Added Sugars:
Why It is Added to Food and the Challenges of Labeling It

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Dedication

This thesis is dedicated to my husband. I have been working on my degree for a good portion of our marriage, and without his unconditional love, sacrifice and patience, I would have never reached my dream of earning my Master's degree. I look forward to many more years together.

My husband has made me laugh. Wiped my tears. Hugged me tight. Watched me succeed. Seen me fail. Kept me strong.
My husband is a promise that I will have a friend forever.
-Fawn Weaver

Abstract

Avoiding too much sugar is an accepted dietary guidance throughout the world. The U.S. Nutrition Facts panel includes information on total sugars in foods. A focus on added sugars is linked to the concept of discretionary calories and decreasing consumption of added sugars as a means to assist a consumer to identify foods that are nutrient-dense. On March 14, 2014, the U.S. Food and Drug Administration proposed that including “added sugars” on the nutrition facts panel would be another tool to help consumers. This thesis discusses the functions of sugar in food and shows that the methods used to replace added sugars in foods can result in no reduction in calorie content or improvement in nutrient density. Without clear benefit to the consumer for added sugars labeling, this thesis highlights the complex business obstacles, costs, and consumer confusion resulting from the proposed rule.

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Chapter 1
Introduction

The rates of chronic diseases are on the rise in the Unites States, and many of these diseases are the direct results of poor quality diet and physical inactivity. According to the 2015 Dietary Guidelines for Americans, "half of all American adults have one or more preventable, diet-related chronic diseases, including cardiovascular disease, type 2 diabetes, and obesity"(U.S. Department of Agriculture [USDA] and U.S. Department of Health and Human Services [HHS] 2015b). This increased rate of chronic diseases among Americans also translates to greater healthcare costs. In 2008, the estimated annual medical cost of obesity alone was $147 billion (Finkelstein and others 2009). Thus, modifying the American diet is a key public health issue that is in the forefront of the United States government to address.

The *Dietary Guidelines* is one government strategy to encourage healthy eating patterns among Americans since it is the basis for developing Federal food, nutrition, and health policies, programs, and educational materials (USDA and HHS 2015b). In the 2015 Dietary Guidelines for Americans, one of the five overarching guidelines is decreasing the consumption of sugar-sweetened foods, more particularly limiting the nutrient "added sugars." This guideline is supported by the Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Within this report, the committee stated that the overconsumption of added sugars has been associated with negative health outcomes, such as obesity, type II diabetes, and cardiovascular disease (USDA and HHS 2015a).

On March 3, 2014, the U.S. Food and Drug Administration (FDA) released its proposal for the nutrition and supplement facts labels, the biggest reform since the nutrition facts label was introduced 20 years ago. Given the recent public attention on added sugars, it is not a surprise to see that "added sugars" is one of the suggested changes. Currently, “sugars” are required to be labeled on packages, but FDA’s proposal would require the declaration of “added sugars” indented under “sugars” so that both would be listed. FDA is suggesting that the mandatory declaration of added sugars will assist consumers in maintaining health-beneficial dietary practices (FDA 2014b). However, there is no clear correlation that the labeling of added sugars will benefit the consumer, and the challenges of labeling added sugars will fall onto the food industry.

The objectives of this thesis are to review (1) the relationship of carbohydrates, sugars, added sugars, and sweeteners; (2) the purpose of sugar in food products; (3) the challenges of labeling added sugars; (4) the issues with current food technology to replace added sugars in products; and (5) a discussion on whether labeling added sugars is an appropriate public health strategy to change the American diet.

Chapter 2

Carbohydrates, Sugars, Added Sugars, and Sweeteners

Sugars and Carbohydrates

The most commonly understood added sugar is sucrose or table sugar. Sucrose is a simple carbohydrate and occurs naturally in plants because they make sucrose via photosynthesis (Kitts 2010). The highest concentrations of sucrose are found in sugar cane and sugar beets, which are the main sources for making commercial sugar (Kitts 2010).

Sucrose is one of many different types of carbohydrates that are widely distributed in nature. Structurally, carbohydrates are molecules of carbon, hydrogen, and oxygen, and there are 3 major classifications of carbohydrates: monosaccharides, oligosaccharides, and polysaccharides (Varzakas and others 2012). The term saccharide is a synonym for carbohydrate.

As the name implies, a monosaccharide consists of a single molecular unit and is the fundamental unit of almost all carbohydrates. Common monosaccharides found in nature include glucose (dextrose), galactose, mannose, and fructose. Honey and fruit juices are common food sources of free glucose and fructose.

Oligosaccharides consist of more than one monosaccharide usually, 2 to 10 monosaccharides. Disaccharides are the most common type of oligosaccharides found in food. Sucrose is officially classified as a disaccharide, and it is composed of one molecule of glucose and fructose and occurs naturally in fruits and vegetables. Lactose is composed of galactose and glucose and occurs naturally in milk. Maltose is composed of 2 glucoses, and it is a byproduct of the enzymatic degradation of starch by amylase. With a similar chemical structure as maltose, trehalose also contains 2 glucose molecules, which are linked by an α1,1-glyosidic bond versus an α1,4-glyosidic bond in maltose (Wilson 2007). It is widely distributed in nature, and mushrooms can contain up to 10% to 25% of trehalose by dry weight (Varzakas and others 2012).

Polysaccharides are composed of a large number of monosaccharides, and the most common ones in nature are starch (energy storage for plants), glycogen (energy storage in animals), and cellulose (supporting material and structural component in plants). Dietary fiber is another common polysaccharide discussed in the human diet.

Monosaccharides and disaccharides are also known as sugars, simple carbohydrates, or simple sugars. Sugars occur naturally in food or can be added during the processing of foods. Naturally occurring sugars in food can also be termed intrinsic or inherent sugars. Natural sources of sugars include vegetables, fruits, milk, and honey (Figure 2-1). The most common sugar added to food is sucrose, also called table sugar.

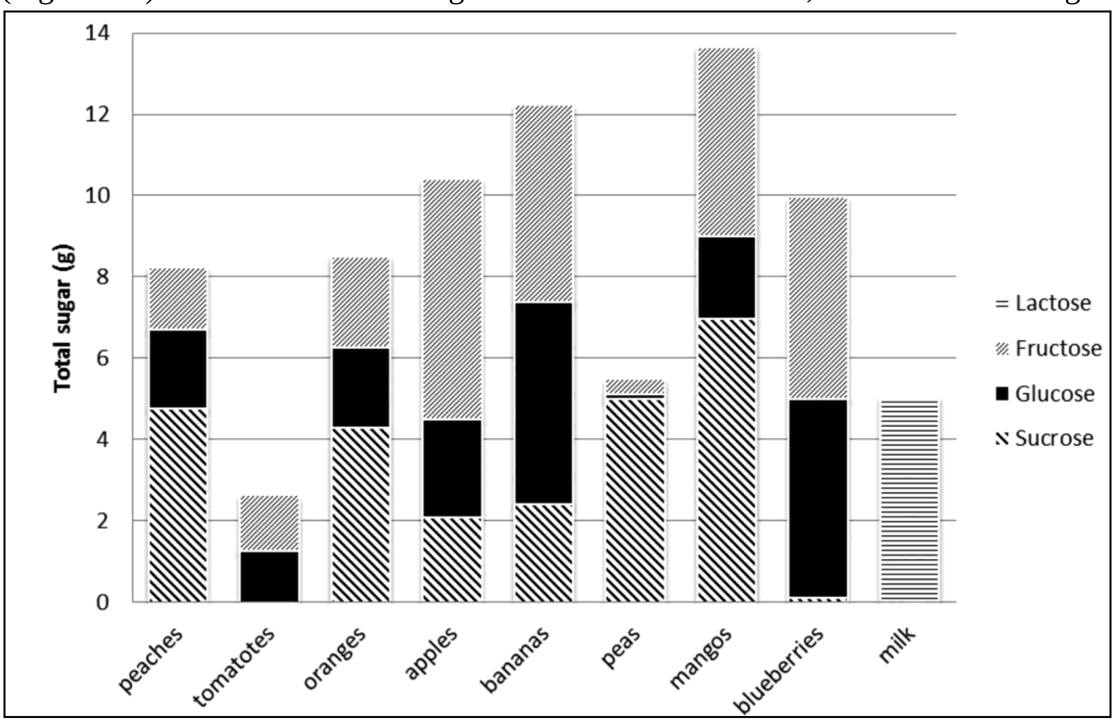

Figure 2-1 Naturally occurring sugar in foods per 100 g (USDA Natl. Nutrient Database for Standard Reference 2014)

The Role and Metabolism of Carbohydrates

Carbohydrates are an integral part of a “healthy” diet. Once consumed, carbohydrates are digested and broken down into glucose. Carbohydrates (starch and sugar) are the primary source of energy for the human body providing on average 4 calories per gram, and glucose is essential for the central nervous system to function (Slavin and Carlson 2014). Because of this, the Institute of Medicine (IOM) set an RDA for carbohydrates of 130 g/d for adults and children aged ≥1-year-old and an acceptable macronutrient distribution range for carbohydrates at 45% to 65% of total calories (IOM 2005).

Because all sugars are carbohydrates, the body metabolizes them similarly by breaking them down into glucose to be used for energy (USDA and HHS 2010). Regardless, if the sugars are naturally occurring or added during food processing, the molecular structure and nutritional value are the same, providing 4 calories per gram. In other words, the human body does not distinguish between added sugars and naturally occurring sugars in foods, so whether a person consumes 10 g of added sugars or 10 g of inherent sugars, it makes no difference to the body (Hess and others 2012).

Sweeteners

A sweetener is any naturally occurring or synthetically made substance that provides a sweet taste in food and beverages. Sucrose (table sugar) is regarded as the “gold” standard for sweet taste and is the most common sweetener in the food industry (Varzakas and others 2012). Sweeteners can generally be classified as nutritive or nonnutritive. Nutritive or caloric sweeteners are usually made by fruits, sugar cane, and sugar beets and on average provide 4 calories per gram (Varzakas and others 2012). Common nutritive sweeteners include sucrose, the other simple carbohydrates, liquid sugars, honey, syrups, and fruit juice concentrates. Nonnutritive or high-intensity sweeteners provide sweetness to food but very little or no calories, or glycemic response in the body, when they are metabolized, unlike carbohydrates (Varzakas and others 2012). Some nonnutritive sweeteners are not metabolized and are excreted unchanged by the body (Varzakas and others2012). Other nonnutritive sweeteners can be partially metabolized, to a limited degree, and their metabolites are readily excreted (Varzakas and others 2012; Carakostas and others 2012). Nonnutritive sweeteners can be derived from plant sources, such as monk fruit or stevioside, or synthetically made, such as acesulfame K, aspartame, sucralose, or saccharine. Synthetically made nonnutritive sweeteners are also known as artificial sweeteners.

Sugar Alcohols

Sugar alcohols could be placed in the nutritive sweetener group because they technically provide calories and taste similar to sucrose. However, they deserve their own discussion because of their reduced caloric value ranging from 0.2 to 3 kcal/g (Varzakas and others 2012). Unlike nutritive sweeteners, their digestion requires little or no insulin synthesis, and they are noncariogenic. When consumed in excessive, some of the sugar alcohols such as mannitol and sorbitol can have a laxative effect unlike nutritive sweeteners. Sugar alcohols are derivatives of monosaccharides, disaccharides, and other oligosaccharides, and they can occur naturally in many fruits and vegetables (Varzakas and others 2012). Because they can contribute to sweetness with fewer calories, they are commonly used as bulk sweeteners in some food products. Other products that use sugar alcohols include mouthwash, toothpaste, breath mints, chewing gum, and special foods for diabetics.

FDA Definition of Added Sugars

In FDA’s proposal, the term “added sugars” is defined as “sugars that are either added during the processing of food, or are packaged as such, and include sugars (free monosaccharides and disaccharides), syrups, naturally occurring sugars that are isolated from a whole food and concentrated so that sugar is the primary component (such as fruit juice concentrates), and other caloric sweeteners" (FDA 2014b). In other words, FDA is proposing that nutritive sweeteners that are added during the processing of food are considered added sugars. Names for added sugars in the proposal included brown sugar, corn sweetener, corn syrup, dextrose, fructose, fruit juice concentrates, glucose, high-fructose corn syrup, honey, invert sugar, lactose, maltose, malt sugar, molasses, raw sugar, turbinado sugar, trehalose, and sucrose. FDA further specified that sugar alcohols are not considered added sugars.

Chapter 3
Functional Properties of Sugar

Introduction

Sugar (sucrose) has several functional properties in food and, so far, no other sweetener has been found or developed to duplicate all or even many of them. These functional properties are derived from the sensory and physical properties of sugar and its many reactions and interactions with the other food ingredients present (Spillane 2006). Understanding the function of sugar in a food product is an important point to consider when reducing or removing sugar from the product.

Sweetness, Flavor Enhancement, and Flavor Balance

The most notable function of sugar in food is its sweet taste. Sweet taste serves as a sensory cue for energy as well as a source of pleasure. Sweetness is one of a few tastes which are innate, and it has been argued that a preference for sweet taste evolved to ensure that animals and humans chose foods that are high in calories and nontoxic (Spillane 2006). During infancy, the heightened preference for sweet tastes may have ensured the acceptance of nature's first food—mothers' milk. Human breastmilk naturally contains 2.12 g of sugar per 1 fluid ounce (USDA 2014). Therefore, these taste mechanisms apparently had a significant effect on survival.

Sweetness improves the palatability of food. Thus, adding sugar to foods with high nutrient quality may increase the chance that they are consumed. Chocolate milk is an example of increasing the palatability of milk for kids, which provides important nutrients particularly calcium, potassium, and vitamin D (Slavin 2014). Sweetness from sugar can also improve the palatability of foods for the elderly by compensating for the chemosensory losses that the elderly experience (Spillane 2006).

