INVESTIGATING THE CHEMICAL BASIS OF FUNCTIONALITY DIFFERENCES BETWEEN BEET AND CANE SUGAR SOURCES IN MODEL EGG WHITE FOAMS AND OTHER PRODUCTS

BY

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THESIS

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ABSTRACT

Though often used interchangeably, researchers have identified differences in functionality between beet and cane sugar sources in some food products. For example, previous research reported sensory differences between meringue cookies made with beet and cane sugar. Beet sugar meringue cookies were more marshmallow-like than cane meringue cookies. However, these sensory differences have not been instrumentally quantified and the underlying cause has not been determined. Thus, the objective of this research was to instrumentally quantify and investigate the chemical basis for the sensory differences between beet and cane meringue cookies. To instrumentally quantify differences between beet and cane meringue cookies, moisture content and water activity was obtained for unbaked meringues and meringue cookies. Additionally, texture profile analysis, three point break analysis, and differential scanning calorimetry was carried out on beet and cane meringue cookies. To gain insight into factors causing differences between beet and cane meringue cookies, heat denatured sugar-egg gel texture, unbaked foam stability, and water loss during simulated baking were obtained. No meaningful difference was found between beet and cane meringues in moisture content, water activity, and foam stability prior to baking. After baking, however, beet meringue cookies where shown to have higher moisture content, water activity, and cohesiveness values, and lower hardness and force to break values. During simulated baking, cane meringues were shown to lose water notable faster than beet meringues, causing moisture and textural differences after baking. These differences during and after baking are likely associated with higher amounts of sulfite in beet sugar compared to cane sugar. Sulfite has been shown to inhibit browning and cleave disulfide bonds, which may causes functional differences egg white proteins during the baking of meringues. To account for differences between beet and cane sugar functionality, a longer baking time for beet sugar meringue cookies is required, though this may lead to undesirable browning and loss of foam volume. Additionally, functional differences between beet and cane sugar were explored in rock candy, crème brûlée, and angel food cakes. This work highlights differences in functionality between beet and cane sugar sources, and raises issues regarding sugar source choice, in addition to market price.
ACKNOWLEDGEMENTS

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Appendix A: Exploratory Experiments
Chapter 1: Introduction

1.1 Rationale and Significance

Sucrose is utilized throughout the food and culinary industries for a variety of uses. While sucrose is most often associated with contributing sweetness, sucrose’s effect on the functional properties of other ingredients, such as water, starch, and proteins, is widely utilized to enhance the overall flavor, texture, and stability of food products.

Sucrose, commonly referred to as sugar or table sugar, is extracted from two major plant sources: sugarbeets (Beta vulgaris) and sugarcane (Saccharum officinarum). Both beet and cane sugars are greater than 99% sucrose. The remainder consists of water, compounds from the sugar plant and growing environment, and compounds introduced unintentionally during processing (Asadi 2007, Godshall 2013). Though small in concentration, these impurities have been shown to be associated with thermal, sensory, and functional differences.

Lu and others (2013) reported that beet and cane sugars exhibit different Differential Scanning Calorimetry (DSC) thermograms, with cane sugar exhibiting two endothermic peaks and beet sugar exhibiting only one endothermic peak. Lu and others (2015) attribute these differences in thermal behavior to a sulfitization step, which occurs in the processing of beet sugar, but not in the processing of cane sugar. This sulfitization results in small amounts of sulfite (approximately 6 to 11ppm) being trapped in the mother liquor occlusions in white refined beet sugar (Lu 2016).

Urbanus and others (2014a) reported that “as is” beet and cane sugar cannot be differentiated by taste alone. However, sensory panelists were able to differentiate the aroma, aroma by mouth, and aftertaste of beet and cane sugars. Specific sensory characteristics associated with these aroma, aroma by mouth, and aftertaste differences were identified through descriptive analysis and the chemical compounds associated with these differences have been identified. Despite differences in aroma and aroma by mouth of “as is” sugars, panelists did not detect differences between sugar cookies, pudding, whipped cream, and iced tea made with beet and cane sugar (Urbanus 2014b). Differences were found, however, in baked meringues cookies (called pavlova cookies in Urbanus and others 2014b) and simple syrups. The functionality differences between beet and cane sugar when used in products, such as crème brûlée and angel food cake, have also been the subject of considerable debated in the media and online forums.
The research herein focuses on bridging the gap between fundamental research into differences between beet and cane sugar and differences in products as reported in the literature, media, and web sources.

Recently, a good deal of research has been conducted on the physical, chemical, and thermal differences between beet and cane sugar (Lu and others 2013; Urbanus and others 2014abc; Lu and others 2015; Lu 2016). Though sensory differences in beet and cane meringue cookies have been identified, these sensory differences have not been instrumentally quantified and the underlying chemical cause of these differences have not been determined.

1.2 Objectives
The major objective of this research was to instrumentally quantify and investigate the underlying cause of differences seen in model egg white foam systems made with beet and cane sugar before and after baking.

1.3 References
Urbanus BL, Schmidt SJ, Lee S. 2014c. Does Information about Sugar Source Influence
Consumer Liking of Products Made with Beet and Cane Sugars? J. Food Sci.
Chapter 2: Literature Review

2.1 Introduction to Sucrose

Sucrose, also termed sugar or table sugar, is considered the “gold standard” of sweet taste and is used as a reference for other sweeteners, both nutritive and non-nutritive. While sucrose is often recognized for its sweetness, sucrose also affects the functionality of other ingredients, such as protein and starch. Additionally, sucrose’s powerful water binding properties add to the stability of many products by lowering water activity. Sucrose’s state in foods, whether glassy, rubbery, or crystalline, often has a large impact on textural attributes. Sucrose also plays a major role in browning through caramelization and Maillard browning reactions. These functions make sucrose a widely utilized ingredient throughout the food and culinary industries (Godshall 2013).

Though pure sucrose is sold and stored almost exclusively in its crystalline state, when used in product, these crystals are most often melted into their amorphous state or dissolved in product matrices. In products, sucrose often occupies multiple states, including crystalline, glassy amorphous, rubbery amorphous, and dissolved.

2.2 Sucrose Source

Sucrose (C₁₂H₂₂O₁₁) is produced from the products of photosynthesis, glucose and fructose, through an enzymatic process (Asadi 2007). Though found in a number of different plant materials, two plants in particular store large amounts of sucrose for later use: sugarbeet (Beta Vugaris) and sugarcane (Saccharum officinarum) (Meade and Chen 1977; Godshall 2013). Though granulated beet and cane sugars are greater than 99% sucrose, recent research has identified thermal, sensory, and instrumental aroma differences between these two sugars (Asadi 2007; Godshall 2013; Lu and others 2013; Urbanus and others 2014abc: Lu and others 2015). Many of these sensory and functionality differences are attributed to differences in plant matter, growing conditions, or processing (Marsili and others 1994; Moore and others 2004).

2.2.1 Sugarbeet

2.2.1.1 Sugarbeet Growth

Sugarbeets can grow in a wide range of cool climates. In the United States, sugarbeets are
grown in California, Oregon, Idaho, Utah, Montana, Wyoming, Colorado, North Dakota, South Dakota, Nebraska, Minnesota, and Michigan (Asadi 2007; Commodity Costs and Returns 2016). Sugarbeets can be grown with or without irrigation depending on the growing climate, and grow best in good water holding soils, such as sandy, clay, and peaty loam soil (Asadi 2007).

In cool climates, sugarbeets are planted in the spring and harvested in late fall or early winter. In warmer climates, sugarbeets can grow through the winter and, thus, are planted in the fall and harvested in the spring. While sugarbeets in the wild take two years to reach full maturity, agricultural sugarbeets are normally harvested after six to eight months. Like most crops, sugarbeets require fertilization for optimum growth. Polyacrylamides are applied to the soil to prevent erosion and carbonation-lime residue, a byproduct of beet sugar production, is applied to control soil pH and enhance lime content. Sugarbeets are fertilized with nitrogen before planting, during planting, and/or during growth (Asadi 2007).

Glucose and fructose are produced in the leaves of sugarbeet plants through photosynthesis. For transportation and storage, these monosaccharides are converted to sucrose. Sucrose is stored in the vacuole of the sugarbeet root cells. Each sugarbeet usually produces about one seventh of its weight in white refined sugar and 7 tons of sugar per hectare (Asadi 2007).

During harvest, the sugarbeet is dug out of the ground by mechanized harvesters and the foliage and crown of the sugarbeet is removed. Considerable effort is expended in cleaning sugarbeets during harvest and early processing. Storage times of cleaned, defoliated sugarbeets can range from weeks to months depending on climate and storage conditions. During storage, sugarbeets consume valuable sucrose through the process of respiration (Asadi 2007).

2.2.1.2 Sugarbeet Processing

The overall goal of sugarbeet processing is to separate sucrose from sugarbeet plant matter and crystalize that sucrose into granular white refined sugar. Sugarbeet processing typically takes place in one factory through a number of unit operations.

Because impurities can form crystal defects, off flavors, and particulate matter in the final granulated sugar, cleaning is a large part of sugarbeet processing. Before processing, sugarbeets are dry cleaned through screens and rollers to remove dirt, sand, and rocks. Sugarbeets are then further cleaned though trash separators and vibrating chip separators. An additional wet beet
washing station further removes dirt. Water from this step is clarified and reused and mud from this step is sometimes used for biogas production (Asadi, 2007).

Now thoroughly cleaned, the sugarbeets are ready for to be sliced into thin beet chips called cossettes. The sole purpose of this step is to increase the surface area of sugarbeet matter to assist with diffusion. Beet cossettes are mixed with hot water (70°C) to breakdown cellular structure and release sucrose and impurities. During this process, called diffusion, 98% of the sucrose in the sugarbeet cossettes is extracted and a concentrated solution of sucrose and impurities forms, called diffusion juice. Spent cossettes are pressed and sold as a byproduct.

