds in all the treatments, only acesulfame K and neotame sweetened treatments contained all of the aroma compounds as the control (Table 3.7). In comparison to the control, camphene and pinene are the volatile aroma compounds that had percentages near equal to the control. Camphene in the neotame treatment was 96% near equal to the control. Pinene in the acesulfame K was 103% near equal to the control. The distinguishing factor that determined which non-nutritive sweetener, acesulfame K or neotame, in Cha-Cha was closer to the control was the treatment with the most volatile aroma compounds that had similar percentages to that of the control. Any percentage of volatile aroma compounds over 100% is an indication the experimental treatment had more of that specific volatile aroma compound than the control treatment. The analysis showed acesulfame K has all the volatile aroma compounds as the control, similar to neotame, but the percent of volatile aroma compounds were closer to the control than the neotame treatment. In Table 3.10, the percent comparison of ethyl decanoate in the acesulfame K treatment was higher than in the neotame treatment compared to the control. The percent comparison of isoxazole, 5-methyl was higher in acesulfame K treatment, than the neotame, sodium saccharin, and aspartame treatments, compared to the control. Ethyl decanonate and isoxazole, 5-methyl were not identified in the aspartame and sodium saccharin treatments, but were identified in the control, this highlights acesulfame K and the neotame sweetened treatments to be more similar to the control. In evaluating the percent comparison in the volatile aroma compounds between the control and the neotame and acesulfame K treatments, acesulfame K has more volatile aroma compounds with higher percentage values showing similarities to the control in dimethyl sulfide, 3-methylbutanal, isoxazole 5-methyl, pinene, linalool, disulfide dipropyl, 2-hydroxymethylbenzoate, ethyl decanoate, and phenol, 2-methoxy-4(1propenyl). Neotame has a

higher percentage value in only furfural, camphene, trans-2-heptenal, terpinyl acetate, 1-phenoxypropan-2-ol, 6-ally-4-methoxy-1,3 benzodioxide. Acesulfame K and neotame had similar percent comparison to the control in hexanal. Therefore, the percent of volatile aroma compounds present in acesulfame K treatment is higher than in other treatments (neotame, aspartame, and sodium saccharin treatments). This analysis is suggestive and has not been repeated for confirmation.

Table 3.7 Percent of Volatile Compounds In Treatments w/Sweeteners Compared to Control

Compounds	Aspartame	Acesulfame K	Sodium Saccharin	Neotame
Dimethyl Sulfide	59	54	27	19
3 Methylbutanal	8	53	52	28
Hexanal	8	7	19	8
Furfural	166	513	145	190
Camphene	33	46	22	96
Isoxazole, 5 methyl	0	80	0	71
Trans - 2-Heptenal	50	190	118	169
Pinene	175	103	189	147
Linalool	85	37	84	11
Disulfide Diproply	518	460	2744	826
Terpinyl Acetate	179	77	142	112
2-Hydroxymethylbenzoate	136	59	198	18
1- Phenoxypropan-2-ol	0	0	0	0
Ethyl Decanoate	0	31	0	2
Phenol, 2 Methoxy - 4 (1 propenyl)	4200	11700	3000	15600
6-allyl-4-methoxy-1,3 benzodioxide	757	943	709	726

The GC/MS analysis clearly demonstrates the carbohydrate sweetener, sucrose, reacts differently in an acidic system with apple cider vinegar, vegetables, and high fructose corn syrup than the chemically derived sweeteners. The difference in the reaction with sucrose vs the reaction with the chemical derived non-nutritive sweeteners showed a difference in headspace volatiles resulting in higher concentrations of the volatile compounds and different volatile compounds. The difference in the volatiles between the treatments may be due to a couple

factors. One being that sucrose has more hydrogen bond donors and acceptors than the non-nutritive sweeteners allows it to have more reaction sites to generate more hydrophilic free volatiles to be released at higher concentrations than the treatments with non-nutritive sweeteners. The volatiles have to be hydrophilic to be released. Lastly, sucrose in this low acidic product may have undergone hydrolysis and converted to an invert sugar allowing a Maillard type reaction to occur generating higher concentrations of volatile compounds.