In food products, sugar plays an important and unique role in contributing to the flavor profile by interacting with other ingredients to enhance or lessen certain flavors. The addition of sugar enhances flavors by increasing the aroma of the flavor. A flavor aroma possesses no taste properties, but once combined with sugar, the sweetness of sugar and the flavor aroma work synergistically (Spillane 2006). For example, if a peach aroma is added to a solution with no sugar, the solution would have no taste, but with sugar added in the solution, sweetness and the peach flavor can be perceived. Small amounts of sugar can be added to cooked vegetables and meat to enhance the food's natural flavors without making them taste sweet (Kitts 2010). The addition of sugar also balances the sweetness and acidity in fruit-based products such as beverages, sauces, and preserves (Gwinn 2013). In reduced-fat ice cream, sugar is added to balance out flavor (Varzakas and others 2012), and the sweetness of sugar balances the bitterness of cocoa in chocolate (Spillane 2006).

Color and Flavor Formation

The Maillard browning reaction and caramelization are fundamental to the formation of color and flavor in several food products. Caramelization occurs when sugars are heated above their melting point in the absence of proteins causing the sugars to degrade (Varzakas and others 2012). This produces a dark brown color and imparts caramel taste and aroma in food products. Caramelization is used in a wide range of products including sauces, candies, desserts, breads, jams, and dessert wine (Kroh1994). This reaction can also be used to commercially produce caramel colors and flavors (Kitts 2010).

The Maillard reaction is another form of nonenzymatic browning, which is the result of a reaction between an amino acid and a reducing sugar (Hwang and others 2011). This is a complex reaction that depends on several factors: reactant types and concentrations, temperature, reaction time, pH, and water activity (Hwang and others 2011). Besides color formation in food, the Maillard reaction provides desirable flavor formation in several food products, such as baked goods, chocolate, coffee, and meat (Danehy and Wolnak 1983). In bread-baking, the early stages of the Maillard reaction are responsible for the pleasant aroma whereas the late-stage reactions produce the recognizable brown crust (Kitts 2010).

Bulk and Texture

Because sugar can be used as one of the primary ingredients in products, it affects the physical characteristics of food to a significant degree. Sugar provides bulk which impacts the mouthfeel and texture of many food products. Instead of being used for their sweetening properties, sometimes specific sugars are used as bulking agents or carriers for other ingredients, especially the sugars that are less sweet than sucrose (Spillane 2006). Sucrose is given an arbitrary sweetness level of 1 to allow its comparison with other sweeteners, and there is a 4 to 5-fold change in relative sweetness between the various sugars, as shown in Table 3-1.

Sugar	Relative sweetness	Reference
Sucrose	1	Varzakas and others 2012
Fructose	1.7	Varzakas and others 2012
Glucose	0.75	Varzakas and others 2012
Maltose	0.30	Varzakas and others 2012
Galactose	0.30	Varzakas and others 2012
Lactose	0.15	Varzakas and others 2012
Mannose	0.60	Tamime and Robinson 1999
Trehalose	0.45	Wilson 2007

Table 3-1 Relative sweetness of various sugars

Sugar plays an important role in the texture of bakery products. It tenderizes bakery products by competing with starch molecules and proteins for liquid components in the dough, which prevents overdevelopment of gluten and slows down gelanization (Varzakas and others 2012). During the mixing and baking of a cookie dough, the sugar dissolves, and the dough transitions into the amorphous rubbery state as water is partially evaporated via baking resulting in a soft cookie (Labuza and others 2010). Sugar also influences the spread and surface cracking of cookies (Pareyt and Delcour 2008). In foam-type cakes, sugar can help stabilize the beaten egg foam and allow the air cells to expand (Varzakas and others 2012).

In the manufacturing of ice cream and other frozen desserts, sugars are important ingredients in the ice cream mix not only for taste but for impacting the final texture and body of the finished product. Numerous small, discrete ice crystals enhance the smooth texture of ice cream, but if the ice crystals increase via recrystallization, the ice cream texture becomes coarse and undesirable. Ice recrystallization inhibition results from a highly concentrated, viscous, and heterogeneous layer surrounding the ice crystals, which prevents water mobility and ice crystals from colliding into each other (Goff 2002). Sugars not only provide sweetness but contribute solids to the liquid serum phase in order to produce this viscous and heterogeneous layer. This in turn improves ice crystal stability against ice recrystallization (E and others 2010). In addition to contributing solids, sugars also impact ice crystallization by depressing the freezing point of the ice cream mix, so that less water is frozen into ice crystals. "As a result, the consistency of the ice cream is softer and its mouthfeel less cold" (Walstra and others 2006).

Similar to ice crystallization in ice cream, sugar crystallization is a major determinant of the texture for candies. A pre-candy mix is a system that is high in sugars, and as the concentration of sugar increases, the boiling point of this mixture also increases, which allows more sugar to be dissolved and removes water (Labuza and others 2010). Upon cooling this mixture back to room temperature, the sugar is no longer in its crystalline state but has transitioned into the amorphous rubbery or glassy state (Labuza and others 2010). The more water that is removed by further boiling will determine whether the candy is in the rubbery or glassy state upon cooling with the latter being the result of a higher boiling temperature (Labuza and others 2010). The rubbery state gives gummy candies and caramels their soft texture, and the glassy state gives hard-ball candy and peanut brittle their hard glass-like texture. On the contrary, it is desirable to have sugar crystals in fudge and fondants as long as the sugar crystals are tiny, which makes for a thick, smooth candy (Kitts 2010).

Having numerous and small sugar crystals are also important in the manufacturing of sweetened condensed milk. In order to ensure the rapid formation of small-sized lactose crystals, tiny seed crystals of lactose are added during manufacturing (Walstra and others 2006). Otherwise, without seeding, large lactose crystals can form causing the product to have a sandy mouthfeel versus the desired smooth one (Walstra and others 2006).

In beverages, the high solubility of sucrose contributes to the mouthfeel of the product by giving the product body (Gwinn 2013). Sugar is also essential in the gelation of jams, preserves, and jellies. Pectin, a natural component of fruits, has the ability to form this gel, but only in the presence of sugar and acid (Varzakas and others 2012).

Fermentation

Fermentation is a process in which microorganisms in the absence of oxygen generate energy by oxidizing carbohydrates. In other words, carbohydrates including sugars are the food sources for these microorganisms. Fermentation has a long history of being used in food production. Common food and beverages produced from fermentation include yogurt, vinegar, sour cream, wine, beer, bread, cheese, soy sauce, and sauerkraut.

Lactic acid bacteria fermentations are among the most ancient and important fermentations in the world (Steinkraus 2004). This type of fermentation was significant for increasing the shelf-life of milk and preventing pathogens from growing in it. Today, it is well known for its application in fermenting dairy products. Lactic acid bacteria utilize the sugar lactose in the milk as a food source and produces lactic acid and other organic molecules. These metabolic products contribute significantly to flavor development and the final aroma and taste of fermented dairy products such as sour cream, yogurt, and cheeses. The bacteria can also produce compounds that contribute to the viscosity, body, and mouthfeel of the product (Gürakn and Altay 2010).

Yeast fermentation is another type of food fermentation. It is used in the production of yeast-leavened bakery products. Yeast can utilize starch as a food source but prefers simple sugars, such as glucose or sucrose, in the dough (Poitrenaud 2004). The fermentation of the carbohydrates produces gas causing the product to rise. This, in turn, affects the volume, crumb texture, and softness of the final product (Varzakas and others 2012).

Preservation

The addition of sugar can lower the water activity of a food, and the lowering of the water activity can restrict microorganism growth by inhibiting biochemical and enzymatic reactions (Labuza and others 2010). Because of this, water activity has been incorporated into government regulations, such as FDA 21 C.F.R. §113 (Labuza and others 2010). Under this regulation, foods with a water activity ≥ 0.85 and a pH ≥ 4.6 must be thermally processed and packaged into hermetically sealed containers so that the food is free of pathogenic microorganisms (FDA 2014a). Traditional jams, jellies, and preserves do not fall under this regulation because of their high solids content (sugar) to lower the water activity and high acid content to lower the pH. Thus, in producing sugar-free versions of jams, jellies and preserves, it is important to test the pH and water activity of the finished product to ensure its safety. For example, in one study that looked at the physical and chemical properties of sugar-free grape jellies found that the average water activity to be 0.968 (Khouryieh and others 2004), while in another study that used a regular grape jelly determined its average water activity to be 0.82 (Stamp and others 1984).

Besides helping from a food safety perspective, sugar can also aid in preserving the quality of a food product. The addition of sugar to cut fruit can slow the browning reaction caused by polyphenol oxidase due to lowering the water activity and slowing down the oxygen diffusion rate. In addition to preserving color, adding sugar can help preserve the texture of a food product. Raisins or other dried fruits are coated with sugar in breakfast cereals in order to inhibit the net moisture transfer rate from the raisins to the cereal, which keeps the cereal crisp and the raisins soft (Labuza and others 2004).

Pharmaceuticals

In addition to sweetening food, the sweetness of sugars can help the palatability of medicine to ensure patient compliance (Spillane 2006). Sugars also provide other desirable functional properties in pharmaceuticals due to its low toxicity, high purity, and diverse physicochemical properties. Most importantly, they are approved by FDA to be used. Sugars can act as an excipient by which the active ingredient of medication is introduced to the body (Spillane 2006). Lactose is a common sugar used since it can be modified for various drug delivery applications, such as compressing it into tablets or milling it for aerosol drugs (Natoli and others2009). In glucose tablets, dextrose (d-glucose) is the primary ingredient, and they are used by diabetics to quickly raise their blood sugar levels in the event of uncomfortable or disabling hypoglycemia. Given the desirable functional properties of sugars, there will always be opportunities for sugar-based products in the pharmaceutical industry.

Chapter 4

Challenges of Labeling Added Sugars

Analytical Challenges: Differentiating Added Sugars from Naturally Occurring Sugars

There are no chemical structure differences between added sugars and naturally occurring sugars in foods. Added sugars and inherent sugars are both simple carbohydrates composed of molecules of carbon, hydrogen, and oxygen. FDA's overall approach is to rely on chemical definitions of nutrients as the basis for regulatory definitions for food labeling. This was noted in FDA's proposal for the inclusion of stearic acid in the definition of saturated fat (2014b). FDA had received comments to exclude stearic acid from the definition of saturated fat because there is evidence indicating that stearic acid does not raise LDL-cholesterol levels or the risk of cardiovascular heart disease unlike saturated fat (2014b). However, FDA responded that "the definitions of nutrients for food labeling purposes have traditionally been based on chemical definitions, rather than individual physiological effects" (2014b). Thus, "added sugars" will be a unique nutrient on the proposed food label because it will be not be chemically or physiologically different than the nutrient "sugars" listed on the current food label.

FDA is not only deviating from a definition standpoint for added sugars but is steering away from an analytical-based method to verify compliance of added sugars values under FDA 21 C.F.R. § 101.9 (2014a). In FDA's proposal, it states that "there are currently no analytical methods that are able to distinguish between naturally occurring sugars and those sugars added to a food" (2014b). As a result, FDA recognizes that they will not be able to rely on an analytical method to determine compliance with the declaration of added sugars in foods that contain both added sugars and naturally occurring sugars. Therefore, FDA is proposing to require manufacturers to create and keep certain records necessary to verify the amount of added sugars present in a food that could be requested for review by the FDA (2014b).

However, there are potential analytical opportunities with stable isotope ratio analysis. This technology is commonly used to determine food fraud by adulterating the product with another ingredient to make it cheaper. For example, cane or corn syrup can be added to adulterate honey, which is a relatively expensive commodity, and this can be verified by looking at the ^{13}C to ^{12}C isotope ratio of the suspected sample (Kelly 2003). Since corn and cane syrups are derived from plants that assimilate photosynthetic CO_2 via the C_4 pathway, the ^{13}C:^{12}C ratio will be distinctly different in comparison to unadulterated honeys, which are derived from plants that utilize the C_3 pathway instead of the C_4 pathway (Kelly 2003). Yet, further research is needed especially with complex multi-ingredient food matrixes and the other added sugars defined by FDA. It will require extensive data collection in order to build robust databases and understand detection limits. There will also be confounding variables in order to determine distinct isotope ratio ranges due to natural variations of ingredients (i.e. seasonal) and the uniqueness of product formulations. Nonetheless, this technology is definitely an area opportunity for future research studies especially if the mandatory declaration of added sugars is finalized by FDA.

Overall, FDA straying away from an analytical-based method to differentiate nutrients on the Nutrition Facts label will lead to challenges for implementation across the food industry and potentially inaccurate declaration of added sugars values. More research is needed to determine validated analytical methods that will be applicable to all food product types.

No Universal Definition for Added Sugars

There is no universal definition for added sugars. Table 4-1 summarizes the various definitions of added sugars across organizations. Compounded by the fact there is no analytical method to determine the amount of added sugars, multiple definitions for added sugars can result in inconsistencies and misinterpretations by consumers, scientists, food manufacturers, ingredient suppliers, and regulators alike. This would be true for any nutrient not just for added sugars.

For example, FDA uses the term “added sugars” whereas the World Health Organization (WHO) uses the term “free sugars” (WHO 2015). WHO definition of “free sugars” includes fruit juices whereas the FDA definition of “added sugars” is limited to fruit juice concentrate (WHO 2015). This could create a challenge for food manufacturers that use international suppliers. An international supplier may mistake that “free sugars” and “added sugars” are identical and count any fruit juice in the formulation as added sugars, which would result in over declaring the amount of added sugars on the ingredient’s nutrition information as proposed by the FDA regulations.