Gypsum, calcium sulfate, calcium chloride, aluminum chloride, and aluminum sulfate are often added to the sugarbeet pulp during the pressing process to increase press water yield. The use of gypsum releases $\text{SO}_4$ molecules into the beet juice and lowers the pH of the beet juice. In some factories, liquid sulfur dioxide is used in this step to prevent microbial activity (Asadi, 2007).

Diffusion juice and press water are mixed and moved to the juice purification step. Juice purification is the most important step in sugarbeet processing. During purification, non-sugars are precipitated out through the addition of milk of lime, which is composed mainly of $\text{Ca(OH)}_2$. The lime is, in turn, precipitated out by the use of $\text{CO}_2$. Improperly purified juice can cause clogged filters and fouled evaporators. The precipitates are filtered out through a complex process, producing a pure juice called thin juice. Thin juice is condensed through a series of evaporators, which utilize low pressure to lower the boiling point of water in the juice. After evaporation and concentration, the resultant solution is called thick juice (Asadi, 2007).

Thick juice from the evaporation stage is moved to the decolorization and sulfitzation steps. Colors are formed in juice mainly due to decomposition of sucrose and invert sugars at high temperatures. These colors are removed through ion-exchange resin and activated carbon. Additionally, sulfur dioxide is added to thin juice before evaporation and thick juice before crystallization at a rate of 50 ppm and 25ppm, respectively. At this point, juice can either be stored or moved to the next process, crystallization (Asadi 2007).

Crystallization is the transition of sucrose molecules from solution to a solid crystalline form. Many beet sugar factories use a three stage process to optimize sucrose crystallization. During the first stage, the standard liquor is supersaturated and seeded with crystals. At the end of this process, sucrose crystals are grown, centrifuged, and washed to produce white refined sugar. During the second stage of crystallization, syrup drained during centrifugation is
crystallized. The crystals produced are centrifuged and recycled to stage one, where they are dissolved into the starting standard liquor. In stage three, the syrup drained from centrifugation in stage two is cooled and crystallized over 2-3 days to produce crystals which are melted back into the standard liquor entering stage one of crystallization. The liquor drained during stage three is considered molasses. Though rarely used in foods, beet sugar molasses is valuable as cattle feed supplement or as a feedstock for alcohol and yeast product (McGinnis 1982). The crystals grown in stage one are dried and sold as white refined sugar (Asadi 2007).

### 2.2.2 Sugarcane

#### 2.2.2.1 Sugarcane Growth

Sugarcane is grown in warm tropical and subtropical areas, and is planted between fall and early winter (August through January in Florida). Sugarcane is a hybrid of multiple species from the *Saccharum* genus, causing wide variation in traits when planted by seed (Baucum and others 2002). Because of this, sugarcane stalks with desirable traits are cut into sections and planted, often by hand. This planting method produces nearly identical sugarcane plants within and between plantings (Meade and Chen 1977; Baucum and others 2002).

After planting, sugarcane fields are weeded by hand or through the use of herbicides. Depending on rainfall, water is either drained from or added to field side ditches. This soil diffusion based water regulation system is made possible by the porosity of the soil in which the sugarcane is planted (Baucum and other 2002).

Sugarcane is harvested between late fall and mid spring depending on climate, logistical, and economic factors (Baucum and others 2002). Harvest is completely automated and consists of removing the sugarcane tops and cutting off the stalks just above ground level (Meade and Chen 1997; Baucum and others 2002). Sugarcane usually produces approximately 10 tons per hectare and each sugarcane contains roughly 15% sucrose containing juice (Asadi 2007). After harvest, fields are maintained and the stalks regrow and are harvested the next year. Sugarcane can be harvested an average of three times before the fields must be plowed and replanted (Meade and Chen 1997; Baucum and others 2002).

#### 2.2.2.2 Sugarcane Processing

Despite having the same objectives, substantial differences exist between sugarcane and
sugarbeet processing. Sugarcane processing typically takes place in two separate steps, rather than just one as in beet processing.

Once harvested, sugarcane must be processed as soon as possible to eliminate microbial growth. The process begins with shredding harvested sugarcane stalks. Sucrose is then extracted from shredded sugarcane stalks through a rolling or soaking process. Lime and flocculants are added to the juice to precipitate out impurities. Lime is normally added in the form of powdered hydrated lime, which contains approximately 97% Ca(OH)$_2$, as well as trace amounts of other minerals including silica, iron, aluminum, sulfur, and magnesium (Meade and Chen 1977). The juice is run through presses, which filter out plant parts and soil. The purified juice is then concentrated by a series of evaporators. This thick syrup is sent to a final vacuum pan where it is concentrated to supersaturation and crystallization begins. The supersaturated syrup is seeded with sucrose crystals and the process of vacuum concentration and crystallization is repeated until no more sucrose can be obtained from the syrup. The final syrup is molasses and is used in baking, ethanol production, and animal feed. The crystals produced during this process are centrifuged to remove residual syrup and the dry crystals are considered raw sugar. Raw sugar can either be stored and processed into granulated white refined sugar, or packaged and sold as raw sugar (Godshall 2013).

To convert raw sugar (about 98% pure sucrose) into white refined sugar (greater than 99% sucrose), raw sugar is first mixed with hot saturated syrup to remove molasses from the crystal surface. The raw sugar crystals are then dissolved into water and the water is clarified. During the clarification process, lime and either phosphoric acid or carbon dioxide are added to precipitate impurities and reduce coloration (Meade and Chen 1977, Godshall 2013). The limed juice is then mechanically filtered and filtered through activated carbon, ion exchange resin, and/or bone char. The resultant syrup is then seeded and crystallized in vacuum pans. The crystals produced are centrifuged and dried. The resultant sugar is considered white refined sugar (Godshall 2013).

### 2.3 Comparison of Sucrose Sources

There has been considerable debate, both in academic literature and popular media, over the chemical, thermal, and functional differences between beet and cane sugar. The following is a discussion of the economic, chemical, sensory, and public opinion differences concerning beet
and cane sugar in its crystalline state, in solution, and in product matrices.

2.3.1 Economic Differences

The type of sucrose grown and consumed in a particular area is, in most cases, a matter of climate. Areas with mainly temperate climates, such as Europe, tend to grow and consume mostly beet sugar; whereas areas with tropical climates tend to grow and consume mostly cane sugar (Vlitos 1995). There are a few countries, such as the United States, Pakistan, and Spain, which have suitable climates for either type of sugar growth and production. World production of sugar is split at 23% beet sugar and 77% cane sugar (Godshall 2013). The maximum efficiency of production between beet and cane sugar is roughly equal (Vlitos 1995). Despite lower sugarbeet price and sugarbeet yield per acre, beet sugar is often sold at a lower price than cane sugar because of the low production cost of beet sugar (USDA 2016; Haley and McConnell 2011). One example of an area that has optimal conditions for the production of beet sugar is the Red River Valley. In this section of Minnesota and Eastern North Dakota, irrigation is not needed, beets can be stored for longer periods of time due to cold winters, and the processing season is long. Because of these optimal factors, the Red River Valley is responsible for more than 50% of United States sugarbeet production (Haley and McConnell 2011).

2.3.2 Chemical Differences

Though both beet and cane sucrose are greater than 99% sucrose, there are notable differences between the chemical composition of the two sources (Godshall 2013). Some of these compositional differences are due to the source plant itself. Because sugarbeets are dicotyledonous and sugarcane is monocotyledonous, differences exist in the way these two plants fix carbon through photosynthesis (Vlitos 1977; Godshall 2013). This carbon fixation difference results in different carbon isotope ratios between sugar sources, with beet sugar having a C13/C12 ratio of 2.595 and cane having a ratio of 1.147 (Godshall 2013). Differences in carbon isotope ratio do not produce functionality differences, but can be used to identify the sucrose source and detect sugar adulteration (Rodushkin and others 2011).

Beet sugar and cane sugar have been shown to differ in raffinose and theanderose content. High raffinose content during processing can alter crystallization morphology and is associated with beets having been subjected to periods of cold storage, having higher amounts of nitrogen.
fertilization, and experiencing differences due to growing location (McGinnis 1978; Asadi 2007). Theanderose, on the other hand, has been identified in cane sugar, but not in beet sugar (duBoil 1996). Both the high levels of theanderose in cane sugar and high levels of raffinose in beet sugar have been proposed as ways of identifying the sucrose source (duBoil 1996).

Because beet and cane sugar are processed in substantially different ways, the non-sugar impurities present in the crystal matrix differ between beet and cane sugar. These impurities are often trapped in tiny pockets of supersaturated sucrose solution within the crystal, known as mother liquor inclusions or occlusions. In particular, beet sugar processing contains a sulfitation step (see Sugarbeet: Processing) to eliminate coloration. This process leads to a higher sulfur content in beet sugar than cane sugar (Lu and others 2015). Additionally, processing differences between beet and cane sugar cause beet sugar to often have a higher pH than cane sugar (Godshall 2013, Lu and others 2015). Table 2.1 contains moisture content, pH, conductivity ash content, and sulfite content values compiled by Lu and others (2015). Table 2.2 is a similar table published by Godshall (2013) with a number of additional comparison parameters.
Table 2.1: Moisture content, pH, conductivity ash, and total sulfite content of select sugar brands (Lu 2016).