Conclusion - Effects of Non-Nutritive Sweeteners in Cha-Cha

Sucrose is a functional sweetener in Florence's HomeStyle Cha-Cha that provides a sweet clean taste to the palate without a bitter aftertaste. Once sucrose is substituted with a non-nutritive sweetener, a bitter aftertaste is generally perceived, impacting the product's taste and aroma qualities. However, the results on this study suggested cha-cha sweetened with acesulfame K is more similar to the control than the other sweeteners evaluated. The optimal sweetness of acesulfame K is normally delivered when used in combinations with other low or high-potency sweeteners. It tends to have a synergistic effect with other sweeteners resulting in optimal taste, sweetness, and stability; especially in a blend with sucrose or fructose (Linden and Lorient, 1999). With high fructose corn syrup being part of the Cha-Cha formulation, the synergistic effect with acesulfame K was more pronounced in optimal flavor that it out performed the other non-nutritive sweeteners being evaluated in the sensory analysis.

The taste characteristics normally associated with acesulfame K consist of its rapid onset and its lingering aftertaste that tends to be much shorter than the other sweeteners evaluated in this application (Deis, 2005). In the sensory analysis, the panelist scored the acesulfame K treatment to be less bitter with minimal aftertaste that lingers than the other treatments evaluated. This short duration of lingering aftertaste is an indication that the properties of acesulfame K does not continue to stimulate the nerve cells, where the unpleasant bitter notes are perceived for a long duration. For instance, neotame was perceived to have a much longer lingering aftertaste and bitterness than any of the treatments evaluated with the non-nutritive sweeteners. It can be perceived that the lingering bitterness is due to the receptor continuously sending signals to the brain, when the receptor and sweeteners molecule bind at a certain fit. The panelists commented

it took more water to cleanse their palate from the neotame sweetened treatment than the other treatments evaluated. In addition to the taste qualities, there is general knowledge that acesulfame K outperforms in long term stability storage than the other non-nutritive sweeteners (Pszczola, 2003). Acesulfame K is typically added with different sweeteners to provide a sweetener system that possess high stability properties in that application. An unstable sweetener system may cause the product to darken, an increase in viscosity, and off-flavors in the product.

Acesulfame K and neotame sweetened treatments were the only two experimental treatments that contained all of the aroma compounds as the control, but at different concentration levels. Ethyl decanoate was the only volatile aroma compound identified in the control, acesulfame K, and neotame. The distinguishing factor that determined which non-nutritive sweetener, acesulfame K or neotame, in Cha-Cha was more similar to the control the percent comparison of the volatile aroma compounds between the treatments that should acesulfame K treatment was closer to the control. Based on the differences, the acesulfame K treatment had more aroma compounds with more similarities in volatile aroma compounds than the neotame sweetened treatment (Table 3.10).

All the analyses were conducted once and have not been confirmed. For confirmation on the findings, it would be best to repeat the analyses. The research conducted was the first phase of evaluating a "sugar free" version of Cha-Cha. The next phase recommended for research would be to substitute the sucrose and high fructose corn syrup with a non-nutritive sweetener or sweetener blends to develop a product for that consumer market of healthy eaters. The same type of analyses would be conducted, but the sensory analyses will be conducted a week after production and a month later, giving the treatments a time to stabilize. The findings from this study provided Florence's HomeStyle, Inc. with an ideation of the taste and sweetness quality of

Cha-Cha with some of the common non-nutritive sweeteners on the market. The findings suggest there is a possibility to develop a palatable, quality product that is “sugar free” that has similar taste qualities as Cha-Cha sweetened with sucrose.

References

Abegaz, E.G., Tandon, K.S., Scott, J.W., Baldwin, E.A., and Shewflet, R.L. 2004. Partitioning Taste From Aromatic Flavor Notes of Fresh Tomato to Develop Predictive Models as a Function of Volatile and Nonvolatile Components. Postharvest Biology and Technology. 34: 227-235.

Burdock, George A. 2005. : Fenaroli's Handbook of Flavor Ingredients, 5th Edition. pp. 238 -1776. Boca Raton, FL. 33431.