Organization	Definition	Exceptions
FDA 2014b	Sugars that are either added during the processing of food, or are packaged as such, and include sugars (free monosaccharides and disaccharides), syrups, naturally occurring sugars that are isolated from a whole food and concentrated so that sugar is the primary component (fruit juice concentrates), and other caloric sweeteners.	Sugar alcohols and naturally occurring sugars such as lactose in milk or fructose in fruits
IOM 2005	Sugars and syrups that are added to foods during processing or preparation. Specifically, added sugars include white sugar, brown sugar, raw sugar, corn syrup, corn-syrup solids, high-fructose corn syrup, malt syrup, maple syrup, pancake syrup, fructose sweetener, liquid fructose, honey, molasses, anhydrous dextrose, and crystal dextrose.	Naturally occurring sugars such as lactose in milk or fructose in fruits
WHO 2015	Free sugars refer to mono- and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices, and fruit juice concentrates.	Intrinsic sugars (incorporated within the structure of intact fruit and veggies) and sugars from milk (lactose)
AHA 2014	Added sugars include any sugars or caloric Sweeteners that are added to foods or beverages during processing or preparation. Added sugars (or added sweeteners) can include natural sugars such as white sugar, brown sugar and honey as well as other caloric sweeteners that are chemically manufactured (such a high-fructose corn syrup).	Naturally occurring sugars found in foods such as fruit (fructose) and milk (lactose)
DGA 2010 (USDA and HHS 2010)	Caloric sweeteners that are added to foods during processing, preparation, or consumed separately.	Naturally occurring sugars such as lactose in milk or fructose in fruits
DGAC Technical Report 2015 (USDA and HHS 2015a)	Used the same definition proposed by FDA (2014b).	
DGA 2015 (USDA and HHS 2015b)	Added sugars include syrups and other caloric sweeteners.	Naturally occurring sugars, such as those in fruit or milk

Table 4-1 Various added sugars definitions

No Added Sugar Claim and Reconstituted Fruit Juice Concentrates

Current FDA regulations do allow for a "no added sugar" claim under 21 FDA C.F.R. § 101.60 (2014a). Overall, the criteria for this claim and the proposed definition of added sugars are similar. However, there are some inconsistencies that can be emphasized with current food products making this claim, such as juices or other beverages.

According to the current regulations, foods bearing the "no added sugar" claim in food labeling must not contain an ingredient that is added during processing or packaging that is "sugar," as defined in FDA 21 C.F.R. §101.9 (2014a), or is an ingredient that contains sugars that functionally substitutes for added sugars. Sugars are defined in FDA 21 C.F.R. §101.9 (2014a) to mean "the sum of all free mono- and disaccharides (such as glucose, fructose, lactose, and sucrose)," and examples of any other ingredient containing added sugars include jam, jelly, or concentrated fruit juice. FDA further clarified in the 1993 preamble to the final ruling that the mere presence in a food of an ingredient containing intrinsic sugars, such as fruit juice or concentrated juice, would not disqualify a food from bearing a "no added sugar" claim as long as the ingredient was not added to functionally substitute for added sugars (FDA 1993). For instance, the addition of a concentrate of the same juice, to achieve uniformity, or the addition of water to a juice concentrate, to produce a single strength juice, would not preclude the use of a "no sugar added" claim (FDA 1993).

Fruit juice concentrates are often preferred by food manufacturers for several reasons including sustainability, sourcing, and logistics. Essentially, it is a lower-cost ingredient than fruit juice, because removing the water from fruit significantly reduces the volume and weight of the product that must be shipped. Thus, a food manufacturer may purchase fruit juice concentrate and partially or fully reconstitute it back to single strength fruit juice as a step in the manufacturing of the finished product. In FDA's proposal, fruit juice concentrates are considered added sugars whereas single strength fruit juice is not. This presents the important question whether a fruit juice concentrate that has been fully reconstituted back to juice would be considered added sugars under the proposal.

For example, a manufacturer is making a 100% apple juice product with apple juice concentrate and water. The ingredient apple juice concentrate contains approximately 39% sugar, and in order to make a 100% apple juice product with the minimum Brix level of 11.5 per FDA 21 C.F.R. § 101.30 (2014a), at least 29.5% of the finished product formula needs to be apple juice concentrate. Because water is the only other ingredient used in the formula, all the sugar in the finished product is coming from the apple juice concentrate, and on an 8 fluid ounce serving size, the total sugars label at a rounded value of 29 g. Because the apple juice concentrate has been fully reconstituted back to single strength, the product qualifies for the current "no added sugar claim," but it could nonetheless be required to declare 29 g of added sugars on the label under the proposed rule because a fruit juice concentrate has been added during the processing of the finished product.

Thus, this example illustrates that it is imperative that the proposed definition of added sugars be consistent with the current "no added sugar" claim definition. Overall, the individual consuming the food with fruit juice or reconstituted fruit juice concentrate will be consuming the same amount of sugar from the same source, fruit (GMA 2014).

Functionality of Added Sugars

As discussed in the previous section, sugars are added to food for various reasons in addition to providing sweetness. Therefore, this poses the question on whether sugars added to food for other reasons than sweetening should be considered added sugars. Under FDA 21 C.F.R. § 101.60 (2014a), one could argue it would be inappropriate to include sugars that are added to food for purposes other than sweetening as "added sugars" (GMA 2014). For example, some natural flavor preparations may analyze for a trace amount of sugars but their intended purpose is not to function as a sweetener but as a flavor enhancer. On the contrary, one could argue any substance with calories that can contribute to the sweetness of a product could be considered a "caloric sweetener" (General Mills 2014).

For yeast-leavened bread, the yeast prefers the simple sugars glucose, sucrose, and fructose over starch during fermentation (Poitrenaud 2004). Additional sugars are added during bread fermentation because the composition of flour is insufficient in these simple sugars. If no sugars are added, then maltose from the starch must be degraded once the naturally present simple sugars in flour are exhausted. Not all yeast types can break down maltose without a lag adjustment, which results in a depression of gas production during the fermentation process (Poitrenaud 2004). Thus, the addition of a small amount of sugars (about 2% to 7%) is added to dough to increase the effectiveness of yeast during fermentation (Poitrenaud 2004). The added sugars act as a temporary leavening agent, while being used by the yeast, causing the dough to rise at a quicker and more consistent rate.

As stated earlier, sugars such as dextrose or lactose are also used as a carrier for other ingredients because of their bulking properties. The sugars associated with these ingredients are incidentally added to the finished product with no intention of them to function as a sweetener. Isomaltulose and tagatose could be classified as sugars from a structural standpoint, and they are commonly used as bulking agents, like lactose, but are also used for their sweetness (Wilson 2007; Sentko and Bernard 2012). When comparing relative sweetness, both are sweeter than lactose (Table 3-1and Table 6-2) and, therefore, a more accurate description of them is "bulk sweeteners" versus "bulking agents." However, they were not named in the FDA's proposal as added sugars, even though tagatose and isomaltulose could be added to a food product as a free monosaccharide or disaccharide, respectively.

Perhaps FDA did not include these sugar-like compounds because they differ from a physiological standpoint when compared to a typical sugar. They do not promote tooth decay and have lower glycemic values compared to sugar (Sentko and Bernard 2012; Vastenavond and others 2012). Thus, they are more like sugar alcohols than typical sugars, so it brings up the question whether they should be considered "added sugars."

The various functionalities of added sugars become more evident with the other nutritive sweeteners that FDA classified as "added sugars" and multi-component ingredients that can contain both inherent sugar and sugar added during processing. For some food products, fruit juice concentrates are added to food for the sole function of providing color, following section FDA 21 C.F.R. § 73.250 (2014a). Juice concentrates are also commonly used to adjust the Brix levels of directly expressed juice, and these juice concentrates are not required to be reflected in the common or usual name of such juices under FDA 21 C.F.R. § 102.33(2014a). Fruit and fruit puree ingredients that contain some sugar added during processing are often added to foods for several purposes, such as providing texture, flavor, nutrients, sweetness, or adjusting soluble solids (GMA 2014).

One example with fruit purees is applesauce that may be used as a substitution for oil in baked goods in order to reduce the fat content (GMA 2014). Applesauce exists in both sweetened and unsweetened forms. In both, there are inherent sugars from the apples, but in the sweetened form, there are also sugars added during processing. Both the inherent sugars and the added sugars may contribute to the sweetness of the product. In this case, the main functionality of the applesauce is an oil substitution, but because it may also contribute to sweetness, it brings up the question whether the sugars, inherent or added, in the applesauce should also be considered added sugars.

Conversely, sweetened condensed milk is added to dessert products for sweetness and flavor. It is made from milk that has been heated to remove some of the water, and this evaporation of the water concentrates the inherent sugar (lactose) in the product. A nutritive carbohydrate sweetener such as sucrose is added during its manufacture, hence, the name sweetened condensed milk (FDA 21 C.F.R. § 131.120 2014a). As discussed earlier in Chapter 3, seed crystals of lactose are also added in order to prevent a sandy mouthfeel in the finished product. Thus, it contains a mixture of inherent concentrated lactose sugar and added sucrose and lactose sugar. This manufacturing process highlights whether the inherent lactose sugar, which has been concentrated similar to fruit juice concentrates, and the addition of the free disaccharide lactose via seed crystals, with no intention as functioning as a sweetener, should be considered "added sugars" in addition to the added sucrose. This is further complicated when the manufacturer adds water in conjunction with the sweetened condensed milk in the finished product, and then the water would fully or partially reconstitute the concentrated lactose.

In summary, depending on how a food manufacturer interprets the definition and the functionality of the "added sugars" in the product, various added sugars values could be declared on the label. FDA not only needs to specify the functionality of added sugars in the definition but may need to specify the physiological characteristics as well.

Some Sweeteners are Not 100% Sugars

FDA's proposed definition includes syrups, honey, and molasses as "added sugars," but these sweeteners are not 100% sugars. They are always mixtures of sugars and water, and some of them also include other nutrients or substances. For instance, molasses naturally contains high levels of vitamins and minerals, such as calcium, potassium, iron, and B vitamins (Varzakas and others 2012). Figure 4-1 summarizes the amounts of water, sugars, and other substances in these types of sweeteners. Thus, the "added sugars" definition should be clarified further that the sugars in syrups, honey, molasses, and similar products should be considered on a dry weight basis ensuring that the contribution of the sugars in these foods is represented accurately (GMA 2014).

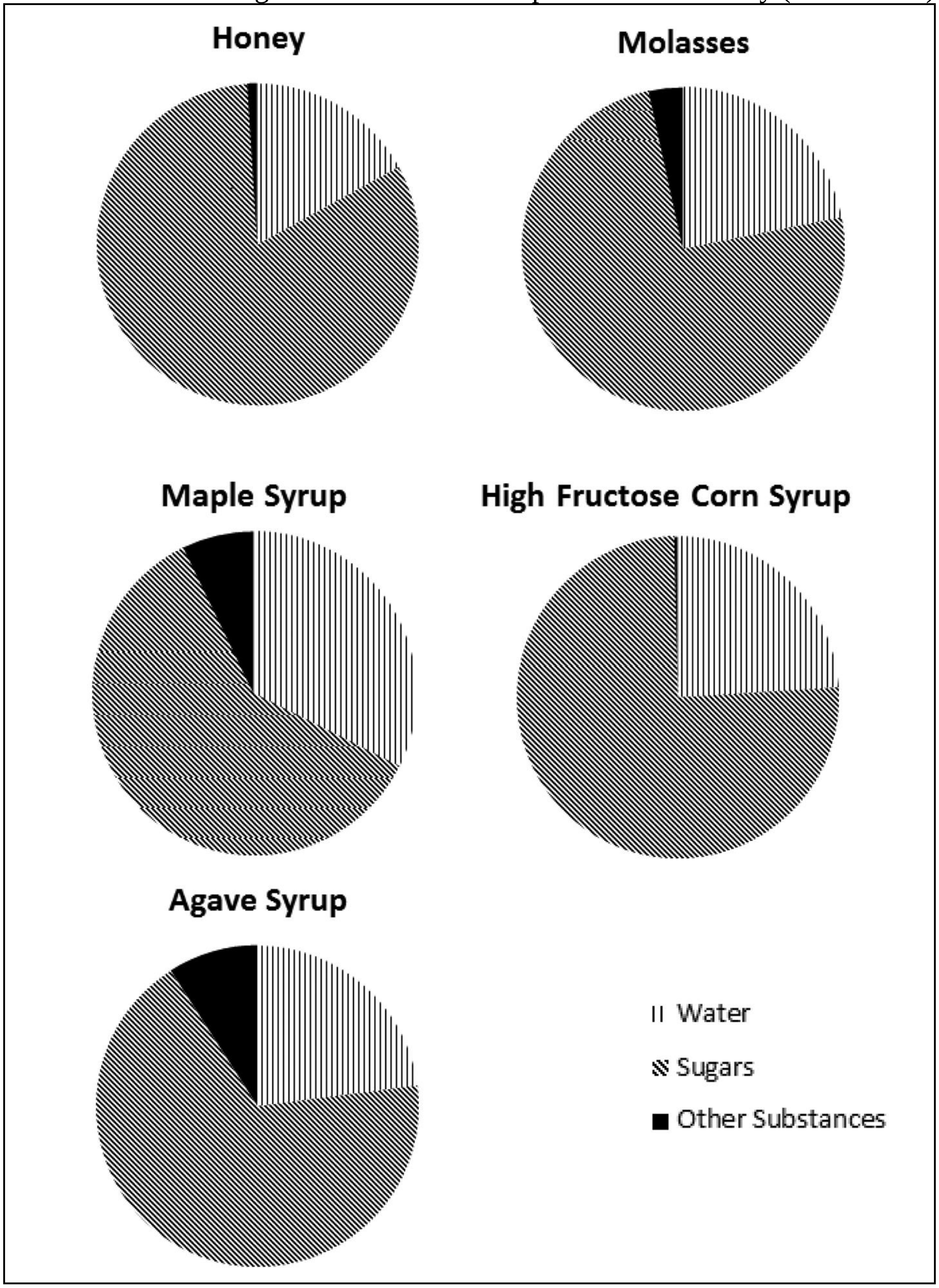

Figure 4-1 Composition of different sweeteners (USDA Natl. Nutrient Database for Standard Reference 2014)

Ingredient Supplier Complications

The lack of a recognized analytical method for added sugars not only causes challenges for the FDA but for food manufacturers as well. Food manufacturers would need access to certain records or information from ingredient suppliers in order to determine the finished product added sugars values. This could be challenging for food manufacturers that use hundreds, or many more, of ingredients. Some ingredient suppliers are small and do not have the current databases or resources to provide this information immediately. Furthermore, suppliers may not want to over share information, due to proprietary reasons, because there is the potential risk of formula information getting in the hands of competitors.