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Source</th>
<th>Karl Fischer titration M.C. (%)</th>
<th>pH (ICUMSA)</th>
<th>Conductivity Ash (ppm)</th>
<th>Total sulfite (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigma Sucrose</td>
<td>Cane</td>
<td>0.025±0.007</td>
<td>5.32 ± 0.34</td>
<td>9.0 ± 0.1</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>Fisher Sucrose</td>
<td>Cane</td>
<td>0.070±0.014</td>
<td>5.40 ± 0.27</td>
<td>9.8 ± 0.6</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>US cane</td>
<td>Cane</td>
<td>0.030±0.000</td>
<td>5.86 ± 0.15</td>
<td>193.5 ± 12.3</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>C&amp;H</td>
<td>Cane</td>
<td>0.090±0.014</td>
<td>5.65 ± 0.11</td>
<td>120.4 ± 11.6</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>Dixie crystal</td>
<td>Cane</td>
<td>0.040±0.014</td>
<td>6.30 ± 0.05</td>
<td>131.0 ± 3.0</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>Domino</td>
<td>Cane</td>
<td>0.035±0.007</td>
<td>6.80 ± 0.07</td>
<td>298.1 ± 13.7</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>Sugar in the Raw</td>
<td>Cane</td>
<td>0.140±0.014</td>
<td>7.73 ± 0.07</td>
<td>1576.7 ± 5.5</td>
<td>8.64±5.54</td>
</tr>
<tr>
<td>Chinese sugar</td>
<td>Cane</td>
<td>0.140±0.000</td>
<td>6.10 ± 0.06</td>
<td>243.5 ± 0.3</td>
<td>6.53±2.22</td>
</tr>
<tr>
<td>US beet</td>
<td>Beet</td>
<td>0.060±0.014</td>
<td>5.95 ± 0.19</td>
<td>116.1 ± 4.8</td>
<td>11.16±4.85</td>
</tr>
<tr>
<td>Pioneer</td>
<td>Beet</td>
<td>0.115±0.007</td>
<td>7.02 ± 0.07</td>
<td>72.1 ± 0.8</td>
<td>10.16±3.51</td>
</tr>
<tr>
<td>Meijer</td>
<td>Beet</td>
<td>0.090±0.000</td>
<td>6.77 ± 0.21</td>
<td>78.1 ± 0.9</td>
<td>7.39±2.08</td>
</tr>
<tr>
<td>Market pantry</td>
<td>Beet</td>
<td>0.055±0.021</td>
<td>6.23 ± 0.18</td>
<td>133.9 ± 2.6</td>
<td>8.66±2.34</td>
</tr>
</tbody>
</table>

* Detection Limits (DL) = 5.28 ppm
** The pH of HPLC grade water = 5.83 ± 0.34
Table 2.2: Composition of cane and beet refined white sugar (Godshall 2013)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Cane</th>
<th>Beet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pol</td>
<td>99.95</td>
<td>99.95</td>
</tr>
<tr>
<td>Color, pH 7</td>
<td>15-35</td>
<td>20-45</td>
</tr>
<tr>
<td>Absorbance ratio pH9/pH4</td>
<td>1.5-4.0</td>
<td>1.3</td>
</tr>
<tr>
<td>pH</td>
<td>6.2-6.7</td>
<td>6.5-8.0</td>
</tr>
<tr>
<td>Conductivity Ash %</td>
<td>0.01-0.03</td>
<td>0.01-0.03</td>
</tr>
<tr>
<td>Moisture %</td>
<td>0.01-0.02</td>
<td>0.01-0.02</td>
</tr>
<tr>
<td>Polysaccharides, ppm</td>
<td>70-200</td>
<td>20-50</td>
</tr>
<tr>
<td>Dextran, ppm</td>
<td>20-60</td>
<td>rarely present</td>
</tr>
<tr>
<td>Starch, ppm</td>
<td>30-50</td>
<td>0</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0</td>
<td>30-50 ppm</td>
</tr>
<tr>
<td>Kestoses</td>
<td>30-50 ppm</td>
<td>0 to trace</td>
</tr>
<tr>
<td>Flocating potential</td>
<td>Low to none</td>
<td>Low to none</td>
</tr>
<tr>
<td>Causes of floc</td>
<td>Protein &amp; ISP*</td>
<td>Saponins</td>
</tr>
<tr>
<td>SO₂, ppm</td>
<td>Not detected</td>
<td>ND in USA, low in Europe</td>
</tr>
<tr>
<td>Sediment, ppm</td>
<td>10-20</td>
<td>15-20</td>
</tr>
<tr>
<td>Turbidity, IU</td>
<td>2-25</td>
<td>1-5 (Higher outside US)</td>
</tr>
<tr>
<td>Turbidity, NTU</td>
<td>0-1.5</td>
<td>0-1.0</td>
</tr>
<tr>
<td>Glucose, %</td>
<td>0.005</td>
<td>0.001-0.003</td>
</tr>
<tr>
<td>Fructose, %</td>
<td>0.005</td>
<td>0.001-0.003</td>
</tr>
<tr>
<td>Volatile compounds odor**</td>
<td>Caramel, molasses</td>
<td>Earthy, VFA</td>
</tr>
<tr>
<td>Total plate count, CFU/10 g</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Yeast &amp; mold, CFU/10 g</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Notes: * ISP is indigenous sugarcane polysaccharide, an arabinogalactan polymer, found in cane cell walls; ** Odors are rarely noted in either cane or beet white sugar; Source: Sugar Processing Research Institute, Inc., New Orleans, Louisiana, USA

2.3.3 Differences in Thermal Properties

Lee and others (2011abc) demonstrated that the initial loss of crystalline structure in analytical grade crystalline cane sucrose is associated with thermal decomposition. Lu and others (2013) reported that 17 samples of crystalline beet sugar and 20 samples of cane sugar exhibited different Differential Scanning Calorimetry (DSC) thermograms, with cane sugar exhibiting two endothermic peaks, with onset temperatures of 155.1 ± 5.92 °C and 187.3 ± 1.77 and beet exhibiting one endothermic peak, with an onset temperature of 188 ± 0.38°C, at a heating rate of 10°C/min. Lu and others (2015) attributed these differences in thermal behavior to a sulfitation step, which occurs during the processing of beet sugar, but not during the processing of most
cane sugars. These thermal differences, coupled with evidence from high performance liquid chromatography (HPLC) and Ramen Spectroscopy, strongly suggests that thermal decomposition of beet sugar is initiated at a higher temperature than thermal decomposition of cane sugar due to chemical differences in the occluded mother liquor solutions (Lu 2016). These thermal behavior differences highlight that even miniscule differences in chemical composition can have substantial impact on thermal behavior, which, in turn, could affect functionality.

2.3.4 Differences in Public Opinion

Though beet and cane sugars are nearly identical in chemical composition and are often used interchangeably in the food industry and in the kitchen, public sentiment is, at times, fiercely divided. Many online forums are hotspots for differing opinions over the matter of taste differences and functionality between these two sugar sources (Urbanus 2014). Opinions range from complete indifference toward sugar source to adamant support for one sugar over the other. In general, cane sugar is preferred to beet, particularly in baking applications. Despite strong opinions on both sides, one study (Urbanus and others 2014c) revealed that consumer knowledge of sugar source had no effect on preference of sweetened orange-flavored beverage.

2.3.5 Differences in Sensory Properties

Beet sugar has long been described in the literature as having a musty or earthy smell, harming consumer acceptance and causing rejected beet sugar batches (Marsili and others 1994; Moore and other 2004; Urbanus and others 2014a). The compounds responsible for beet sugar’s off aroma have been investigated through analytical instrumentation. Marsili and others (1994) identified geosmin, produced by soil molds, to be responsible for a musty/mildew smell. Additionally, butyric and isovaleric acids, produced by soil bacteria, were identified as producing off dairy aromas. Moore and others (2004) found a number of volatile fatty acids associated with rancid and fatty odors to be present in beet sugars.

Urbanus and others (2014a) found that, while beet and cane sugar cannot be differentiated by taste alone, the aroma, aroma by mouth, and aftertaste of beet and cane sugar can be differentiated by R-index sensory testing (Urbanus and others, 2014a). Urbanus and others (2014a) also found, though descriptive analysis, that two commercial beet sugar brands were high in earthy, barnyard, off dairy, and oxidized aromas, burnt sugar aroma by mouth, and
burnt sugar aftertaste. Cane sugar, on the other hand, was associated with a fruity aroma by mouth and sweet aftertaste. Despite differences in aroma and aroma by mouth of “as is” sugars, consumer panelists did not detect differences between sugar cookies, pudding, whipped cream, and iced tea made with beet and cane sugar (Urbanus and others 2014b). Differences were detected, however, in pavlova cookies and simple syrups. Urbanus and others (2014b) reported beet sugar pavlova as being softer and more marshmallow-like than pavlova made from cane sugar, which exhibited the desirable textural characteristics of crunchy, hard, and foam-like. After increasing the baking time at 300°F (149°C) from 22 minutes to 38 minutes, beet sugar pavlova exhibited similar texture to that of cane sugar pavlova. The cause of differences between beet and cane pavlova, however, was not determined during this study and requires further investigation.

2.4 Meringues and Pavlova

Meringues are widely used in the food and culinary industries for the textural and flavor properties offered by a sweetened protein foam matrix (Foegeding and others 2006). Meringues are used as a topping for desserts, mixed as leavening agents for products such as angel food cake and soufflés, or bake alone to form meringue cookies and pavlova. Urbanus and others (2014b) studied pavlova as a model product matrix because the egg foam matrix allows for investigation of sugar source’s effect on foam stability.

Pavlova was invented to honor ballet dancer Anna Pavlova during her 1920s world tour (Goldsmith 2008). A considerable and heated debate still rages over whether the dessert was invented in New Zealand or Australia. Pavlova and meringue cookies are simple product matrixes consisting of sucrose, water, egg whites, and flavoring. While recipes vary in flavoring, coloring, inclusions, shape, and size, all pavlovas consist of sweetened egg white foams that are baked until the outside is crispy, but not completely dry in the center (Pavlova Recipes, allrecipes.com). Meringue cookies, on the other hand, are almost always baked until completely dry in the center. While produced in a similar manner, pavlova and meringue cookies are differentiated by size, shape, and application.

2.5 Egg White Protein

Egg whites are utilized throughout the food industry for their gelation and foaming
properties. Egg whites contain the following proteins in descending order of percent composition: Ovalbumin, ovotransferrin, ovomucoid, ovomucin, lysozyme, and globulins (Mine 1995). Figure 2.1 shows a Differential Scanning Calorimetry (DSC) thermogram of fresh egg white. Egg white proteins exhibiting endothermic peaks in Figure 2.1 include: ovotransferrin (60°C), lysozyme (67°C), ovalbumin (78°C), and s-ovalbumin (82°C) (Ferreira and Raemy 1997).