Burg, J. 1998. Generating Yummy Gummies. Food Product Design. May. http://www.foodproductdesign.com/articles/465/465_0598DE.html.

Buttery, R. G., Seifer, R. M., Guadagni D. G., and Ling L.C. 1971. Characterization of Additional Volatile Components of Tomato. J. Agric. Food Chem.19(3):524-529.

Buttery, Ron G., Teranishi, Roy, and Ling, Louisa C. 1987. Fresh Tomato Aroma Volatiles: A Quantitative Study. J. Agric. Food Chem. 35(4):540-544.

Buttery, Ron G., Teranishi, Roy, and Ling, Louisa C. 1988. Quantitative Studies on Origins of Fresh Tomato Aroma Volatiles. J. Agric. Food Chem. 36(6):1247-1250.

Colonna, William, J., Samaraweera, Upasiri., Clarke, Margaret, A., Cleary, Micahel, Godshall, Mary An, White, J.S., 2001. Sugar. Chpt. 5 *in Kirk-Othermner Encyclopedia of Chemical Technology*. pp.1-15. Hoboken, NJ.

Corliss, G., Prakash, I., Ponakala,R., and Ishikawa,G. 2002. Neotame: The Next Generation Sweetener. Food Technol. 56(7):36-40.

Davis, Eugenia A. 1995. Functionality of Sugars: Physicochemical Interactions in Foods. Am. J. Clin. Nutr. 62:170S-7S.

deMan, John, M. 1999. : Flavor. Chpt 7 in *Principles of Food Chemistry, 3rd Edition*. pp. 263-291. Springer, Verlag.

Deis, Ronald C. 2005. How Sweet It Is Using Polyols and High-Potency Sweeteners. Food Product Design. October:57-68.

Delarue, J. and Giampaoli, P. 2006. : Carbohydrate-Flavour Interactions. Chpt.10. in *Flavour in Food*, ed. Etievant, Patrick and Voilley, Andree. pp.209-228. Woodhead Publishing Ltd., England.

FDA Consumer Magazine. 2006. Artificials Sweeteners: No Calories…Sweet!. July-August (40)4. http://www.fda.gov/fdac/features/2006/406_sweeteners.html. (Accessed May 2009).

Gallo-Torres, Julia. 2005. Neotame-ing the Beast. Prepared Foods. May:88-89.

Frank, Paula. 2006. Sweet Combinations. Prepared Foods. October. http://www.preparedfoods.com/Articles/Feature_Article/5f9cc2d63983e010VgnVCM100000f932a8c0. (Accessed May 2009).

Kemp, S.E. 2006. : Low Calorie Sweeteners. Chpt. 9 in *Optimising Sweet Taste in Foods,* ed. Spillane, William J. pp.175-251. Woodhead Publishing Ltd., England.

Khan, Riaz and Konowicz, Paul, A. 2001.: Sugar, Sugar Derivatives. Chpt.3 in *Kirk-Othmermner Encyclopedia of Chemical Technology*. pp.3-12. Hoboken, NJ.

Knehr, Elaine. 2005. Carbohydrate Sweeteners. Food Product Design. May.http://www.foodproductdesign.com/articles/463/463_0505CARB_5.html#. (Accessed May 2009).

Kroger, M., Meister, K., and Kava, R. 2006. Low-Calorie Sweeteners and Other Sugar Substitutes: A Review of the Safety Issues. Comp.Review Food Sci.Food Safety. 5:35-47.

Kuntz, L. 1995. Achieving Flavor Parity with Alternative Sweeteners. Food Product Design. August. http://www.foodproductdesign.com/archive/1995/0895cs.html. (Accessed May 2009).

Kuntz, L. 1993. Sweeteners In Confections. Food Product Design. February. http://www.foodproductdesign.com/archive/1993/0293DE.html. (Accessed May 2009).

Lee, Thomas, D. 2006. : Sweeteners. Chpt.2 in *Kirk-Othermner Encyclopedia of Chemical Technology*. pp.1-31. Hoboken, NJ.