Besides these issues, ingredient suppliers can also interpret the definition of added sugars differently. For example, nonfat dry milk is a common ingredient sourced in food manufacturing. Similar to fruit juice concentrates, nonfat dry milk is often preferred by food manufacturers because of the decreased cost and increased shelf-life compared to traditional pasteurized milk. It is made by removing most of the water from pasteurized skim milk in order for it to contain no more than 5 percent by weight of moisture (FDA 21 C.F.R. § 131.125 2014a). As discussed earlier with condensed milk, this removal of the water concentrates the inherent sugar (lactose), which results in about 52% of the ingredient to be sugar (USDA 2014). Therefore, an ingredient supplier may interpret the definition that nonfat dry milk is "added sugars" because it has been concentrated so that sugar is the primary component, but another supplier may rationalize that this ingredient has not been isolated from its original source milk since other nutrients are present, such as protein. As a result, this ingredient supplier concludes it is not "added sugars." Of course, this will cause confusion if a food manufacturer is sourcing nonfat dry milk from both of these suppliers, especially since under FDA 21 C.F.R. § 101.4 (2014a), nonfat dry milk may be declared as "skim milk" or "nonfat milk" in the ingredient deck, which is excluded from the definition of added sugars.

As stated earlier, this becomes even more complicated with other functional uses of added sugars. When milk powders are made via spray drying, the lactose sugar is generally in the amorphous glassy state after drying (Labuza and others 2010). Because amorphous sugars are hygroscopic, these milk powders are highly susceptible to caking if exposed to high humidity, and caking interferes with the powder's ability to dissolve or be free flowing (Labuza and others 2010). Whey powder is especially prone to caking given the high amount of amorphous lactose, but this issue can be considerably reduced if the lactose is crystallized prior to spray drying (Walstra and others 2006). This crystallization can be done by seeding with lactose crystals just like in the manufacturing of sweetened condensed milk. Even though lactose is added as a free disaccharide during manufacturing, its purpose is to reduce caking issues and also increase the yield of the milk powder versus functioning as sweetener.

Depending on how the definition is interpreted, ingredient suppliers may declare various added sugars values for the exact same ingredient, and this is further complicated with minor differences in calculations and rounding-off numbers. Thus, this could be a time-consuming process for food manufacturers and ingredient suppliers to agree on an added sugars value for an ingredient.

Complexity of Chemical Reactions

There are significant difficulties calculating the added sugars in products subjected to fermentation, carmalization, and Maillard reactions. These reactions metabolize or transform sugars into other compounds that are no longer detectable as sugars through conventional analytical methods (Perez-Locas and Yaylayan 2010). FDA acknowledges these complications from these reactions and requested more information from manufacturers of such products because FDA (2014b) does not have adequate data to assess the degradation themselves.

Yeast-leavened bread shows all 3 reactions during breadmaking, and the American Bakery Association in its comments to FDA's proposal responded that it would be extremely difficult to calculate the reduction in added sugars in yeast-leavened products with both naturally occurring and added sugars. As discussed earlier, additional sugars are added for improved fermentation because the inherent composition of flour is insufficient in simple sugars. This fermentation process in yeast-leavened bread is influenced by several "factors including but not limited to, variations within ingredients (such as yeast activity and flour quality), ingredients (such as the type and/or amount of sugar, salt, malt, vinegar, spices, and other components), varying types of fermentation systems (water brews, flour brews, sponge and dough, straight dough), and variation in make-up and proof times and temperatures" (American Baking Association 2014). Besides sugar loss during fermentation, sugars can also disappear during the baking process through caramelization and Maillard reactions, which cause the browning of the crust in bread (Purlis 2010).

Other fermented products, such as certain types of alcoholic beverages and yogurts, are other examples that will have potential challenges in quantifying the amount of added sugars lost as the result of fermentation. Alcoholic beverages, such as wine and beer, use fermentation to convert sugars into alcohol. Majority of alcoholic beverages fall under the labeling provisions of the Federal Alcohol Administration Act (FAA Act), but in certain circumstances where an alcohol beverage is not covered by the FAA Act, the beverage is subject to the ingredient and other labeling requirements under the FDA (Alcohol and Tobacco Tax and Trade Bureau [TTB] 2008). FDA labeling requirements apply to beers that do meet the definition of a malt beverage under the FAA Act, such as beers that are brewed from a substitute for malt (rice or corn), and wines with an alcohol content of less than 7% alcohol by volume, such as some types of cider beer (TTB 2008). Cider beer is commonly made from apples, which naturally contain the sugars sucrose, glucose, and fructose, as shown in Figure 2-1. However, sweeteners can be added during manufacturing because the sugar content is lower than expected in the apples due to seasonal and/or variety reasons or the manufacturer simply wants to increase the alcohol content or add “back carbonation” during bottling (Watson 2013). If sucrose is added during manufacturing, the microorganisms will consume either the added sucrose and/or naturally occurring sucrose in the apples. It makes no difference to the microorganisms; as of now, it is impossible to determine which is being consumed during fermentation.

In the case of diary yogurt, this will be discussed further in Chapter 5, but fermented soymilk to produce a dairy alternative yogurt is another example that will have issues with sugar loss via fermentation. Sucrose occurs naturally in soymilk, but sucrose can also be added to the soymilk prior to fermentation. Cultures, such as *S. thermophilus*, are able to grow in soymilk, which can use either the naturally occurring or added sucrose (Garro and others 1998). Again, it would be impossible to know which one the cultures are metabolizing, and how this fermentation would impact the labeled “added sugar” value is brought to life in the last section of this chapter with a case study.

Without a validated analytical test to distinguish added sugars from those naturally occurring in all food matrixes, manufacturers will not be able to discern where the sugar loss is occurring as result of these reactions. These chemical reactions depend on several variables, which are unique to each formula and process, and it would be impossible to come up with a standard equation that could be applied across each similar food product. Therefore, it would be a time-consuming process to research each unique product formula, and manufacturers may resign themselves to declaring all added sugars without a reduction from these reactions, thereby resulting in an overstatement of the amount of added sugars in these types of products.

Fruit-flavored Soy-based Yogurt Alternative Case Study

The following is a case study summarizing the complexities and challenges of labeling added sugars in a fruit-flavored soy-based yogurt alternative. A typical ingredient deck for this type of food product is below, and the ingredients underlined could be or could not be considered “added sugars” depending on the various interpretations of FDA’s proposed definition. This example also highlights the impact of fermentation. Table 4-2 summarizes the interpretations and how different added sugars labeled values could be derived for the same product.

Ingredients: Cultured Pasteurized Soymilk, Sugar, Blueberries, Pectin, Calcium Carbonate, Elderberry Juice Concentrate (for color), **Natural Flavor.**

In this particular case, the manufacturer is receiving a sweetened plain soymilk with sucrose. According to the USDA database (2014), an unsweetened plain soymilk naturally contains 1 g of sucrose per cup (243 g). The serving size of the soy yogurt is 6 oz (170.1 g). The formula assumptions of the finished product are listed in Table 4-3, and the amount of sugar from each ingredient was determined by using the USDA database (2014). The amount of sugar lost as a result of fermentation was estimated to be 2 g (Farnworth and others 2007; Hou and others 2000).

	Interpretation 1	Interpretation 2	Interpretation 3	Interpretation 4
Ingredient(s) considered added sugars	Sugar, fruit, soymilk, natural flavor, juice concentrate	Sugar, natural flavor, juice concentrate	Sugar	Sugar (fermentation loss from added sugars only)
Ingredient(s) excluded from added sugars calculation		Inherent sugar in soymilk and fruit	Inherent sugar in fruit and soymilk, natural flavor, juice concentrate	Inherent sugar in fruit and soymilk, natural flavor, juice concentrate
Added sugars (unrounded)[a]	22.78 g	22.1 g	21.92 g	21.22 g
Total sugars (unrounded)[b]	22.78 g	22.78 g	22.78 g	22.78 g

Interpretation one: The manufacturer interprets that any substance with calories that contributes to the sweetness of a product could be considered a "caloric sweetener."

Interpretation two: The manufacturer subtracts the inherent sugars from the soymilk and the blueberries from the added sugars value, but keeps the trace amount of sugars in the natural flavor and the fruit juice concentrate as added sugars.

Interpretation three: The manufacturer only counts the added sugars from the sweetened soymilk, the fruit blend, and the entire amount of sugar directly added in the formula. In this case, the manufacturer rationalizes that the functionality of the natural flavor and fruit juice concentrate is not for sweetening. The natural flavor is for flavoring, and the fruit juice concentrate is for coloring.

Interpretation four: The manufacturer is following the same rationale as Interpretation three, but also assumes that the cultures only metabolize the added sucrose versus the inherent sucrose in the soymilk.

[a] This is the unrounded figure in order to show the variation.

[b] This is the total with a 2 g fermentation sugar loss.

Table 4-2 Summary of various labeled added sugars values based on different interpretations for a fruit-flavored soy-based yogurt alternative product

		Grams per serving size (170.1 g)		
Ingredient	% of Finished product	Inherent	Added	Total
Sweetened plain soymilk	80	0.7	3.5	4.2
Sugar	10		17	17
Blueberry fruit blend (50% fruit and 20% sugar)	8	0.678	2.72	3.40
Pectin	1			0
Calcium carbonate	0.5			0
Elderberry juice concentrate	0.3			0.1
Natural flavor	0.2			0.08
Total	100			24.78[a]

[a] This is the total before the 2 g are subtracted for fermentation.

Table 4-3 Formula assumptions for fruit-flavored soy-based yogurt alternative case study

Chapter 5

The Impact of Fermentation on Added Sucrose in a Nonfat Yogurt

Introduction

In the United States, yogurt, lowfat yogurt and nonfat yogurt are standardized foods, and within these standard of identities, the bacterial cultures used for culturing the dairy ingredients must contain the lactic acid-producing bacteria: *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (21 C.F.R. §§ 131.200, 131.203, 131.206 2014a). These bacteria grow better together than alone forming a mutualistic relationship based on the exchange of growth enhancing metabolites (Sieuwerts and others 2010). *S. thermophilus* releases formic acid and carbon dioxide, which stimulates *L. bulgaricus*, and the proteolytic activity of *L. bulgaricus* produces stimulatory peptides and amino acids for *S. thermophilus* (Gürakn and Altay 2010). Both of these bacteria metabolize the naturally occurring sugar lactose in milk, which is about 5% in regular, unsweetened milk (USDA 2014). During fermentation, the bacteria convert a portion of the lactose into 0.9% to 1.4% lactic acid, which results in the pH drop of the yogurt product (Walstra and others 2006; Gürakn and Altay 2010).

Because a portion of the lactose is converted to lactic acid, this also decreases the total sugar content of the yogurt product in regards to labeling. Thus, the “Total Sugar” value must be adjusted on yogurt products. Yet, with the recent proposed rule by FDA (2014b), “Added Sugars” will be required to be declared on the nutrition facts panel in addition to the “Total Sugar” value, and yogurt is commonly sweetened with ingredients that FDA has defined as “added sugars,” especially sucrose. Sucrose (dry or liquid) is frequently added to the milk blend prior to fermentation, but it is unknown on how fermentation will impact added sucrose in a dairy yogurt.

Prior to this proposed rule, it was not of importance if the lactic acid bacteria were only consuming the inherent lactose from the milk. Therefore, previous studies that added sucrose prior to fermentation were more concerned about the impact of various amounts of added sucrose on the growth of the bacteria cultures and the sensory characteristics of the finished product. Fernández-García and others (1998) and McGregor and White (1986) compared yogurts sweetened with 0%, 2%, 4%, and 6% sucrose in regards to fermentation time, bacterial counts, and sensory scores. Vinderola and others (2002) examined the effect of various food additives, including sucrose at 5%, 15%, and 20% concentrations, on the growth of lactic acid bacteria commonly used in fermented dairy products. Other studies have measured similar variables in regards to bacterial growth and sensory characteristics in order to compare sucrose with other sweeteners including nutritive and high potency sweeteners (Keating and White 1990; McGregor and White 1987; Popa and Ustunol 2011).

Now, with the potential mandatory declaration of added sugars, it is significant to understand if the initial amount of the added sucrose remains after fermentation. It is well established that *S. thermophilus* and *L. bulgaricus* metabolize lactose, but both can utilize other sugar sources in addition to lactose. *S. thermophilus* is highly adapted to grow on lactose and only ferments the glucose moiety of lactose, while the galactose moiety is excreted into the medium (van den Bogaard and others 2004). However, studies with various strains of *S. thermophilus* have shown that *S. thermophilus* is capable to metabolize sucrose as a carbon source in addition to lactose and glucose (van den Bogaard and others 2004; Thomas and Crow 1983). *L. bulgaricus*, on the other hand, cannot utilize sucrose as a pure culture (Amoroso and others 1989; Amoroso and Manca de Nadra 1992). This phenomenon has also been seen with **s**tudies utilizing similar cultures as in dairy yogurt to produce a fermented soymilk product (Murti and others 1993; Mital and Steinkraus 1974). Sucrose, which naturally occurs in soymilk, is the main fermentable sugar in soymilk, and *S. thermophilus* is well able to grow in soy beverages because of its ability to use sucrose (Garro and others 1998; Farnworth and others 2007; Chumchuere and Robinson 1999).

Since it is well established that S. *thermophilus* can metabolize sucrose, the objective of this study is to investigate the impact of fermentation by commercial yogurt cultures, *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, on sucrose added prior to fermentation in the production of a nonfat yogurt. Overall, the significance of this study is to understand the implications of added sugars labeling for dairy yogurts.

Materials and Methods

Preparation of Yogurt Mix

The same amount of organic nonfat dry milk powder (Frontier, Norway, IA) was used to make each batch, so that each batch had the same amount of initial lactose prior to fermentation. Compositional analysis of the nonfat dry milk powder showed that the milk solids nonfat (MSNF) of the powder was 96.42%, which was used to determine the amount of nonfat dry milk powder to add to each batch in order to target an initial 10% MSNF level prior to the water addition. Granulated sucrose (Crystal Sugar, Minneapolis, MN) was added at a level of 4%, 8%, and 12% by weight, and the control batch was devoid of added sucrose. The amount of water that was used in each batch to reconstitute the nonfat dry milk powder was adjusted accordingly based on the amount of added sucrose in the batch. Table A-5.1 in the appendix shows the formulation for each batch used in this study. Once the ingredients were added and blended together, each yogurt mix was then batch pasteurized for 30 minutes at 65°C (Tamime and Robinson 1999) using the HotmixPRO, a thermal mixer (Vitaeco S.R.L, Modena, Italy). After the batch was pasteurized, the yogurt mix was transferred to an ice bath until a temperate of 43°C or lower was reached in preparation for the culture inoculation.