Figure 2.1: DSC curve of fresh egg white showing individual protein denaturation (Ferreira and Raemy 1997)

Because heating can drastically affect protein conformation and functionality, considerable study has been carried out on the effect of processing on egg white functionality (Lechevalier and others 2005; Lechevalier and others 2007). The addition of sucrose to egg white solutions drastically increases egg white denaturation temperature, as shown in Figure 2.2, (Christ and others 2005). The onset temperature of denaturation for ovalbumin without sugar and with 60% sugar is 76.8 and 98.4°C, respectively. The elevated temperatures needed to denature egg proteins in the presence of sucrose make for stronger protein-protein interactions and
stronger heat induced gels (Christ and others 2005).

Figure 2.2: Differential scanning calorimetry thermograms of egg white protein solutions with increasing amounts of sucrose (Christ and others 2005).

![Figure 2.2: Differential scanning calorimetry thermograms](image)

### 2.6 Foam Formation

The surfactant and bonding properties of native, partially denatured, and denatured egg white proteins allow egg foams to trap air bubbles and form a foam matrix (Foegeding 2006; Lomakina and Mikova 2006). While each egg white protein exhibits different functionality with respect to foam formation, egg white proteins generally arrange themselves at the gas-liquid interface with their hydrophobic portions oriented toward the air phase and hydrophilic portions oriented toward the liquid phase (Lomakina and Mikova 2006).

The synergistic action of egg white proteins together produces a high volume foam. Globulins have been shown to be excellent foaming agents, but interaction with ovomucin and lysozyme reduce their foaming capacity (Johnson and Zabik 1981ab; Lomakina and Mikova 2006). During foaming, ovalbumin has been shown to migrate to the gas liquid interface, where
it changes conformation and participates in disulfide bonding (Kitabatake and Doi, 1987). Antipova and others (1999), however, demonstrated that more ovalbumin is likely to remain in the liquid phase in sucrose solutions during whipping due to hydrogen bonding between ovalbumin and sucrose. Ovalbumin contains the only free sulfhydryl groups in native egg white proteins. Before denaturation, however, these free sulfhydryl groups are buried within the hydrophilic protein core. During conformation change at the gas-liquid interface and during heating, these sulfhydryl groups are exposed and can interact within and between proteins, particularly during heating (Kitabatake and Doi 1987; Mine 1990; Lechevalier and others 2003; Raikos and others 2007). Electrostatic interactions help improve foaming properties of egg white proteins. For example, lysozyme, a more positively charged protein at neutral pH, participates in electrostatic interaction with other egg white proteins, which are generally more negatively charged at neutral pH, creating a network, improving foam formation (Mine 1995). In addition to electrostatic interactions, foam formation is positively affected by protein flexibility and surface hydrophobicity (Mine 1995).

2.7 Foam Stability

Egg foam stability and quality are crucial to consumer acceptance in a number of products. Foam stability refers to how a foam holds its shape and liquid phase against the constant pressure of gravity (Bovskova and Mikova 2011; Licciardello and others 2012). High quality egg foams are associated with numerous, regularly sized small bubbles. Kato and others (1983) demonstrated that foam quality is also positively correlated with conductivity. Kato and others (1990) showed that dry heating egg whites powder, can decrease the enthalpy of protein denaturation and increase the foam conductivity. Another common parameter measuring the quality of the whipping material and method is overrun. Overrun is the measure of the initial solution volume compared to the final foam volume (Licciardello and others, 2012). In egg white foams, an increased sugar to egg white ratio has been shown to produce a larger number of small bubbles, creating a smooth texture (Licciardello and others, 2012).

Egg white foam and sugar combinations are an integral part of the structure of a number of products, such as soufflés and angel food cakes. Because beet and cane sugar sources have been shown to produce different results in pavlova cookies, it stands to reason that other egg white foam products may show similar differences when made with beet versus cane sugar
sources. Prior research has been done pertaining to the functional properties of egg whites in angel food cake systems (Johnson and Zabik 1981ab; Berry and others 2009; Mleko and others 2010), but these studies have not specifically investigated the effect of sucrose source on foam stability or cooked egg products such as pavlova or angel food cake.

Some suggest that whipping pure egg white foams for a fixed amount of time is an unreliable test of egg white protein functionality and foam stability due to the variability between batches of spray dried egg whites (Baker 1974). Because of this unreliability between batches, efforts should be made to use the same batch of spray-dried egg whites when testing an ingredient’s effect on egg white functionality.

2.8 Introduction of Methods

2.8.1 Moisture Content

Moisture content is a measure of the total amount of water in a food system. Moisture content can be measured in a number of different ways, including simple gravimetric determination through loss on drying, chemical determination using Karl Fischer Titration, nuclear magnetic resonance spectrosocpy, near-infrared spectrophotometry, or distillation. For gravimetric determination, a vacuum and desiccant are often used to help extract water at lower temperatures (Cauvain and Young 2008). One new instrument used for gravimetric determination of moisture content, the TrueDry moisture analyzer, utilizes desiccated air to ensure samples are fully dried, allowing for reproducible moisture content measurements regardless of the ambient relative humidity. Additionally, the TrueDry uses a combination of convection and conduction for gentler heating and thus reduced loss of volatiles due to decomposition (TrueDry CV9 Operator’s Manual 2014).

2.8.2 Water Activity

While moisture content measured the total amount of water is in a food, it does not provide insight into the availability of that water in a food product. Water activity, on the other hand, has been shown to be highly correlated with chemical reactions, microbial spoilage, and textural properties in food systems (Scott 1957; Schmidt 2004). In ideal systems, water activity is equal to the mole fraction of water and is a measure of the chemical potential of water in the system. Many foods, however, do not act as ideal systems and, thus, it is often best to measure
water activity in foods experimentally (Schmidt 2004).

When a food is placed in a closed system, the water in the food transitions between the liquid and vapor state until equilibrium is reached. Once equilibrium is reached, the chemical potential of the water in the food and the water vapor in the headspace above the food are equal. At equilibrium, under constant temperature and pressure, water activity can be estimated from the ratio of the vapor pressure above a food and the vapor pressure above pure water. Because percent relative humidity is the vapor pressure of a given atmosphere compared to that of pure water, water activity can be determined by measuring the relative humidity of the headspace above a food and dividing by 100 (Schmidt 2004). Water activity is most often determined using a capacitance or chilled mirror system.

It is important to consider that many food products have multiple components that are not in equilibrium with each other. One common example of this phenomenon is freshly baked bread, which has a dry outer crust and a soft inner foam matrix. These two component have very different water activity values, and measuring their combined water activity may be a less effective test than measuring each component separately (Cauvain and Young 2008). During storage in sealed containers, water in multiple component foods moves from areas of high water activity to areas of low water activity in a well-studied and modeled phenomenon commonly called moisture migration (Guillard 2003; Guillard 2004; Schmidt 2004). In baked products, such as breads and biscuits, this occurrence often causes the loss of the desirable crisp outer texture.

### 2.8.3 Texture Analysis

Texture is composed of a variety of sensations and parameters and can be measured by both sensory and instrumental methods (Szczesniak 1975). Instrumental texture analysis is used to produce quantified parameters, which can quickly and conveniently describe the texture of a food for research or quality control purposes. Instrumental methods of texture analysis were first explored at the General Foods (now General Mills) company and later published by Friedman and others (1962). Figure 2.3 shows a typical two bite texture profile analysis curve and how to analyze the resulting force-distance curve to determine cohesiveness, springiness, hardness, and adhesiveness.
Figure 2.3: Annotated example of a typical texture profile analysis curve (Friedman and others 1962).

Though texture profile analysis is used widely throughout the food industry, there are many foods for which texture profile analysis is not recommended. These misuses of texture profile analysis have elicited recommendations from instrument manufacturers (An Overview of Texture Profile Analysis n.d.) and critical comments from founding experts in the field (Bourne 1998). To ensure meaningful results, the parameters being analyzed should be matched to the physical properties of the food being tested. Some parameters are best measured using different probes and methods. One example is the measure of fracturability. While fracturability can be measured using texture profile analysis, Texture Technologies Corp. and Food Technology Corporation both recommend measuring fracturability and crispness of using a three point break test (An Overview of Texture Profile Analysis n.d.; TMS Lightweight 3-Point Bend 2016). The three point break test measures the force needed to break a sample by supporting a sample on
two ends and applying a force to the center. This method is often used for the textural analysis of baked goods (Gaines 1992ab)

2.8.4 Foam Stability

Because foam stability is a measure of how well a foam holds its shape and liquid phase against the constant pressure of gravity, one commonly used foam stability measure is drainage of liquid phase from foams (Lomakina 2006; Raikos and others 2007; Berry and others 2009; Henningsen Foods, Omaha, NE). This method provides a measure of how well a foam holds its liquid phase and give insight into the functionality of egg white proteins during whipping.

2.8.5 Simulated Baking

The Aqualab TrueDry CV9 moisture analyzer (Decagon, Pullman, WA), can analyze up to 9 samples at once. Because the TrueDry has an adjustable analysis temperature and measures moisture at various time points during the drying process, a simulated baking environment can be created inside the TrueDry and moisture content trends can be tracked throughout the baking process.

2.8.6 Differential Scanning Calorimetry

In addition to water activity and moisture content, the physical state is an important property to consider when studying a food product. Differential Scanning Calorimetry (DSC) is the process of measuring the heat flow to a material over a range of temperatures. By analyzing changes in heat flow, DSC can be used to determine the glass transition of a sample, the gain and loss of crystalline structure during heating, and the denaturation and gelatinization temperatures and enthalpies of some proteins and starches, respectively (Thomas and Schmidt 2010). Figure 2.4 shows an example thermal profile produced by DSC with common transitions labeled.