Lewinsohn, Efraim; Schalechet, Fernond; Wilkinson, Jack; Matsui, Kenji; Tadmor, Yaakov; Nam, Kyoung-Hee; Amar, Orit; Lastochkin, Elena; Larkov, Olga; Ravid, Uzi; Hiatt, William; Gepstein, Shimon; and Pichersky, Eran. 2001. Enhanced Levels of Aroma and Flavor Compound S-Linalool by Metabolic Engineering of the Terpenoid Pathway in Tomato Fruits. Plant Physiol. 127 :1256-1265.

Lichtenthaler, Frieder and Immel, Stefan. 1993. *Sucrose, Sucralose, Fructose, and some Non-Carbohydrate High Potency Sweeteners : Correlations Between Hydrophobicity Patterns and AH-B-X Assignments*. ed. M. Mathlouthi, J.A. Kanters, and G.G.Birch. Elsevier Application Science. 21-53. London, New York.

Linden, G. and Lorient, D. 1999. Extraction and Modifications of Biomolecules. Chpt.10 in *New Ingredients in Food Processing*, pp.211-218.Woodhead Publishing Ltd., England. Lindemann, Bernd. 2001. Receptors and Transduction in Taste. Nature. September 413:219-221.

Lindley, M. 2004. A Sweet Solution For Dieters. Functional Ingredients. September. http://www.functionalingredientsmag.com/fimag/articleDisplay.asp?strArticleId=552&strS. (Accessed May 2009).

Lindsay, Robert C. 2008. : Food Additives. Chpt 12. in *Food Chemistry*, 4th Edition, ed. Fennema, Owens. pp 768-794. Marcel Dekker, Inc., New York.

Meyers, B. and Brewer, M.S. 2008. Sweet Taste in Man: A Review. J Food Sci. 73(6):R81-R90.

Meyer, S. and Riha, W. 2002.Optimizing Sweetener Blends for Low Calorie Beverages. Food Technol. July 56(7):42-46.

Nofre, C., Tinti, J. M. and Glaser, D. 1996. Evolution of the Sweetness Receptor in Primates. II. Gustatory Responses of Non-human Primates to Nine Compounds Known to be Sweet in Man. Chemical Senses. 21:747-762.

Pszczola, D. 2003. Sweetener + Sweetener Enhances the Equation. Food Technol. November 57(11):48-57.

Purves, William K. 2006. How Can an Artificial Sweeter Contain No Calories. Scientific American.http://www.sciam.com/article.cfm?id=how-can-an-artificial-swe. (Accessed May 2009).

Reineccius, Gary. 2006. : An Overview of Flavor Perception. Chpt.1 in *Flavor Chemistry and Technology,* 2nd Edition. pp. 6-9. Boca Raton, Florida.

Rorie, Somolynn. 2006. The Sweet Thereafter. Insider. March:48-54.

Salant, Aber. 1972.: Nonnutritive Sweeteners. Chpt.13 in Handbook of Food Additives, ed. Thomas Furia.pp.523-575.Cleveland, Ohio.

Selim, Jocelyn. The Chemistry of Artificial Sweeteners. Discover. August. 26 (8). http://discovermagazine.com/2005/aug/chemistry-of-artificial-sweeteners/. (Accessed May 2009).

Theodore, S. 2006. New Options For Diet Drinkers. http://www.bevindustry.com/content.php?s=BI/2006/03&p=14. (Accessed May 2009).

Tragash, K. and Tomiyama, Y. 2005. Asparatme Revisited. Food Product Design. October:73-76.

Walters D.E., Rosalind Franklin. 2006. : Improving the Taste of Sweeteners. Chpt 15 in Optimising Sweet Taste in Foods, ed. Spillane, Willam. pp.344-348. Woodhead Publishing Ltd., England

Wang, L., Xu, Y., Zhao, G., and Li, J. 2004. Rapid Analysis of Flavor Volatiles in Apple Wine Using Headspace Solid-Phase Microextraction. J. Inst. Bre. 110(1): 57-65.

Yoo, S.R., Min, A., Prakash, A., Min, D.B. 2003. Off –Odor Study with γ-irradiated Orange Juice Using Sensory and Volatile Compound Analyses. J Food Sci. 68(4):1259-1264.