Cultures and Inoculation

Commercial starter cultures (Yoflex ® Mild 2.0), which contained a majority of *Streptococcus salivarius* subsp. *thermophilus* bacteria than *Lactobacillus delbrueckii* subsp. *bulgaricus* bacteria, was provided by Chr. Hansen Inc. (Milwaukee, WI) in Direct Vat Set form. The storage, maintenance, and preparation of the cultures were carried out as per the recommendation of the manufacturer. A 1:10 dilution of the cultures was prepared using a portion of the pasteurized milk base after it cooled down to 43°C or lower. Then, each batch was inoculated at a 0.02% rate by adding 2 mL/L of the 1:10 dilution culture mixture.

Fermentation and Storage Conditions

After inoculation, each batch was poured into 5 sample containers (125 mL), and then, incubated in a water bath at 43°C for fermentation until a pH of 4.6 was obtained. One of the sample containers was used for pH checks starting at 3.5 hours into the fermentation period and then at intervals of 30 minutes until a pH of 4.6 was reached. The time taken to reach pH = 4.6 was recorded for each batch in order to study the effects of the various sucrose concentrations on fermentation time. The sample used for pH checks was then discarded, and the remaining samples were quickly cooled to 25°C in an ice bath and then placed in a blast freezer at -10°C to prevent any further fermentation. For reference, step by step instructions for the yogurt manufacturing in this study can be found in the appendix.

Experimental Design

The experiment was arranged in a completely randomized design with two replications (Batch A and B) for each sucrose concentration: 0%, 4%, 8% and 12% by weight. Thus, a total of eight yogurt batches were produced in the study. Two samples from each batch were randomly labeled and blocked into two different submissions for sugar analysis, so that there were analytical duplicates for each batch prepared in the study. The remaining two samples per batch were thawed to 25°C to re-check the pH and determine the titratable acidity.

Analytical Methods

Compositional analysis of the nonfat dry milk powder included ash (AOAC 930.30), protein (Kjeldahl, AOAC 930.29), fat (Mojonnier, base hydrolysis, AOAC 932.06), moisture by vacuum (AOAC 927.05) and carbohydrate by difference (Rtech Laboratories, Arden Hills, MN). The pH values of the samples were measured using a pH meter (Fisher Scientific, Pittsburgh, PA). The titratable acidity of the samples was determined by titration with 0.01 N NaOH solution using phenolphthalein as a color indicator and expressed as percent lactic acid (AOAC 947.05).

Samples that were submitted for sugar analysis were analyzed by HPLC for lactose, sucrose, and fructose (AOAC 982.14; Rtech Laboratories, Arden Hills, MN). Since the peaks for glucose and galactose cannot be satisfactorily separated under the HPLC assay as shown in previous studies (Hou and others 2000; Richmond and others 1987), glucose and galactose were determined via enzymatic methods. Glucose was measured using the enzyme D-Glucose test kit from Boehringer Mannheim/R-Biopharm, and galactose was determined using the rapid enzymatic assay procedure from Megaenzyme for Lactose and D-Galactose, K-LACGAR 03/14 (Rtech Laboratories, Arden Hills, MN). The raw data of the sugar analysis is presented in Table A-5.2 in the appendix.

Statistical Analysis

Statistical analysis was performed using Minitab 17 (version 17.2.1; Minitab Inc., State College, PA). The means and standard deviations were calculated for each of the sugars analyzed per the initial added sucrose level (%) of the batches and then further broken down by Batch A and B (Table 5-1). A standard regression analysis was performed on the average of the analytical sucrose values per each batch made versus the initial added sucrose (%) of each batch in order to determine the regression line equation.

This regression line was then compared to a perfect line (m = 1; y = 0) where analytical sucrose equals initial added sucrose ([0,0], [4,4], [8,8], [12,12]) by determining the 95% confidence intervals based on t-distribution for the slope and y-intercept of the regression line.

Results and Discussion

Effect of Added Sucrose on Fermentation Time

In general, as the amount of added sucrose increased, the fermentation time to an end pH of 4.6 also increased as shown in Figure 5-1. Fernández-García and others (1998) and McGregor and White (1986) found similar results in regards to fermentation time when comparing yogurts sweetened with 0%, 2%, 4%, and 6% sucrose.

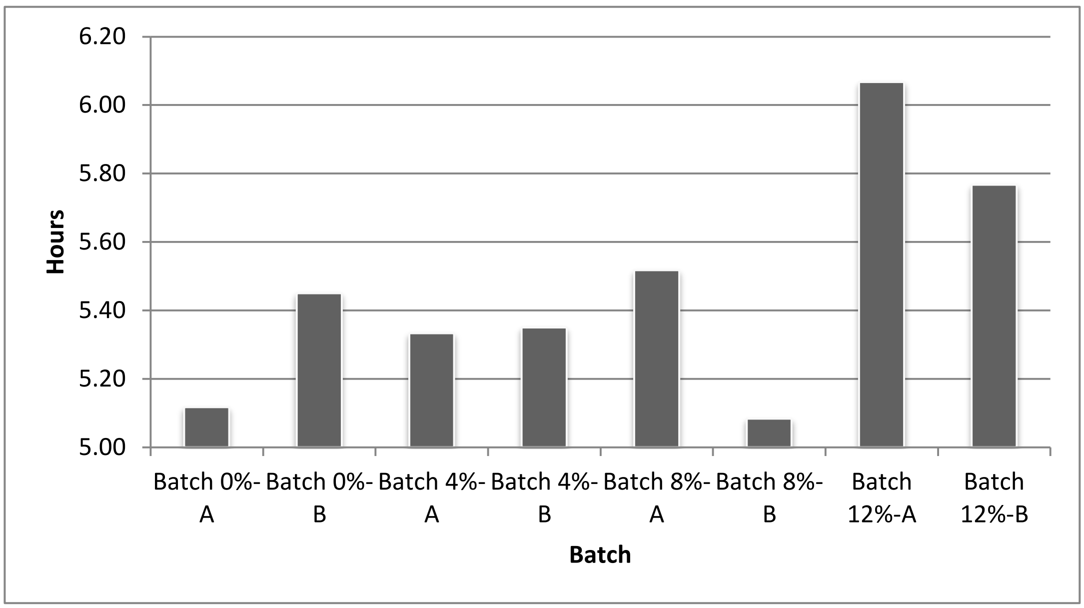

Figure 5-1 Fermentation times of batches

McGregor and White (1986) also saw that the fermentation time for the samples with no added sweetener were significantly shorter when compared with those containing sweeteners and concluded that this difference was the result of increased total solids instead of the presence of a sweetener. Increased total solids in the yogurt mix can cause slower growth of the cultures with a critical inhibitory concentration around 22% (Tramer

1973). This would explain the much higher fermentation times for the 12% added sucrose batches since the total solids concentration was above 23% (Table A-5.1). Because of the slower growth of the yogurt cultures, the total amount of sugar solids in the yogurt mix should not exceed 10-11% (Chandan and O'Rell 2006; Özer 2010).

Changes of Added Sucrose after Fermentation

The regression analysis was used to determine the regression equation: Analytical Sucrose (%) = -0.1100 + 0.9788 Initial Added Sucrose (%). The rest of the regression analysis can be seen in Table A-5.3 in the appendix. This regression line was then compared to a perfect line where analytical sucrose equals initial added sucrose by calculating the 95% confidence intervals for the slope and y-intercept of the regression line equation. The results showed that the slope (m = 1) and y-intercept (y = 0) of a perfect line falls within the 95% confidence intervals of the slope (0.9556, 1.0020) and y-intercept (-0.2837, 0.0637) of the regression line. The 95% confidence intervals are visually shown in Figure 5-2. Thus, analytical sucrose values (%) are close within the initial added sucrose values (%) indicating that added sucrose remained after fermentation.

Another indication that added sucrose remained after fermentation is because there was no significant accumulation of fructose in the medium. *S. thermophilus* prefers to metabolize lactose, glucose or sucrose over fructose or galactose (van den Bogaard and others 2004; Thomas and Crow 1983). As for *L. bulgaricus*, it can metabolize fructose but prefers the sugars glucose and lactose (Sobowale and others 2011). As a result, if any of the added sucrose was metabolized during fermentation, it would have been expected to see fructose to accumulate in the medium similar to how galactose accumulates in the medium since it is released when lactose is metabolized (Zourari and others 1992; O'Leary and Woychik 1976). According to the raw data of the sugar analysis (Table A-5.2), fructose was only reported on one of the analysis days, but the sister samples had none reported on a different day. Presumably, this is due to the different interpretations of the peaks on the chromatograms. Given what is understood about sugar metabolism in *S. thermophilus* and *L. bulgaricus*, the samples with no fructose reported are more likely.

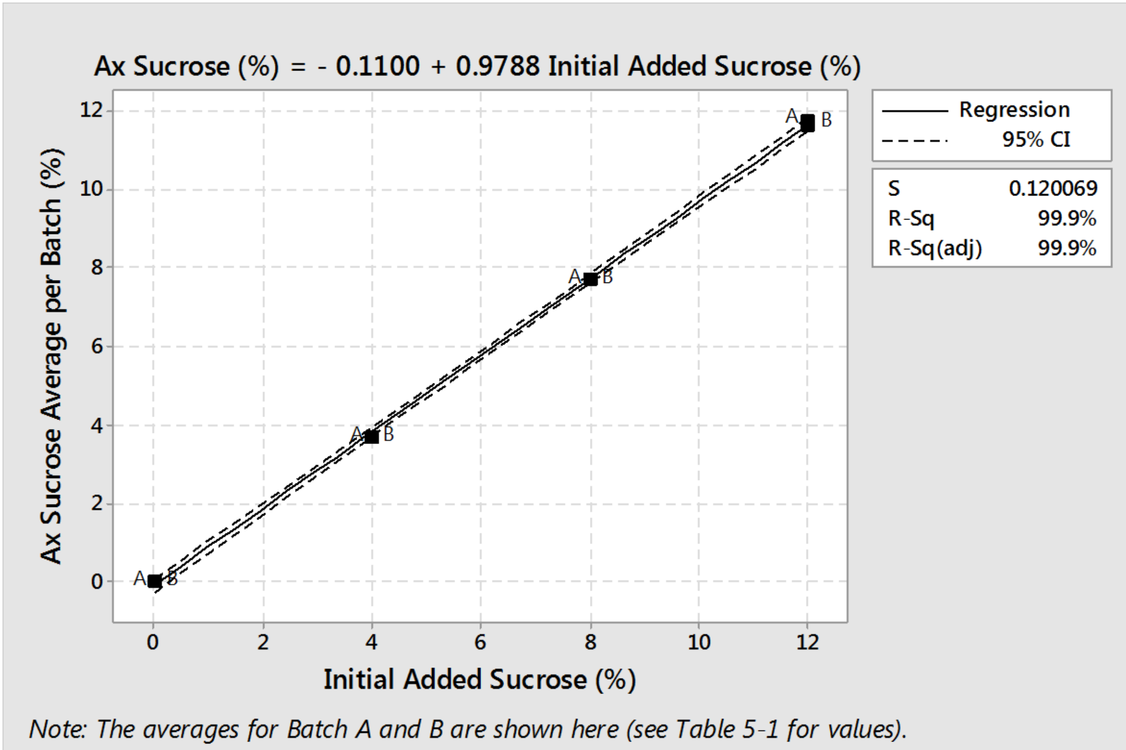

Figure 5-2 Fitted line plot after fermentation

As shown in Table 5-1, the average analytical sucrose values (%) for the 4%, 8%, and 12% batches are slightly lower than the initial sucrose concentrations added to these batches. This could be partially explained by the fact that anhydrous sucrose was not used in the study. Experimental variability is another potential contributing factor since it was the main source of variability in the study in comparison to analytical variability (CV% = 1.33; Retch Laboratories, Arden Hills, MN). In a similar study with fermented soymilk, HPLC analysis had an 87% to 100% recovery of added sucrose depending on the initial added sucrose amount and sample preparation prior to analysis (Buono and others 1990). Thus, perhaps the percent recovery of the added sucrose was slightly lower than 100% in this particular study as well. Even though the analytical sucrose values (%) are slightly lower than expected, the results overall indicate that the cultures will consume the inherent lactose versus the added sucrose.

Initial Added Sucrose (%)	Batch	Statistic	Fructose	Lactose	Sucrose	Galactose	Glucose
0%	Both A & B	mean	0.000	3.800	0.000	0.550	0.020
		s.d.	0.000	0.082	0.000	0.018	0.000
	A	mean	0.000	3.800	0.000	0.535	0.020
		s.d.	0.000	0.141	0.000	0.007	0.000
	B	mean	0.000	3.800	0.000	0.565	0.020
		s.d.	0.000	0.000	0.000	0.007	0.000
4%	Both A & B	mean	0.075	4.600	3.650	0.165	0.023
		s.d.	0.096	0.082	0.129	0.006	0.005
	A	mean	0.100	4.600	3.650	0.160	0.025
		s.d.	0.141	0.141	0.212	0.000	0.007
	B	mean	0.050	4.600	3.650	0.170	0.020
		s.d.	0.071	0.000	0.071	0.000	0.000
8%	Both A & B	mean	0.050	4.600	7.700	0.183	0.025
		s.d.	0.058	0.000	0.000	0.010	0.006
	A	mean	0.050	4.600	7.700	0.185	0.025
		s.d.	0.071	0.000	0.000	0.007	0.007
	B	mean	0.050	4.600	7.700	0.180	0.025
		s.d.	0.071	0.000	0.000	0.014	0.007
12%	Both A & B	mean	0.100	4.600	11.700	0.198	0.055
		s.d.	0.115	0.082	0.424	0.010	0.006
	A	mean	0.100	4.600	11.750	0.200	0.055
		s.d.	0.141	0.141	0.636	0.014	0.007
	B	mean	0.100	4.600	11.650	0.195	0.055
		s.d.	0.141	0.000	0.354	0.007	0.007

Table 5-1 Descriptive statistical analysis of sugar results after fermentation

Changes of Lactose after Fermentation

As expected, lactose was consumed in all the batches and glucose was hardly detected while galactose accumulated in the medium (Table 5-1). When lactose is metabolized, *S. thermophilus* and *L. bulgaricus* prefer the glucose moiety of lactose while the galactose moiety is released into the medium (Zourari and others 1992; O'Leary and

Woychik 1976). Since the same amount of nonfat dry milk powder was used in the yogurt mix preparation, each batch had the equivalent amount of lactose prior to fermentation, and this initial amount of lactose was then used to calculate the average percent of lactose consumed within the batches, as shown in Table 5-2.