In particular, the glass transition temperature (Tg) of a food and its location relative to storage temperature or intended consumption temperature and relative humidity can be used to predict the stability and texture of a food (Slade and Levine 2009). The transition from an amorphous glassy to an amorphous rubbery state is associated with increased molecular mobility and a softer texture. The glass transition temperature is a function of both temperature and relative humidity (Thomas and Schmidt 2010). The amount of crystalline material within a food
is important for stability and texture, with higher crystalline content often producing a grainier, but more stable product. The size of the crystallization peak and crystalline melting peak can provide insight into the amount of crystallizable amorphous material and initial crystalline material (Thomas and Schmidt 2010).

Differential Scanning Calorimetry is the process of measuring the heat flow to a material over a range of temperatures. By analyzing changes in heat flow, DSC can be used to determine the glass transition of a sample, the gain and loss of crystalline structure during heating, and the denaturation and gelatinization temperatures and enthalpies of some proteins and starches, respectively (Thomas and Schmidt 2010).

Figure 2.4: Example differential scanning calorimetry thermogram with common material transitions (TA Instruments DSC Brochure 2012)

2.9 Justification
Throughout the food and culinary industries, consistent ingredient functionality is vital because differences in ingredient functionality can cause safety and consumer acceptance concerns. Significant research has been done on the physical, chemical, and thermal differences between
beet and cane sugar. Though sensory differences of select product matrices have been identified, the underlying cause of these functionality differences has not been determined. Thus, the purpose of this research is to investigate the nature of the functionality differences between beet and cane sugars in a model egg white system.

2.10 References


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Raikos V, Campbell L, Euston SR. 2007. Effects of sucrose and sodium chloride on foaming


Chapter 3: Investigating the chemical basis of functionality differences between beet and cane sugar sources in a model egg white foam

Abstract

Though often used interchangeably, researchers have identified differences in functionality between beet and cane sugar sources in some food products. For example, previous research reported sensory differences between meringue cookies made with beet and cane sugar. Beet sugar meringue cookies were observed to be more marshmallow-like than cane meringue cookies. However, these sensory differences have not been instrumentally quantified and the underlying chemical basis has not been determined. Thus, the objective of this research was to instrumentally quantify and investigate the chemical basis for the sensory differences between beet and cane meringue cookies. To instrumentally quantify differences between beet and cane meringue cookies, moisture content and water activity was obtained for unbaked meringues and meringue cookies. Additionally, texture profile analysis, three point break analysis, and differential scanning calorimetry was carried out on beet and cane meringue cookies. To gain insight into factors causing differences between beet and cane meringue cookies, heat denatured sugar-egg gel texture, unbaked foam stability, and water loss during simulated baking was investigated. No meaningful difference was found between beet and cane meringues in moisture content, water activity, and foam stability prior to baking. After baking, however, beet meringue cookies were shown to have higher moisture content, water activity, and cohesiveness values, and lower hardness and force to break values. During simulated baking, cane meringues were shown to lose water notably faster than beet meringues, likely causing moisture and textural differences after baking. These differences during and after baking are likely associated with higher amounts of sulfite in beet sugar compared to cane sugar. Sulfite has been shown to inhibit browning and cleave disulfide bonds, which may cause functional differences in egg white proteins during the baking of meringues. To account for differences between beet and cane sugar functionality, a longer baking time for beet meringue cookies is required, though this may lead to undesirable browning and loss of foam volume. This work highlights differences in functionality between beet and cane sugar sources, and raises issues to consider when choosing a sucrose source.
3.1 Introduction

Sucrose, commonly termed sugar or table sugar, is used in a wide variety of applications in the food and culinary industries. The two major sources of sugar, beet sugar and cane sugar, are each greater than 99% sucrose. The remainder consists of water, compounds from the sugar plant and growing environment, and compounds introduced unintentionally during processing (Asadi 2007, Godshall 2013). Though small in concentration, these impurities have been shown to be associated with thermal, sensory, and functional differences between these two sucrose sources.

Lee and others (2011abc) demonstrated that the initial loss of crystalline structure in analytical grade crystalline cane sucrose is associated with thermal decomposition. Lu and others (2013) reported that crystalline beet and cane sugars exhibit different Differential Scanning Calorimetry (DSC) profiles, with cane sugar exhibiting two endothermic peaks and beet sugar exhibiting only one endothermic peak. Lu and others (2015) attribute these differences in thermal behavior to a sulfitization step, which occurs in the processing of beet sugar, but not in the processing of cane sugar. This sulfitization results in small amounts of sulfate (approximately 6 to 11ppm) being trapped in the mother liquor occlusions in white refined beet sugar (Lu 2016). In addition to producing thermal differences between beet and cane sugar, sulfite has been shown to reduce browning (Wedzicha and others 1991) and cleave disulfide bonds in proteins (Clark 1932; Stricks and Kolthoff 1951; Thannhauser and others 1984).

Off aromas in beet sugar has been the subject of instrumental analysis. Marsili and others (1994) identified geosmin, produced by soil molds, to be responsible for a musty/mildew aroma in beet sugar samples. Marsili and others (1994) also found that butyric and isovaleric acids, produced by soil bacteria, are responsible for producing off dairy aromas in beet sugar. Moore and others (2004) identified a number of volatile fatty acids associated with rancid and fatty odors to be present in beet sugars.

Urbanus and others (2014a) found that, while beet and cane sugars cannot be differentiated by taste alone, the aroma, aroma by mouth, and aftertaste of beet and cane sugar can be differentiated though consumer testing. Though descriptive analysis testing, Urbanus and other (2014a) found that trained panelists described two commercial beet sugar brands as being higher in earthy, barnyard, off dairy, and oxidized aromas, burnt sugar aroma by mouth, and burnt sugar aftertaste. On the other hand, the two cane sugar brands tested were described has
having a fruity aroma by mouth and sweet aftertaste. Despite differences in aroma and aroma by mouth of “as is” sugars, panelists did not detect differences between sugar cookies, pudding, whipped cream, and iced tea made with beet and cane sugar (Urbanus 2014b). Differences were detected, however, in baked meringues cookies (called pavlova cookies by Urbanus and others 2014b) and simple syrups. Through R-index testing, Urbanus and others (2014b) observed beet sugar meringue cookies as being softer than cane sugar meringue cookies. However, the underlying cause of the textural differences between beet and cane meringue cookies has not been investigated.

Meringues are produced by incorporating air into a mixture of egg whites and sugar. Ovalbumin, ovotransferin, ovomucoids, ovoglobulins, and lysozyme are key proteins in foam formation and stabilization. The collective action of these proteins together allows for high volume foam formation during whipping, and foam stabilization during heating. Sucrose has been shown to decrease egg foam volume, but increase foam stability (Raikos and others 2007). Factors such as pH, trace mineral content, and sulfite have been shown to affect the functionality of egg white proteins (Turner and others 1959; Lomakina and Mikova 2006). Lu (2016) reported differences in pH, conductivity ash, and sulfite content between various beet and cane sugar sources. Thus, chemical differences between beet and cane sugar may cause functional differences when combined with egg white proteins.

Recently, a good deal of research has been done on the physical, chemical, and thermal differences between beet and cane sugar (Lu and others 2013; Urbanus and others 2014abc; Lu and others 2015; Lu 2016). Though sensory differences in beet and cane meringue cookies have been identified, these sensory differences have not been instrumentally quantified and the underlying chemical cause of these differences have not been determined. Thus, the purpose of this research is to investigate the nature of the functionality differences between beet and cane sugars in a model egg white system.

3.2 Materials and Methods
3.2.1 Materials
3.2.1.1 Sucrose

Two sugar sources, C&H cane sugar (Domino Sugar, Yonkers, NY) and Pioneer beet sugar (Michigan Sugar Company, Bay City, MI), were used in this study. These brands were
selected because they are representative of commercially available beet and cane sugars and have been used in prior research (Urbanus and others 2014abc; Urbanus 2014; Lu 2016). While a large number of beet and cane sugar brands exist within the United States, there is a much smaller number of separate manufacturers. C&H cane sugar, from the ASR group (Crockett, CA), and Pioneer beet sugar (Michigan Sugar Company, Bay City, MI) are from separate, but representative sugar manufacturers. Both sugars were purchased at a local grocery store (Urbana, IL), and were stored at room temperature in standard factory packaging before and during use. No caking during storage was observed.

3.2.1.2 Egg White Powder

Spray dried egg white powder (Product P-19-J #428) was donated by Henningsen Foods (Omaha, NE). This product is described by Henningsen Foods as “Whipping egg white without additives for use in bakery and confectionary applications” (Egg Products - Dehydrated Egg Whites n.d.). Spray dried egg white, rather than raw or pasteurized egg white, was selected due to its successful use by Urbanus and others (2014abc), as well as its safety and extended shelf life. The egg white powder was stored in a sealed plastic bag at 4°C. During preliminary testing in this study, high variability was observed between spray dried egg white powder batches from different manufacturers. Thus, all reported results were obtained using the same lot of egg whites from Henningsen Foods to eliminate possible differences due to lot-to-lot variation in egg white powders.

3.2.2 Methods

3.2.2.1 Sample Preparation

3.2.2.1.1 Meringue

Meringues were produced based on pavlova methods used by Urbanus and others (2014b). Egg white protein (5.7% total weight) and sugar (48.4% of total weight) were combined until homogeneous using the bowl and metal whisk attachment of a KitchenAid 5qt tilt stand mixer (KitchenAid, St. Joseph, Mich., U.S.A.). Water (45.9 of total weight) filtered through a reverse osmosis system (Ecowater systems, St Paul, MN) was added and the sides of the mixing bowl were scraped using a rubber spatula. Ingredients were mixed for 2 min at speed 4. The bowl was scraped before mixing again for 2 min and 30 sec at speed 4. The bowl was scraped
and the mixer was turned to speed 8 for 7.5 min, stopping at 4 min to scrape the sides of the bowl. The meringue was used immediately to avoid loss of foam volume and liquid phase separation.