Initial Added Sucrose of Batch (%)	Average Lactose Consumed of Batch A & B (%)
0%	25.911
4%	10.314
8%	10.314
12%	10.314

Table 5-2 Average lactose consumed after fermentation

The batches with no added sucrose had the highest percentage of lactose consumed compared to the batches with added sucrose, and these control batches (no added sucrose) were closed to what it is expected to be metabolized during fermentation. In general, about 30% of lactose is consumed through fermentation (Chandan 2006; Nauth 2004). Thus, the batches with added sucrose had a lower than expected amount of lactose metabolized during fermentation, but these results reinforced the impact that total solids can have on the cultures. A yogurt mix of 16-20% total solids may slow culture growth (Nauth 2004). As shown in Table A-5.1, the added sucrose increased the total solids in the 4%, 8%, and 12% batches to 14.5%, 18.9%, and 23.4% respectively, which could explain the lower amount of lactose consumed in these batches.

Since the batches with no added sucrose had more lactose consumed, the assumption was that there was more lactic acid production in these batches. In order to assess this, the percent lactic acid of each batch was determined by titration, and the pH of the each batch was also re-checked (Figure 5-3). As expected, the batches with the lower pH's had more lactic acid (%), but it was unexpected to see that the batches with no added sucrose to have a lower lactic acid (%) and a higher pH.

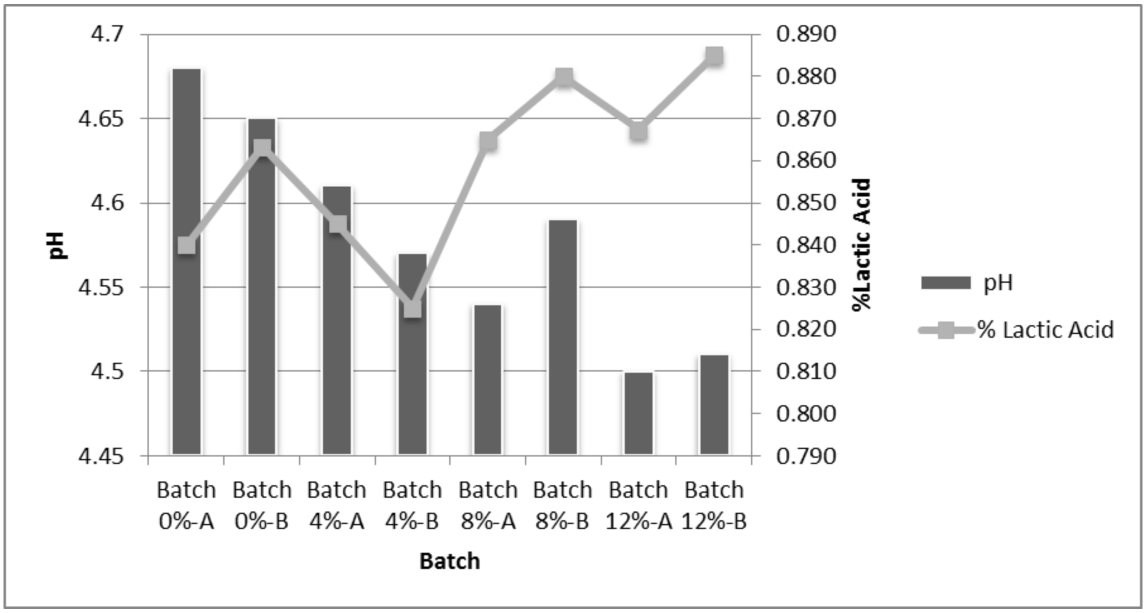

Figure 5-3 pH and % lactic acid of batches @ 25°C post fermentation

This simply could be explained by the longer fermentation times for the 8% and 12% batches, as shown in Figure 5-1, allowing more lactic acid production. Yet, this would be unexpected since the fermentations of the batches were terminated around the same pH of 4.6. Yazici and others (1997) found similar results in their study when comparing calcium-fortified soymilk yogurts with non-fortified soymilk yogurts (control). The pH reduction was considerably slower in the calcium-fortified ones than in the control yogurts, but the calcium-fortified yogurts had significantly higher titratable acidity values than the control yogurts at a given pH. Yazici and others (1997) concluded that the addition of the calcium to the soymilk increased buffering capacity. Similar results are also seen in yogurts with increasing levels of MSNF. The titratable acidity of the yogurt mix is greater due to the buffering action of the additional proteins, phosphates, citrates, lactates, and other miscellaneous milk constituents (Tamime and Robinson 1999). Possibly, the added sucrose and the resulting increase in total solids impacted the buffering capacity of the yogurt mix causing the titratable acidity to be higher, but more research is needed to understand this further, which is out of scope of this present study.

Since it appears that not all the lactose consumed was converted into lactic acid in the control batches, it is possible that some of the lactose was lost during the pasteurization of the yogurt mix. In one study, Richmond and others (1987) found a 7.3% decrease in the lactose content after the initial pasteurization and heat treatment. They tentatively concluded that some of the lactose isomerized into lactulose in the heated milk, but no lactulose peak was found on the HPLC chromatogram due the limit of sensitivity for the instrumentation. In addition to isomerization to lactulose, lactose can participate in the Maillard and caramelization reactions during heat treatment (Walstra and others 2006). Consequently, perhaps the presence of the added sucrose in the other batches interfered with these reactions so that less lactose was lost, but again additional research is needed to understand this further, which is out of scope of this present study.

Because the Mild 2.0 cultures are high texturing cultures due to the increased production of Exopolysaccharides (EPS), it also reasonable to believe that part of the hydrolyzed lactose is used to produce EPS versus lactic acid (Chr. Hansen Inc., Milwaukee, WI). EPS are polysaccharides composed of monosaccharides from catabolized sugars, which is lactose in the case of yogurt, and their function in yogurt is to improve the rheology and consistency of the product (Gürakan and Altay 2010). Their production can be influenced by several factors, such as composition of the medium (carbon and nitrogen sources) and incubation conditions (Yuksekdag and Aslim 2008). Purwandari and Todor Vasiljevic (2009) studied the influence of various amounts of added sucrose on EPS production by *S. thermophilus* strains in fermented milk, and they found that higher concentrations of sucrose impaired bacterial growth, which was weakly and positively correlated to EPS concentration. Hence in this study, the presence of added sucrose potentially interfered with EPS production, so that the control batches (no added sucrose) possibly had a greater amount of EPS production accounting for the increased lactose consumption in the control batches.

Labeled Sugar Values: Formulation versus Analytical

Table 5-3 summarizes the labeled sugar values for each of the nonfat yogurt samples produced in this study based on the initial formulation of added sucrose or the average analytical results for sucrose. Even though the analytical results for sucrose were slightly lower than what was initially added to the batches, this does not impact the "Total Sugar" or "Added Sugars" values significantly, and in most cases, these values are exactly the same for either the formula- and analytical-based values once rounding is taken into account to the nearest gram (21 C.F.R. § 101.9). "Added Sugars" only differs by 1 gram for the 4% and 8% added sucrose batches, but this difference falls within normal variability allowed by 21 C.F.R. § 101.9. Overall, this means that either the theoretical formulation or analytical results for sucrose will represent the labeled sugar values appropriately for these nonfat yogurt samples.

Initial Added Sucrose for Batch (%)	Based on Initial Added Sucrose Values (%)				Based on Average Analytical Sucrose Values (%)			
	Total Sugar		Added Sugars		Total Sugar		Added Sugars	
	Raw	Rounded	Raw	Rounded	Raw	Rounded	Raw	Rounded
0	6.46	7	0	0	NA	NA	NA	NA
4	14.63	15	6.80	7	14.03	14	6.21	6
8	21.43	21	13.61	14	20.92	21	13.10	13
12	28.24	28	20.41	20	27.73	28	19.90	20

Table 5-3 Labeled sugar values of a nonfat yogurt based on a 170.1 g serving size

Conclusion

The significance of this study shows when labeling dairy yogurt products that the amount of sugar loss due to fermentation should be subtracted from the "Total Sugar" value not the "Added Sugars" value if sucrose is added to the product. The results indicate that the cultures prefer the inherent lactose in the milk versus the added sucrose, so that the sucrose added prior to fermentation should remain afterwards. In a manufacturing setting, if there is a large discrepancy between the theoretical formulation versus the analytical results for sucrose, the loss of sucrose is most likely caused by the sugar delivery system and equipment at the plant versus fermentation.

However, this may not always be the case. If the proposed rule is finalized to require the declaration of "added sugars," this will encourage advancements in food technology to reduce "added sugars" in products. In the circumstance of fermented dairy products, the cultures could be genetically modified to prefer sucrose over lactose during fermentation. Of course, the appropriate sweetness level of the product is a potential concern, but if the cultures do not metabolize the fructose, the sweetness level of the product would technically increase since fructose is sweeter than sucrose. This highlights an important question if a by-product from fermentation would be considered "added sugars." As the proposed definition of "added sugars" stands today, it only defines "added sugars" as sugars that are added during the processing of foods but does not specify the functionality of "added sugars" (FDA 2014b). Consequently, in this particular case, the "Added Sugars" value would decrease to account for the consumption of sucrose via fermentation even though the sweetness of the product would actually go up due to the accumulation of free fructose. Thus, further clarification will be needed by FDA on the definition of "added sugars."

Finally, even though yogurt is commonly sweetened with sucrose, other nutritive sweeteners with different compositions than sucrose (i.e. honey or high fructose corn syrup) can be used to sweetened yogurt. Furthermore, other optional cultures (i.e. *Lb. acidophilus* or *Lb. casei*) can be incorporated via the starter culture in addition to the required cultures by the yogurt standard identity, and these cultures can differ in their sugar metabolism in comparison to *S. thermophilus* and *L. bulgaricus.* Thus, more studies with different types of cultures and sweeteners are needed in order to fully understand the impact of fermentation on "added sugars" in yogurt products**.**

Chapter 6
Issues with Replacing Added Sugars

Introduction

Sugar (sucrose) has several functional properties in food, which makes it challenging to replace. No other sweetener has been developed to duplicate all of its functional properties. Thus, it is imperative to understand how the sugar is functioning in a particular food product before replacing it. Other nutritive sweeteners, such as honey, high-fructose corn syrup, or fruit juice concentrates, have been used in the past. However, because nutritive sweeteners are considered added sugars, more manufacturers will be looking at using nonnutritive sweeteners to replace sugar in their products.

Synergistic Relationship of Nonnutritive Sweeteners and Bulking Agents

Nonnutritive sweeteners are also called high-intensity sweeteners because of their very intense sweetness compared to sucrose. Sucrose is given an arbitrary sweetness level of 1 to allow its comparison with other sweeteners, as shown in Table 6-1. Given their intense sweetness, nonnutritive sweeteners are used in small amounts in food products, and as stated earlier, many are not completely metabolized by the body. Both of which explain why nonnutritive sweeteners do not provide calories like sugar. Because of their low usage levels, something else needs to replace the reminder of the missing sugar amount in the product, and this is where bulking agents or bulk sweeteners come into play.

Nonnutritive sweetener	Relative sweetness	kcal/g	Source	Aftertaste	Reference
Aspartame	160–220	4	Synthetic	Prolonged sweetness	Varzakas and others 2012
Acesulfame K	150–200	0	Synthetic	Very slightly bitter	Varzakas and
Sucralose	400–800	0	Synthetic	Not unpleasant	Varzakas and others 2012
Saccharin	300—600	0	Synthetic	Bitter, metallic	Varzakas and others 2012
Steviol Glycosides	200-300	0	Plant	Bitter, unpleasant	Varzakas and others 2012
Glycyrrhizin	50–100	0	Plant	Prolonged sweetness, licorice	Varzakas and others 2012
Neohesperidin dihydrochalcone	1000–2000	2	Semi – synthetic	Lingering menthol-licorice	Varzakas and others 2012
Neotame	7000–13000	0	Synthetic	Not unpleasant	Varzakas and others 2012
Thaumatin	2000	4	Plant	Licorice	Varzakas and others 2012
Cyclamate[a]	30–40	0	Synthetic	Prolonged sweetness	Varzakas and others 2012

[a] Banned in the United States due to controversial toxicity studies but permitted in other countries (EU, Australia, Canada, New Zealand).

Table 6-1 Nonnutritive sweeteners comparison to sucrose at a relative sweetness of 1

This is similar technology that is utilized to replace fat in products. Fat, like sugar, provides bulk, mouthfeel, and texture to food products. Without the use of bulking agents, food products would not be appealing to the consumer. For example, if sugar is removed from bran cereal, it would have the consistency of sawdust (Varzakas and others 2012). Bulking agents can provide some sweetness but their primary function is providing bulk (Wilson 2007). Table 6-2 lists some common bulking agents, and their relative sweetness is below that of sucrose with some even at 0. It is important to find bulking agents that can work synergistically with nonnutritive sweeteners.