### 3.2.2.1.2 Meringue Cookies

Meringue was placed in a pastry bag (Wilton, Woodridge, IL) and manually dispensed onto a baking sheet lined with parchment paper. The diameter of each meringue cookie prior to baking was approximately 2.5 cm. The meringue cookies were baked in a GE JSP42 electric oven (General Electric, Fairfield, CN) at 300°F (148.9 °C) for 22 min, rotating pans 180 degrees after the first 11 min. Baking sheets of meringue cookies were removed from the oven and the parchment papers containing the cookies were removed from the baking sheets. Meringue cookies were cooled on the parchment paper for approximately 5 minutes on wire cooling racks before analysis. Figure 3.1 shows an examples of each type of meringue cookie after baking and before analysis.

### 3.2.2.1.3 Heat Denatured Sugar-Egg White Gels

Heat denatured sugar-egg white gels (gels) were made using a mixture of 15% spray dried egg white powder (Henningsen Foods, Omaha, Nebraska), 42.5% sugar and 42.5% filtered water. Solutions were mixed for 2 hours using a magnetic stir bar. The solutions were then poured into 15ml centrifuge tubes (Nalge Nunc International Corporation, Rochester, NY) and incubated for 30 minutes in a 90°C water bath. Centrifuge tubes containing the now solid gels were cooled using cold running water and then chilled in a refrigerator at 4°C for approximately 4 hours. Centrifuge tubes were then left at room temperature for 30 minutes, after which the gels were removed from the centrifuge tubes and cut into 2.5 cm cylinders for texture profile analysis.

### 3.2.2.2 Moisture Content

Moisture content of meringue cookies was measured using an Aqualab TrueDry CV9 moisture analyzer (Decagon, Pullman, WA). Tared aluminum sample cups were filled by hand with 2 to 3 grams of whole crushed meringue cookie (approximately 1.5 to 2 cookies). Sample cups were placed inside the TrueDry moisture analyzer and dried at 130°C until the weight
change of each sample was less than 0.02% per minute. Three batches of meringue cookies were produced from each sugar on different days and four samples from each batch were analyzed.

3.2.2.3 Water Activity

Water activity was measured using an Aqualab 4TE (Decagon, Pullman, WA) in single measurement mode at room temperature (ranging from 24.8 to 26.3°C). Water activity and sample temperature were recorded. The $a_w$ meter was verified using salt solutions of known water activity values.

3.2.2.3.1 Meringue Water Activity

Meringues were placed in a Boulder plastic bag (Aldi inc., Batavia, IL) and a small hole (about 2mm) was made in the bottom corner. Meringue was dispensed into Aqualab plastic analysis cups such that the meringue completely covered the bottom of the cup and reached half the total height of the cup. Lids were placed on sample cups prior to analysis. Three batches of Meringue were produced from each sugar on different days. Three samples from Day 1 were analyzed and four samples Day 2 and Day 3 were analyzed. Figure 3.2 shows meringues in sample cups prior to lidding and analysis.

3.2.2.3.2 Meringue Cookie Water Activity

To gain an accurate water activity measurement of the inside of each meringue cookie, the top and sides of each cookie were removed using a razor blade (American Safety Razor Company, Verona, VA). The resultant internal cookie portion was placed in the center of an Aqualab plastic analysis cup to be analyzed. Three batches of meringue cookies were produced from each sugar on different days. Three samples from Day 1 were analyzed and four samples Day 2 and Day 3 were analyzed. Figure 3.3 shows the inside of a meringue cookie in a sample cup prior to lidding and analysis.

3.2.2.4 Texture Profile Analysis

A two-bite texture profile analysis was completed on heat denatured sugar-egg white gels and meringue cookies using a TA XT2 Texture analyzer (Texture Technologies Corp, Hamilton, MA). Test parameters were 40% compression on both bites, 2mm/s pretest speed, 1mm/s test
speed, 1mm/s pretest speed, and 5 seconds between bites. Data were analyzed for hardness (maximum force in g) and cohesiveness (ratio of area peak 2/area peak 1 in %). Three batches of meringue cookies were produced on different days. Texture profile analysis was completed on 6 samples of each sugar type on Day 1. The sample size was increased to 20 samples each on days 2 and 3 due to larger within day variation.

### 3.2.2.5 Three-Point Break Texture Analysis

A three-point break test was completed on meringue cookies using the TA XT2 Texture analyzer (Texture Technologies Corp, Hamilton, MA). Test parameters were 75% compression, 2 mm/s pretest speed, 1mm/s test speed, 1mm/s posttest speed. The bottom supports were separated by a distance equal to 1/3 the average meringue diameter of each day. Data was analyzed for force to break (peak force during experiment in g), total distance in mm, and distance to break in mm. Three batches of meringue cookies were produced on different days. Three point break was completed on ten samples of each sugar type on each day.

### 3.2.2.6 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) was used to analyze the thermal behavior of meringue cookies. DSC measurements were carried out using a Q2000 DSC and analysis was carried out using TA universal analysis (TA instruments, New Castle, DE). Because textural differences were consistently observed only on the inside of meringue cookies after baking, only the inside portion of beet and cane meringue cookies were analyzed. Beet and cane meringues were analyzed immediately after baking (termed fresh), after 24 hours storage in individual sample cups, and after drying.

For fresh and 24 hr storage meringue cookies, approximately 8mg of meringue cookie inside portion was placed in TA Tzero aluminum pans and sealed with inner and hermetic outer lids. An inner lid was used to improve the contact between the sample and the bottom of the Tzero pan. Samples were equilibrated at 50°C, then equilibrated at -60°C, then ramped from -60°C to 200°C at 20°C/min. On day 2 of analysis for stored meringue cookies, ramp endpoint temperature was lowered to 120°C due to concerns over pans bursting. The thermal profile of meringue cookies was obtained using DSC and analyzed using TA Universal Analysis software (version 4.5A, TA instruments, New Castle, DE) for glass transition onset and ΔCp.
In an attempt to eliminate variation caused by differing moisture contents, beet and cane meringue cookies were dried in a desiccator for a minimum of 12 days. The inside portions of 4 dried meringue cookies were then removed, crushed, thoroughly mixed, and approximately 14mg of material was placed inside unsealed TA tzero aluminum pans. Filled pans were then dried in a desiccator until the cookie weight fluctuated less than 3% over 24 hours. Pans were then sealed with an inner and hermetic outer lid and analyzed. Samples were equilibrated at 75°C, then -60C, then the temperature was then ramped from -60°C to 225°C at a rate of 20°C/min. The thermal profile of meringue cookies was analyzed using TA Universal Analysis software for glass transition onset, cold crystallization onset, and the net area of cold crystallization and crystalline melting peaks.

3.2.2.7 Foam Stability

Foam stability was analyzed by measuring liquid drainage after foam production. This method is used widely in both the literature and industry (Stadelman and Cotterill 1995; Raikos and others 2007; Berry and others 2009; Henningsen Foods, Omaha, NE). Immediately after production, a weighed amount of foam (approximately 100g) was transferred to a drainage apparatus consisting of a plastic mixing bowl (Arrow, Elk Grove, IL) with a 7mm hole cut at the center of the bottom of the bowl. Liquid drainage was collected using a tared 500ml beaker (Pyrex, Greencastle, PA) placed on a Mettler PM3000 or Mettler BB6000 digital scale (Mettler Toledo, Columbus, OH) beneath the drainage apparatus. The drainage weight was measured every hour for the first eight hours and at hours 10, 12, 18, and 24. Meringues from each sugar were produced simultaneously, and the process as a whole was repeated on three different days.

3.2.2.8 pH of Meringue Starting Mixtures

pH of meringue starting mixtures was measured using a Thermo Orion 410 (Thermo Fisher Scientific, Waltham, Massachusetts) pH analyzer calibrated using standards of pH 4, 7, and 10. Mixtures of 5.7% egg white protein, 48.4% sugar (beet or cane), and 45.9% water were combined using a magnetic stir bar for approximately 1 hr. Three samples were analyzed from mixtures of each sugar type.
3.2.2.9 Simulated Baking

The objective of simulated baking was to track water loss during baking to determine when differences in moisture content between beet and cane meringues occur within the baking process. Simulated baking was carried out using an Aqualab TrueDry CV9 moisture analyzer (Decagon, Pullman, WA). Meringue was loaded into piping bags and 2 to 3 grams of meringue was piped into tared metal sample cups. Sample cups were placed inside the TrueDry moisture analyzer and dried at 300°F (148.9°C) until the weight change of each sample was less than 0.02% per minute. This process took around 70 min. Meringue from each sugar was produced simultaneously and four samples from each batch were analyzed. This process was repeated 6 times on different days.

Moisture content was calculated on a wet basis using Equation 1:

$$MC_t = \left(\frac{(W_t - W_{td})}{W_{ti}}\right) \times 100\%$$  \hspace{1cm} (Equation 1)

where $MC_t$ is the moisture content at time $t$, $W_t$ is the sample weight at time $t$, $W_{td}$ is the weight of the dried sample, and $W_{ti}$ is the initial weight of the sample. Drying rate was calculated on a dry basis using Equation 2:

$$R_t = \left[\frac{(W_{t-1} - W_t)}{(W_{td} (t - t_{-1}))}\right]$$  \hspace{1cm} (Equation 2)

where $R_t$ is the drying rate at time $t$, $W_{t-1}$ is the sample weight at time $t_{-1}$, $W_t$ is the sample weight at time $t$, and $W_{td}$ is the weight of the dried sample, $t$ is time $t$, and $t_{-1}$ is the time point immediately before to time $t$.

3.2.2.10 Extended Baking

To create a beet sugar meringue cookie with similar moisture content to cane sugar meringue cookies, beet sugar meringue cookies were made as previously described, but batches were baked an additional 5 and 10 min. Samples were analyzed for moisture content, with a sample weight of 1 to 2 g due to a limited amount of sample, and water activity as previously described in section 3.2.2.2.1.