Bulking agent	Relative sweetness	kcal/g	Reference
Xylitol	1	2.5	Varzakas and others 2012
Maltitol	0.75	2.7	Varzakas and others 2012
Erythritol	0.7	0.2	Varzakas and others 2012
Sorbitol	0.6	2.5	Varzakas and others 2012
Mannitol	0.6	1.5	Varzakas and others 2012
Isomalt	0.55	2.1	Varzakas and others 2012
Lactitol	0.35	2	Varzakas and others 2012
Hydrogenated starch hydrolysates	0.4–0.9	2.4–3.0	Deis 2012; Varzakas and others 2012
Polydextrose	0	1	Varzakas and others 2012
Isomaltulose	0.4	4	Sentko and others 2012; Varzakas and
Tagatose	0.92	1.5	Vastenavond and others 2012
Maltodextrin (depends on DE value)	0.1–0.2	1–3.8	Varzakas and others 2012
Fructooligosaccharides	0.3–0.6	2	Gwinn 2013
Inulin	0	1	Gwinn 2013

Table 6-2 Bulking agents comparison to sucrose at a relative sweetness of 1

Other Functional Considerations

Besides the lack of bulk, there are other functional issues that will need to be addressed when replacing sugar in food products. One of the key issues is taste. Nonnutritive sweeteners may have a bitter, metallic, and licorice aftertaste (Varzakas and others 2012). Blends of various nonnutritive sweeteners can be used to mask these off-flavors and improve the sweetness profile of food products. These blends make use of sweetener synergy where the sweetness intensity of the blend is greater than the total sweetness of the individual components (Wilson 2007). The use of flavor technology is another area that can be utilized to help address these undesirable aftertastes as the result of using nonnutritive sweeteners.

As mentioned earlier, ingredients that are used as fat-replacers can also be utilized in replacing sugar in food products. This is why some of the bulking agents listed in Table 6-2, such as maltodextrins and polydextrose, can also be used as fat-replacers.

Other ingredients, which may be used when replacing sugar or fat in a product, include starches, modified starches, cellulose, guar gum, gelatin, and carrageenan. They are used to modify the physical properties of food, such as acting as a thickener, to help replace the structural characteristics that were originally contributed by sugar or fat. Thus, they are utilized for the same reasons as bulking agents to help improve the mouthfeel of the product. However, these ingredients must be chosen carefully because they can affect the flavor and viscosity of the product. Some have shown to reduce the perceived sweetness of a product (Spillane 2006). Thus, the sweetener blend may need to be modified, such as increasing the level. Others, such as cellulose, have caused the products to become too viscous with a gummy mouthfeel (Varzakas and others 2012).

Another consideration is whether the sugar is participating in any chemical reactions, such as the Maillard reaction in baked goods. In this case, the brown color as the result of this reaction is important to the finished product. Some of the nonnutritive sweeteners may be capable of participating in the Maillard reaction but cannot produce the brown color due to their low usage levels (Kitts 2010). Furthermore, all sweetness is lost if they do participate in the reaction (Varzakas and others 2012). Therefore, it is important to select the right nonnutritive sweetener and a bulking agent that could participate in the reaction versus the sweetener. Finally, there is no known nonnutritive sweetener that can participate in caramelization (Varzakas and others 2012).

Replacing sugar in a product is a trial and error process given all the functional properties that sugar can contribute. Each product formula is unique and one size does not fit all. For example, a study was done to evaluate the effects of fat and sugar replacements in cookies, and one of the combination ingredients was polydextrose as the fat mimetic and maltitol as the sugar substitute (Zoulias and others 2002). The use of these ingredients resulted in very hard and brittle cookies, and the study concluded that the textural properties were improved by either decreasing the amount of alternative sweetener or increasing the concentration of fat mimetic in the gel which was added to the cookies. Thus, it can be a costly and lengthy process to find the right ingredients, the correct amounts, and the manufacturing parameters to create similar-quality products without sugar. In the end, it is not possible to match exactly the same quality characteristics of the nutritive sweetener-containing counterparts, and the consumers will ultimately decide what they find acceptable.

Calorie Reduction May Not Be Achieved

The driving force of replacing added sugars in a product is to reduce the calories. However, in some cases, calorie reduction may be insignificant or may even increase. When sugar is removed, it generally must be replaced with something else, so that the bulk of the product is not affected. As stated earlier, this is why bulking agents are utilized. However, these bulking agents usually provide energy because they are carbohydrate-based, as shown in Table 6-2.

Isomaltulose is a prime example. As discussed earlier in Chapter 4, it is a disaccharide-type carbohydrate that could be classified as a sugar, but it is not a typical sugar from a physiological point of view (Wilson 2007). Isomaltulose is completely absorbed by the small intestine, thus providing the same calorie value as sucrose at 4 kcal/g (Wilson 2007). Other bulking agents, such as maltodextrins or hydrogenated starch hydrolysates, can still contribute above 3 kcal/g, so that calorie reduction may be insignificant in the final product (Deis 2012; Varzakas and others 2012). Depending on the functionality needs in the product, using lower or noncaloric bulking agents may not be an option.

In one particular instance, a sugar-free baking batter was formulated using an increased level of flour and water, a hydrogenated starch hydrolysate (Hystar 5875), and the nonnutritive sweetener aspartame (Wallin 1996). The hydrogenated starch hydroylsate Hystar 5875 that was used had about 4 kcal/g of solids, and the flour of the product was increased, which contributes 4 kcal/g as well. Thus, the total calorie content of the product was not significantly reduced, but it was essentially free of added sugars.

Besides adjusting a carbohydrate-based ingredient, such as flour or starch, fat is another option to replace bulk and mouthfeel of the product. Fat contributes 9 kcal/g. Thus, the calorie reduction of the total product may be negated or even increased depending on how much of the fat is used in the product. One study developed a cookie dough with acesulfame-K instead of sugar and polydextrose as a bulking agent (Bullock and others 1992). Nutrient analysis revealed that the calorie reduction in the formulation was rather insignificant(less than 10%), because the fat proportion increased in the end product. This sugar-fat seesaw effect has also been shown through a systematic review of dietary intake studies for countries with cultural similarities: United Kingdom, Ireland, other European countries, United States, and Australia (Sadler and others 2014). This review demonstrated that there is a strong and consistent inverse association between total sugars and total fat intakes expressed as percentage energy. Thus, multiple guidelines in regards to fat and sugar may be difficult to achieve in practice at the population level and may not result in the calorie reduction as intended.

Other Concerns with Added Sugars Replacements

Another main hurdle that manufacturers will face with replacing added sugars in products is the consumer movement for “cleaner” labels and “natural” ingredients. As discussed earlier, several ingredients may need to be added, such as multiple high-potency sweeteners, fat, bulking agents, thickeners, and flavoring, in order to replace one ingredient (sugar). Thus, the numbers and amount of food additives on the food label will increase, which will be viewed negatively by some consumers. Some of these ingredients are produced synthetically or via chemical modification. Thus, they would not be considered “natural.” In some cases, consumers may prefer naturally occurring nutritive sweeteners such as honey. Even though honey consists mainly of sugars, it also provides vitamins, minerals, and antioxidants (Varzakas and others 2012), which may be regarded more positively compared to other sweeteners by some consumers. These consumers like that they understand what the ingredient is and where it is coming from.

There may also be general public health concerns in regards to the food technology used to replace added sugars. Commonly used nonnutritive sweeteners to replace sugars are artificial sweeteners, such as aspartame, acesulfame K, sucralose, and saccharin. There is a public perception that artificial sweeteners are unsafe to consume. This is mainly driven by animal studies conducted in the 1970s that linked saccharin to cancer (International Food Information Council [IFIC] 2003). However, those studies used extremely high doses compared to what is normally consumed in the human diet, and several epidemiological studies since then have been carried out showing no link between cancer and saccharin consumption (IFIC 2003). The 2015 Dietary Guidelines for Americans also reiterated that these artificial sweeteners have been determined to be safe for the general population based on the available scientific evidence (USDA and HHS 2015b). Yet, sucralose is gaining attention today due to a newly, published study from the Ramazzini Institute, which found that sucralose caused cancer in male mice (Soffritti and others 2016). Thus, the public perception that artificial sweeteners can cause cancer still remains today.

Another health concern with the artificial sweetener aspartame is that it contains phenylalanine. Certain individuals (with a genetic disorder) lack the enzyme phenylalanine hydroxylase (PAH) to metabolize phenylalanine. This accumulation of phenylalanine, which is further converted to phenylpyruvate, can cause serious damage in brain development (Varzakas and others 2012). As a result of this health risk, products that contain aspartame must have a warning label stating that it contains phenylalanine (FDA 21 C.F.R. § 172.804 2014a). Besides products with aspartame carrying a warning label, some products containing sugar alcohols also need one stating that excessive consumption can have a laxative effect (FDA 21 C.F.R. §§ 180.25, 184.1835 2014a).

Like fat, salt may also be increased in foods with reduced or replaced sugar contents. It has been known for some time that the additions of salt and sugar work synergistically to increase the intensity of sweetness (Kilcast and Ridder 2008). Thus, it is a potential tool for manufacturers to increase salt in order to increase the sweetness of the product to compensate for the reduction of sugar. Nonetheless, salt is a nutrient of concern given its link to cardiovascular diseases (Dötsch and others 2009). In a sense, replacing sugar or reducing sodium in a product can become a “lesser of 2 evils game.”

The Effectiveness of Nonnutritive Sweeteners

The theory behind replacing added sugars is to reduce calories, which consequently, could lead to weight loss. Nonnutritive sweeteners can help achieve the similar sweetness characteristics as sweetened foods without adding calories. However, in the recent release of the Scientific Report of the 2015 Dietary Guidelines, the advisory committee cited that there is insufficient evidence to recommend the use of nonnutritive sweeteners as an effective strategy for long-term weight loss and weight maintenance and that added sugars should not be replaced with nonnutritive sweeteners in foods and beverages (USDA and HHS 2015a). This could be related to the fact that calorie reduction in the total product is ultimately not reduced or that consumers use it as an excuse to ingest calories in other forms. Yet, nonnutritive sweeteners in conjunction with bulking agents are the most effective strategies to replace added sugars in the food industry as of now. It is very unlikely that there will be many new sugar replacers developed over the next decade. The time and cost of development alone and the regulatory hurdles for new food ingredients will inhibit their speed to market (Spillane 2006).

Chapter 7
The Effectiveness of Labeling Added Sugars

Consumer Confusion with Proposed Label

FDA's intent to implement changes to the nutrition facts label is to contribute to greater consumer understanding and awareness in order to assist them to choose foods that fit within healthy dietary patterns. Yet, it is not clear that the labeling of added sugars, as one of the proposed changes, will benefit consumers but will result in confusion instead.

The International Food Information Council (IFIC) conducted a national survey of adult U.S. consumers to investigate consumer understanding of the "added sugars" declaration on the proposed label. Consumers were shown 3 Nutrition Facts panels for the same food product. Version S was in the proposed format panel with the "Sugars" designation as shown in the current Nutrition Facts panel. Another version, Version S+A, was the exact format in FDA's proposal with the "Sugars" designation and "Added Sugars" as a subgroup designation. The third version, Version TS+S, matched Version S+A except that the "Sugars" designation included the word "Total" in front of it. When asked to report the total amount of sugars in the product, 92% answered correctly with version S, but only 55% answered correctly with version S+A (IFIC 2014).

IFIC (2014) research also demonstrated that consumers' understanding of the "Added Sugars" line varies with 19% of consumers stating that they did not know what it means. This was further exemplified when consumers were given a list of 23 types of nutritive and nonnutritive sweeteners and asked to indicate which would be included in the "Added Sugars" line on the Nutrition Facts panel. Over one-third of respondents indicated that nonnutritive sweeteners would be considered added sugars: Sweet 'n Low (39%), Splenda (38%), Aspartame (35%), and Stevia (34%) (IFIC 2014).

As part of the supplemental information to the proposed rule, FDA recently released a separate consumer study that showed equivalent results as the IFIC consumer survey. Once more, consumers were shown 3 different formats of the Nutrition Facts panels for the same food product, and this study found that 90% of consumers were able to correctly identify total sugars given the "Control Format" with no "added sugars" declaration versus 65% for the "Sugars+Added Sugars Format" and 76% for the "Total Sugars+Added Sugars Format" (FDA 2015b). It was noted that some of the consumers added the total sugars and added sugars together resulting in them erroneously identifying the amount of total sugars in the product. FDA further tested how the "added sugars" line would impact consumers' perception of the product in regards to being nutritious. Consumers were presented a pair of Nutrition Facts panels for the same type of food, but one of the Nutrition Facts panels indicated a more nutritious product with fewer calories, less fat, and more fiber, vitamins and minerals. When the more nutritious product had more added sugars, the percentage of consumers identifying that product as being healthier decreased (FDA 2015b).

Similar results were also reiterated in the comments to FDA by several food companies and organizations, which conducted their own consumer studies. The Center for Science in the Public Interest (CSPI) conducted an online survey and found that only 44 percent of participants could correctly identify the amount of total sugar in one serving of the food when shown FDA's proposed label (CSPI 2014). General Mills showed that the current Nutrition Facts label format resulted in 92% accuracy for the total sugar content in a product while the proposed label format resulted in 66% accuracy (General Mills 2015). In a joint effort, the American Baking Association, Corn Refiners Association, International Dairy Foods Association, National Confectioners Association, and Sugar Association found that consumer groups are more likely to select the less healthful (low fiber/higher total sugars/no added sugars) product as the healthier product and better choice to maintain a healthy weight when “added sugars” are on the label. Specifically, the percentage of consumers correctly identifying the healthier (higher fiber/lower total sugars) product dropped from 56% to 32% when “added sugars” were declared (American Baking Association and others 2015). Bertino and others (2014) also had similar results after conducting an online survey with consumers. When presented with two labels and asked to choose the healthier option, over one-third of consumers selected a food with lower added sugars although it had higher calories, fat, and saturated fat.

Overall, the results of these consumer studies suggest that including added sugars on the Nutrition Facts panel will result in consumer confusion regarding the total amount of sugars in a product, and even worse, labeling added sugars may result in decreasing healthfulness perception for healthy foods while increasing healthfulness perception for unhealthy foods. Since the goal of the nutrition facts panel is to help consumers make healthier choices, additional consumer research is needed to determine if labeling added sugars will generally benefit consumers or result in unintended consequences.

Food Sources of Added Sugars

In the Scientific Report of the 2015 Dietary Guidelines, food sources of added sugars were broken down by categories. Beverages, excluding milk and 100% fruit juice, accounted for 47% of added sugars consumption in the United States (USDA and HHS 2015a). In these types of beverage products, the current total sugars declaration reflects the amount of added sugars because virtually all the sugar is added. Since 100% fruit juice is excluded from being categorized as added sugars, there is likely an unintentional effect of simply substituting sugar-sweetened beverages for 100% fruit juice. However, 100% fruit juice can have similar or even higher total sugar content compared to sugar-sweetened beverages resulting in no calorie reduction in the American diet.

The food categories dairy and grains, for which labeling added sugars will be more of a challenge, only accounted for 4% and 8% of added sugars consumption, respectively (USDA and HHS 2015a). These categories included the yeast-leavened and fermented products, which transform added sugars into other compounds via chemical reactions. Again, these chemical reactions depend on several variables which are unique to each formula and process, and it will be a time-consuming process to research each unique product formula to understand the amount of added sugars lost. These categories also provide other nutrients besides added sugars, such as vitamin D, calcium, dietary fiber, B vitamins, whole grain, and iron.

As for the category of snacks and sweets, it provided 31% of added sugars consumption in the United States (USDA and HHS 2015a). These included desserts, such as cakes, cookies, and chocolate, and these types of products may also have a challenge in labeling the exact amount of added sugars due to the Maillard and caramelization reactions. However, one could reason that consumers understand and consume these types of products as indulgent treats in their diet. Thus, consumers are enjoying these types of products for pleasure and not for their nutritional value.

Added Sugars and Link with Public Health Concerns

Originally, FDA did not provide a Daily Reference Value (DRV) for added sugars consumption. The reason for excluding a DRV is that there was no sound scientific evidence for the establishment of a quantitative intake recommendation for which a DRV for added sugars can be derived (FDA 2014b). Yet, the Scientific Report of the 2015 Dietary Guidelines Advisory Committee urged FDA that added sugars should be labeled and a DRV should be established. Their recommendation is limiting added sugars to a maximum of 10% of total daily caloric intake based on strong evidence that limiting added sugars intake will reduce health risks, such as cardiovascular disease, excessive body weight and type II diabetes (USDA and HHS 2015a). Because the Scientific Report provided the missing scientific evidence, FDA has reconsidered their initial stance in the recent release of the supplemental information to the proposed rule. FDA (2015a) is now proposing to establish a DRV of 10 percent of total energy intake from added sugars and to require the declaration of the percent Daily Value (DV) for added sugars on the label. Given this latest proposal by FDA, it was also no surprise to see the 2015 Dietary Guidelines for Americans (DGA) following suit with the recommendation to consume less than 10 percent of calories per day from added sugars.

DRV's are established through underlying science on the association between increased intakes of a specific nutrient on either reducing or increasing the risk of chronic diseases (FDA 2014b). By establishing a DRV for added sugars, this indicates that there are health implications of consuming added sugars. Historically, FDA has relied extensively on reports from the Institute of Medicine (IOM) to establish current DRV's for nutrients. The Institute of Medicine uses a comprehensive, rigorous science-based process to determine Dietary Reference Intakes (DRI) for nutrients, and in the 2005 IOM DRI Macronutrient report, there was insufficient evidence to set a DRI for total or added sugars (IOM 2005). However, the report did suggest an Upper Limit (UL) for added sugars, a maximal intake level of 25 percent or less of energy, based on nutrient dilution since consuming too much added sugars could displace essential nutrients in the diet. However, in the case of added sugars, FDA is proposing to establish a DRV for added sugars based on the Scientific Report of the 2015 Dietary Guidelines Advisory Committee versus the IOM DRI Macronutrient report, which is unprecedented for the agency. As a result, the evidence, for which the DRV for added sugars has been derived, has sparked controversy within the industry and the public health and science communities alike.

The 2015 DGA acknowledges that the evidence for added sugars and health is still developing, and their rationale to limit calories from added sugars is based on research examining eating patterns and health (USDA and HHS 2015b). When added sugars in foods and beverages exceed 10% of calories, a healthy eating pattern may be challenging to achieve (USDA and HHS 2015b). However, FDA has previously stated that food modeling data cannot form the primary scientific basis for establishing DRV's (2014b). Thus, FDA specifically stated the Scientific Report provided evidence suggesting a strong association between a reduced intake of added sugars and a reduced risk of cardiovascular disease (2015a). Yet, prospective cohort or observational studies accounted for half of the studies cited in the Scientific Report, and their findings should be interpreted as associations not necessarily a cause and effect relationship.

The etiology of chronic diseases are complex, and confounding factors, such as total energy intake, BMI, sex, age, physical activity, ethnicity, and family culture, can contribute to them. Therefore, it is difficult to establish a cause and effect relationship not only with added sugars but with any specific nutrient. History, as in the case with dietary fat recommendations, has shown that single nutrient approaches are generally ineffective to address public health concerns (Slavin 2015). Rather than isolating added sugars, strategies that focus on establishing healthy dietary patterns and moderating total caloric intake with energy expenditure to achieve a healthy weight are more likely to improve health outcomes in the long-term (USDA and HHS 2010). Sugars or added sugars do not contribute to weight gain any more than any other macronutrient source (Bray and others 2012; de Souza and others 2012; Lowndes and others 2012; Lewis and others 2013). Te Morenga and others (2013), one of the studies cited in the Scientific Report, also concluded that changes in body weight are most likely an effect of excess energy intake since isoenergetic exchange of dietary sugars with other carbohydrates showed no change in body weight.

Finally, majority of the sources cited in the Scientific Report focused on sugar-sweetened beverages, and as discussed earlier, the total amount of sugars would be the same as the amount of added sugars in these types of products. Thus, additional studies are needed with food, especially food with both naturally occurring sugars and added sugars, to determine if added sugars is directly associated to negative health outcomes or whether it is an outcome of excessive calorie consumption from sugar-sweetened beverages. In summary, food companies and organizations, both of which support or against added sugars labeling, are recommending that FDA should implement the traditional IOM process before establishing a DRV for added sugars in order to ensure the scientific evidence linking added sugars intake to chronic diseases is sound.

Cost of Implementation

Sugar consumption is already decreasing in the United States without added sugars being on the Nutrition Facts label (USDA and HHS 2015a; Wittekind and Walton 2014; Welsh and others 2011). Therefore, this raises the important question whether labeling added sugars will outweigh the costs associated with implementation. Of course, it will result in added costs for ingredient and food manufacturers given the challenges around labeling added sugars as discussed earlier, but ultimately, this additional cost will be passed on to the consumer. Besides monetary consequences, there may be other unintended consequences for consumers. By shifting the focus of the consumer to a single nutrient, the consumer will possibly lose sight of selecting products based on overall healthfulness and not choose certain nutrient dense foods given the mere presence of added sugars on the label. Not only will the attention of the consumer change to added sugars, the food industry will most likely be prompted to reformulate products to decrease or replace added sugars as a result of labeling added sugars. As discussed earlier, calorie reduction in the product may be insignificant or even increase, and the use of nonnutritive sweeteners and ingredients with higher fat and sodium levels in products may rise as well. In conclusion, FDA should fully evaluate whether the labeling of added sugars would in fact better enable consumers to follow current federal dietary recommendations and offset the potential unintended negative consequences for consumers.

Chapter 8
Closing Remarks

Chronic diseases are growing at an alarming rate in the United States, and added sugars are being targeted by governmental regulatory agencies in an attempt to reduce the occurrence of chronic diseases among Americans. Yet, labeling "added sugars" will have its challenges in the food industry, and it is not clear that it will benefit the consumer either. The scientific evidence linking added sugars intake to chronic diseases is neither complete nor perfect. Overall, the public health recommendations about "added sugars" must be balanced with the reality that sugar added to food is an important piece in the food science puzzle given its several functionalities in food. Not only can a spoonful of sugar help the medicine go down, but it can help fruits, vegetables and fiber go down as well.

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Appendix

Yogurt Batch Formulation

Step 1-Initial Yogurt Mix (%)				
Initial Added Sucrose (%)	Control	4	8	12
NFDM	10.37	10.37	10.37	10.37
Sugar	0	4	8	12
Water	89.63	85.63	81.63	77.63
Total	100	100	100	100

Step 2-Culture Blend (%)	
Step 1 Milk Blend	90
Thawed Cultures	10
Total	100

Step 3-Adding Cultures to Yogurt Mix (%)	
Milk Blend from Step 1	99.8
Culture Blend from Step 2-0.02% culture inoculation	0.2
Total	100

Total Sorted Aggregated (%)				
Initial Added Sucrose (%)	Control	4	8	12
Step 1-MSNF	9.98	10.39	10.85	11.34
Step 2-MSNF	0.02	0.02	0.02	0.02
Total MSNF	10.00	10.41	10.87	11.36
Step 1-Total Solids	10.03	14.43	18.87	23.35
Step2-Total Solids	0.02	0.03	0.03	0.04
Total solids	10.05	14.46	18.91	23.39

Table A-5.1 Formulation for each batch per initial added sucrose (%)

Yogurt Manufacturing Instructions

1. Measure out dry and wet ingredients per the formula. See batch cards for amounts.
2. Pour pre-weighed water into the Hotmix Pro container (with the stirring paddle) and add 0.05 g Dow antifoam to water (about a small drop size amount).
3. Attach the Hotmix and turn on the mixer to a mixing speed no higher than 2 and hold at room temperature.
    a. Note: Going higher than 2 will cause foaming.
4. Slowly add the pre-weighed NFDM into the vortexing water through the lid opening of the Hotmix.
5. Slowly add the pre-weighed sugar amount.
6. Allow to stir at the slowest slow speed for at least 5 minutes.
    a. Note: Check for clumps of NFDM. If some are noticed, continue mixing a few more minutes and re-check.
7. Heat to 149°F (65°C) and hold for 30 minutes while continually stirring on the slowest speed.
    a. Note: It takes about 10 minutes to hit temperature, so set the timer to 40 minutes.
8. Start thawing cultures 10-15 minutes into the heating.
    a. Shake and rotate the bag of cultures to assure adequate distribution of the pellets.
    b. Thaw the pellets in water bucket filled with warm water around 33°C until the bag is completely melted. Make sure there are no holes in the bag.
    c. Shake the bag periodically.
9. Transfer the yogurt mix to a tared stainless steel container and record the weight. Place the container in an ice bath, and cool with stirring as fast as possible until the base is 43°C/110°F. Record the start and end time to achieve this temp.
    a. Note: It takes about 2 minutes to reach 43°C.
10. When 110°F/43°C is reached, remove the container from the ice bath and pour out 90 mL of the mixture into a sterile bottle to prepare a 1/10 dilution.
    a. Note: Place the 90 mL in the water bath set at 43°C until ready to make the 1/10 dilution.
11. Transfer 998 mL of the remaining base into a tared stainless steel container and record the weight.
    a. Note: Place the stainless steel container into the 43°C water bath until the 1/10 dilution is completed.
12. 1/10 dilution of cultures:
    a. Mix the thawed pellets with a sterile spatula or pipette in the bag.
    b. Weigh/Pipette out 10.00 grams into the sterile bottle with the 90 mL of yogurt product mix.
    c. Swirl the bottle to mix the contents.

d. 1:10 dilution can be used to inoculate laboratory-sized batches of cultured products. If not going to use immediately, place material in an ice batch, but it should be used within 15-30 minutes.

13. 0.02% Inoculation Rate:
    a. Pipette out 2 mL of the 1/10 dilution mixture and place into the 998 mL product mix at 40-43°C (104-110°F). Mix/stir well for about 5 minutes.
    b. Pour about 100 grams into the labeled 125 mL sample containers. Fill 5 sample containers per batch.
    c. Use one of the sample containers for pH checks throughout the fermentation.
    d. Discard any remaining mixture.
14. Get an initial pH of the base in the sample container for pH checks.
15. Put all the sample containers into the 110°F/43°C water bath. Record the start time.
16. Start checking the pH in the sample container for pH checks after 3.5 hours into the fermentation.
    a. Then, continue checking every 30 minutes until the pH is at or below 4.6.
17. Yogurt is done when pH is 4.60. Record the end fermentation time. Estimated 4.5 to 6 hours.
18. Place the sample containers in an ice bath and cool the yogurt to room temp. (25°C). Record the start and end of time for cooling. Re-test and record pH of the sample container for pH checks.
19. Discard the sample container for pH checks and place remaining sample containers in the blast freezer at -10°

Raw Data of Sugar Analysis

				HPLC Sugar Results (%)			Enzymatic Tests (%)	
Analysis Date	Sample Id	Batch	Initial Added Sucrose Level (%)	Fructose	Lactose	Sucrose	Galactose	Glucose
8/24/2015	19	A	0	0	3.7	0	0.53	0.02
8/27/2015	17	A	0	0	3.9	0	0.54	0.02
8/24/2015	42	B	0	0	3.8	0	0.57	0.02
8/27/2015	43	B	0	0	3.8	0	0.56	0.02
8/24/2015	12	A	4	0	4.5	3.5	0.16	0.02
8/27/2015	11	A	4	0.2	4.7	3.8	0.16	0.03
8/24/2015	36	B	4	0	4.6	3.6	0.17	0.02
8/27/2015	35	B	4	0.1	4.6	3.7	0.17	0.02
8/24/2015	7	A	8	0	4.6	7.7	0.18	0.02
8/27/2015	9	A	8	0.1	4.6	7.7	0.19	0.03
8/24/2015	30	B	8	0	4.6	7.7	0.17	0.02
8/27/2015	32	B	8	0.1	4.6	7.7	0.19	0.03
8/24/2015	22	A	12	0	4.5	11.3	0.19	0.05
8/27/2015	21	A	12	0.2	4.7	12.2	0.21	0.06
8/24/2015	28	B	12	0	4.6	11.4	0.19	0.05
8/27/2015	26	B	12	0.2	4.6	11.9	0.2	0.06

Table A-5.2 Raw data of sugar analysis per batch after fermentation

Regression Analysis

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	1	153.272	153.272	10631.60	0.000
Sugar Conc. %	1	153.272	153.272	10631.60	0.000
Error	6	0.086	0.014		
Lack-of-Fit	2	0.081	0.041	32.60	0.003
Pure Error	4	0.005	0.001		
Total	7	153.359			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.120069	99.94%	99.93%	99.90%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-0.1100	0.0710	-1.55	0.172	
Sugar Conc. %	0.97875	0.00949	103.11	0.000	1.00

Table A-5.3 ANOVA of average analytical sucrose(%)/batch vs. initial added sucrose(%)/batch

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