3.2.3 Statistical Analysis

Moisture content, water activity, meringue cookie texture, and gel texture data were analyzed with SAS software version Studio 3.5 (SAS Institute Inc., Cary, NC) using the general
linear model procedure (GLM), with product, day, and product-day interaction in the model. Day and product-day interaction were treated as random effects. P-values from the type I sums of squares estimable function were used for moisture content, meringue cookie texture, and gel texture. For water activity data, P-values from the type III sums of squares estimable function and lsmeans were used to account for uneven sample sizes between days (SAS/STAT 9.2 User's Guide n.d.). No significant interaction between day and sucrose source was observed. Below is an example of the model used in GLM analysis.

Moisture Content = Sugar Day Sugar*Day

In this model, “Moisture content” represents the dependent variable; sugar represents the source of sugar (i.e. C&H cane or Pioneer beet sugar). Day of experimentation was included in the model to investigate and mitigate the effects of day-to-day variation.

Due to the unequal time points during simulated baking, data were analyzed using the ggplot2 package within RStudio (RStudio Inc., Boston, Ma). For sections A and C, a non-parametric regression line with 95 percent confidence intervals were fitted to data from each sugar source using the “Loess” method within the “geom_smooth” function. For section B, the “lm” method with 95% confidence intervals were used. Drying rates during section B were analyzed using a two tailed, unpaired, t-test (P<0.05).

3.3 Results and Discussion

3.3.1 Moisture Content and Water Activity

Before and after baking moisture content and water activity data were determined and are presented in Table 3.1. Prior to baking, the moisture content of cane meringue was significantly higher (α=0.05) than that of beet meringue. While statistically significant, the 0.17% difference in moisture content between beet and cane meringues is too small to be considered practically meaningful. In practical terms, this difference between beet and cane meringues translates to 3.4 milligrams of water in a meringue with a starting weight of 2 grams.

Prior to baking, the water activity values of beet and cane meringues were not significantly different. Because the degree of dissolution of sugar in a solution determines the water activity (Schmidt 2004), lack of significant difference between beet and cane meringues suggests that the sugars were dissolved to a similar degree. The lack of meaningful difference
between both the moisture content and water activity values before baking suggests that, prior to baking, the amount and behavior of water is similar between beet and cane meringues.

After baking, the moisture content water activity of beet meringue cookies were significantly higher than that of cane meringue cookies. Because the water activity and moisture content of beet and cane meringues were the same prior to baking, it can be inferred that a functionality difference between beet and cane sugars is associated with increased retention of water in beet meringue cookies during baking.

Significant day-to-day variation was observed for all moisture content and water activity measurements. This day-to-day variation was attributed to environmental conditions and the variations inherent in the baking process. To limit variation between days, meringue cookies were only baked on low humidity days, with a relative humidity range between 12 to 23% and a temperature range between 21.0°C to 22.6°C. To account for day-to-day variation, day was included in the statistical model during data analysis. It is also worth noting that the variation in measurements of moisture content and water activity data was very low before baking and markedly increased after baking.

3.3.2 Texture Analysis

Results from the texture profile analysis and three-point break analysis are presented in Table 3.2. The mean peak force required to compress a sample during texture profile analysis (Hardness) was significantly higher in cane meringue cookies than in beet meringue cookies. Similarly, the peak force required to break a sample during three point break testing (Force to break) was higher in cane meringue cookies than in beet meringue cookies. Higher hardness and force to break measurements indicate a stronger foam structure in cane meringue cookies than in beet meringue cookies.

Cohesiveness is a measure of “the strength of internal bonds making up the body of the product” (Brookfield n.d.). Cohesiveness was significantly higher in beet meringue cookies than in cane meringue cookies. Urbanus and others (2014b) observed beet meringue cookies to have a notable softer, marshmallow-like texture, suggesting higher cohesiveness when compared to cane meringue cookies, which were observed to have the desirable, crunchy, hard, foam-like texture. It is worth noting that interaction between day and product with respect to cohesiveness was significant, cohesiveness being higher in beet meringues on day 1 and 3, and higher in cane
meringues on day 2. During experimentation, both beet and cane meringue cookies were observed to have both hard and soft textures, with beet meringue cookies having a soft texture more often than cane meringue cookies. Significant interaction between product and day with respect to cohesiveness suggests inconsistent textural attributes between batches. Significantly higher overall cohesiveness in beet meringue cookies than in cane meringue cookies with significant interaction between day and product accurately reflects observations made during experimentation, and suggests that beet meringue cookies are often, but not always, more cohesive than cane meringue cookies. This day to day variation in cohesiveness is likely due to variations in meringue cookie height between days and differing environmental conditions between days.

Final probe distance during three point break testing was not significantly different between beet and cane meringue cookies. Because the distance the probe traveled during each test was equal to 75% of the total height of the sample, the final probe distance can be related to sample height. Lack of a significant difference between beet and cane final probe distance indicates that beet and cane samples had similar heights. Because height influences breaking characteristics, consistent heights between samples are desirable. It is worth noting that final probe distance was significantly different between days, indicating that meringue cookie height differed between days. Differing heights between days is likely due to differences in product production via piping and differences in environmental conditions.

The distance to break during three point break testing between beet and cane meringue cookies was also not significantly different. This suggests that, in addition to being of similar heights, beet and cane meringue cookies broke at similar points during the probing process. Thus, the difference in force to break values between beet and cane meringue cookies is not due to breaking behavior, but the strength of the internal structure of the cookie.

3.3.3 Differential Scanning Calorimetry

Thermograms of fresh beet and cane meringue cookies were obtained using DSC. Example DSC thermograms of fresh beet and cane meringue cookies are presented in Figure 3.4. As can be seen in Figure 3.4, the cane meringue cookie thermogram exhibits a glass transition and the onset of a cold crystallization peak. The beet meringue cookie thermogram exhibits a sloping transition down to a baseline that continues until the beginning of an endothermic event
likely caused by the pan becoming unsealed due to pressure. While these thermograms are representative of what was observed most often for each sugar type, some cane meringue cookies produced thermograms that were similar to that of the example beet meringue cookie and some beet meringue cookies produced thermograms that were similar to that of the example cane meringue cookie. The thermal events shown in the beet example thermogram are likely due to the soft, sticky structure of many beet and some cane fresh samples. This soft, sticky structure was difficult to load into the DSC pan and sample preparation often resulted in significant loss of sample structure, as well as problems with pan sealing integrity. To obtain thermograms that are more conducive to analysis from soft, sticky samples, it may be beneficial to freeze samples with liquid nitrogen or wrap samples in tinfoil prior to placing them in the DSC pan in future studies.

After storage in individual sample cups for 24 hours, beet and cane meringue cookie DSC thermograms were obtained. An overlay of thermograms from stored beet and cane meringue cookies is shown in Figure 3.5. Both beet and cane thermograms exhibited glass transitions, cold crystallization peaks, and crystalline melting peaks. During day 1 of experimentation, highly variable cold crystallization and crystalline melting peaks, as well as multiple pan bursts, were observed. Thus, the endpoint temperature was reduced to 125°C on day 2 of experimentation, which halted sample analysis during cold crystallization and before crystalline melting. As a result, cold crystallization and crystalline melting DSC parameters were not obtained for stored meringue cookies. Thermograms were analyzed for glass transition onset temperature, midpoint temperature, endpoint temperature, and change in heat capacity (ΔCp). Mean and standard deviation results from this analysis are presented in Table 3.3. Glass transition onset temperature, midpoint temperature, and endpoint temperature means were lower in stored beet meringue cookies than stored cane meringue cookies, though these differences were not statistically significant (α=0.05) due to the large standard deviations associated with these measurements. Higher moisture content in beet meringue cookies compared to cane meringue cookies is likely responsible for the differences between beet and cane glass transition temperatures. Large variation was observed in glass transition temperatures for meringue cookies made with both sugar sources, which is consistent with textural observations. Beet and cane stored meringue cookies exhibited similar ΔCp values, with beet meringue cookies ΔCp being slightly lower than cane meringue cookie ΔCp. Similar glass transition ΔCp values suggest that the amount of amorphous material is not substantially different between stored beet and cane meringue cookies.
To eliminate variation due to moisture content, beet and cane meringue cookies were dried over desiccant prior to analysis. An overlay of example dried beet and cane meringue cookie thermograms is presented in Figure 3.6. All dried samples exhibited a glass transition, as well as overlapping cold crystallization and crystalline melting peaks. Due to the overlapping of the cold crystallization and crystalline melting thermal events, individual integration of each peak was not possible, but rather a linear integration of both peaks was carried out. The angle of linear integration was found by extending the baseline between the glass transition and cold crystallization to the end of crystalline melting, as shown in Figure 3.7. DSC results from analysis of dried beet and cane meringue cookie thermograms are presented in Table 3.4. Onset, midpoint, and endpoint glass transition temperatures were lower in dried beet meringue cookies compared to cane meringue cookies, with onset and endpoint differences being statistically significant. These differences may be attributed to differences in decomposition and/or slight differences in moisture content after drying. ΔCp values were not significantly different between beet and cane dried meringue cookies. Similar glass transition ΔCp values between beet and cane dried meringue cookies suggest that the amount of amorphous material is not significantly different. Cold crystallization onset was significantly lower in dried beet meringue cookies compared to dried cane meringue cookies. This difference may be attributed to differences in decomposition and/or slight differences in moisture content after drying. Linear integration of both peaks produces a very small net difference between the areas of cold crystallization and crystalline melting peaks in both beet and cane dried meringue cookies. Differences in thermal behavior (glass transition, cold crystallization, and melting) were observed between stored and dried meringue cookies. These differences could be due higher moisture content in stored samples compared to dried samples or differences in decomposition between batches of meringue cookies. In particular, the difference between stored beet and cane meringue cookie glass transition

The amount of amorphous material is often quantified by either comparing the size of the sample glass transition to the size of the glass transition for a 100% amorphous material (for example 0.78 J/g°C as determined for freeze-dried sucrose by Magoń and others 2014) or by comparing the area under the exothermic cold crystallization peak to the area of the endothermic melting peak (Thomas and Schmidt 2016). For dried meringue cookies made with both sugar sources, these two measures of amorphous content give rather different results. Based on the size
of the glass transition in dried meringue cookies (ΔCp, Table 3.4), the amorphous content is approximately 78%, whereas, based on the area comparison the amorphous content is nearly 100%. While more research would be necessary to determine the true amount content, it is worth noting that there are concerns with both of these measures of amorphous content. In regards the use if the size of the glass transition, it has been reported that composition, method of production, and thermal decomposition greatly affect the change in heat capacity associated with the glass transition (Vanhal and Blond 1999; Lee and others 2011; Roos 2010). Thus, it may not be appropriate to compare the ΔCp of the meringue cookie to the ΔCp of freeze-dried sucrose. In regards to the use of the areas of the cold crystallization and melting peaks, as mentioned previously, it was not possible to individually determine the enthalpy associated with each event, due to their overlap. Thus, a linear integration approach, based on the initial slope of the baseline, was used to determine the net enthalpy difference between the peaks. The net value being close to zero suggested that the material was 100% amorphous, but as can be seen in Figure 3.7, the end of the integration line appears to contact the melting peak in a rather arbitrarily location, rather than smoothly connect with the baseline, potentially resulting in an inaccurate net area. The conflicting results of these DSC amorphous content qualification methods merit additional research.

3.3.4 Foam Stability

Mean and standard deviation values from three replications of the foam stability test are shown in Figure 3.8. Though beet meringues exhibited higher drainage over time, drainage measurements were not significantly different at any time point, suggesting that beet and cane meringues had similar draining behavior over time, and thus similar foam stability. Foam stability is a measure of how well foam can hold its liquid portion in the foam matrix against the force of gravity, and is affected by protein functionality, sugar content, and sugar dissolution (Raikos and others 2007). Similar foam stability between beet and cane meringues indicates that both sugar sources interacted with the egg white proteins responsible for foaming in a similar manner.

A number of compounds have been shown to affect stability in egg white foams, including sucrose (Raikos and others 2007), copper sulfate (Phillips and others 1987), iron, and aluminum (Cotterill and others 1992). While beet and cane sugar are greater than 99% sucrose,
differences exist in the impurities that have the potential to cause differences in foam stability. Values for the pH, conductivity ash and total sulfite content for Pioneer beet sugar and C&H cane sugar are shown in Table 3.5. C&H cane sugar has been shown to have a higher amount of conductivity ash than Pioneer beet sugar. Additionally, Pioneer beet sugar has been shown to have a higher pH and total sulfite content than C&H cane sugar (Lu 2016). pH has been shown to affect foam stability and bubble morphology, with egg foams at pH 4.8 having the best long term stability and smallest bubbles compared to pH 7 (Lomakina and Mikova 2006). Despite differences between beet and cane sugar impurities and pH, no significant difference in foam stability was observed between beet and cane meringues, suggesting that the impurities present in C&H cane sugar and Pioneer beet sugar are not present in high enough concentrations in foams to produce consistent differences over multiple days. An example of the limited effect of differences between beet and cane sugar sources in egg mixtures is pH. Though Pioneer beet sugar and C&H cane sugar significantly different pH values in aqueous solution, Pioneer beet and C&H cane sugar-egg white mixtures have pH values of 6.77±0.16 and 6.69±0.03 respectively.

It is worth noting that both beet and cane meringues exhibited high levels of variation in foam stability between days. Due to equipment limitations, only one replication per day was possible, thus, no measure of the variation of foam stability within day was available. However, moisture content and water activity measurements of unbaked meringues had very little variation within batches (within day standard deviation values for moisture content and water activity were 0.15 and 0.0020, respectively) suggesting that unbaked meringues have very little initial variation within each batch. Variability between days is likely attributable to meringue samples being somewhat exposed to environmental conditions throughout testing. Variation increased throughout testing, suggesting that longer exposure to environmental conditions caused higher variation between days. As shown in Figure 3.9, the bowl holding the meringue during drainage was covered with cellophane, but the drained liquid collection bowl was uncovered. The drainage liquid’s exposure to environmental conditions could result evaporation over time.

3.3.5 Heat Denatured Sugar-Egg White Gels

Heat denatured sugar-egg white gels (gels) were analyzed with texture profile analysis to investigate beet and cane sugar’s effect on egg white protein functionality in an unwhipped
matrix. Results from the texture profile analysis of gels are presented in Table 3.6. No significant difference was found between the hardness of beet and cane gels, suggesting that beet and cane sugars do not have a significant effect on egg white proteins’ ability to form firm gels. Cohesiveness, however, was significantly different between beet and cane gels, with beet sugar gels having a higher cohesiveness value. The creators of texture profile analysis described cohesiveness as “a direct function of the work needed to overcome the internal bonds of a material” (Friedman and others 1963; Rosenthal 2010). This limited difference (1.4%) between beet and cane gel cohesiveness suggests that beet gels formed a stronger internal protein bonding network than cane sugar.

3.3.6 Simulated Baking

A plot of moisture content over time during simulated baking in a TrueDry moisture analyzer is presented in Figure 3.10. The data were divided into 3 sections, A, B, and C, based on the drying rate profile shown in Figure 3.11. Figure 3.11 shows a rising rate period from minute 0 to minute 5 (section A), a transitional period from minute 5 to minute 8 (section B), and a falling rate period from minute 8 until the samples are completely dry (section C).

Data from section A are shown in Figure 3.12. To determine if differences exist in water loss during baking behavior between beet and cane meringues, a non-parametric regression with shaded regions representing 95% confidence intervals for the mean expected value were plotted in Figure 3.12. Beet and cane showed very similar behavior during section A. Because samples are placed in the TrueDry moisture analyzer at room temperature, the first portion of the baking process involves heating samples from room temperature to analysis temperatures. As a result, both beet and cane meringues exhibited a rising rate of water loss between 0 and 5 minutes, as shown in section A of Figure 3.11.

The transitional period between the rising rate period (section A) and falling rate period (section C) is shown in Figure 3.13. This transition spans from minutes 5 to 8 with linear trend lines modeling the generally linear decrease in moisture content during this period of baking. While food products normally exhibit a constant rate drying period after the rising rate period, no clear plateau in drying rate was observed. An analysis of the drying rates during this period shows significant differences between beet and cane meringues with cane meringues loosing water at a faster rate than beet meringues during this period. This higher rate of water loss in
cane meringues compared to beet meringues may be attributed to differing functionality in egg white proteins as they begin to denature due to heating.

Following the transitional period there is a falling rate drying period. The falling rate drying period extends from minute 8 until the samples are completely dry. Figure 3.14 shows a non-parametric regression line for each sugar source during section C. Beet and cane meringues show markedly different moisture contents during the initial period of section C and then converge as samples approach complete dryness. The horizontal lines in Figure 3.14 show the moisture content of beet and cane meringue cookies after baking in oven and given in Table 3.1. Baking in a conventional oven and simulated baking in a TrueDry moisture analyzer show differences in the rate of drying, with a conventional oven drying samples at a faster rate than the TrueDry moisture analyzer. This is likely due to differing heating methods, with conventional oven being a combination of convection and radiation, and the TrueDry moisture analyzer utilizing convection, conduction, and desiccated air to reduce decomposition within samples. The slower drying rate in the TrueDry moisture analyzer is likely attributed to the TrueDry’s gentle heating to avoid decomposition within samples. Additionally, the conventional oven was preheated prior to bake, while the Truedry heats during analysis. Because of the difference in drying rate, moisture content should be used to determine doneness, rather than time. The beet and cane meringue cookie moisture content lines intersect the regression line at minute 26.2 and 28.4 respectively, marking where samples were removed from the oven. As discussed in section 3.3.1, beet and cane meringues significantly differ in moisture contents after baking. Figure 3.14 shows that beet and cane meringues have significantly different moisture contents leading up removal from the oven. Continuing baking produces beet meringue cookies with similar moisture contents.

In order to create beet and cane meringue cookies with similar moisture profiles, beet sugar meringues were baked for an additional 5 and 10 minutes (as described in section 3.2.2.4). Moisture content and water activity measurements are presented in Table 3.7. As with previous experiments, beet exhibited a higher moisture content and water activity after both beet and cane meringues cookies were baked for 22 minutes. However, after 27 minutes of baking, beet meringue cookies showed similar moisture content and water activity to that of cane meringue cookies after 22 minutes of baking. While moisture content and water activity values were similar between beet and cane meringues after 22 and 27 minutes of baking respectively,
additional browning and loss of volume was observed in beet samples after 27 minutes than cane after 22 minutes. These observations suggest that, while beet and cane samples may have similar moisture profiles after baking for 22 and 27 minutes, respectively, consumer acceptance may be compromised in beet meringue cookies with additional baking.

Urbanus and others (2014b) found that after baking for 38 minutes, beet meringue cookies reached the same texture as cane meringue cookies baked for 22 minutes. While the nature of our results are in agreement with those of Urbanus, the extra baking time for beet meringue cookies needed to produce similar moisture profiles beet and cane meringue cookies in our experiment was considerably less than the time needed for Urbanus’s beet meringue cookies to reach similar texture profiles. This difference in time is likely due to differing oven or environmental conditions between our experiment and that of Urbanus and others (2014b).

A number of factors are likely associated with differences between beet and cane meringue cookies, the first of which is sucrose hydrolysis and browning. As meringues loose water during baking, the solids concentration of the liquid portion of the foam matrix increases. As solids content increases, the boiling point of the liquid portion increases, allowing the product temperature to increase. Boiling point elevation is a colligative property of a solution, meaning an increase in the number of molecules dissolved in a solution will result in an increase in the boiling point of the solution. Cane sugar has been shown to hydrolyze at a faster rate than beet sugar (12.38% inversion in beet sugar and 33.98% inversion in cane sugar over three hours) at 120°C under standard pressure (Hubbard and Mitchel 1915). Hydrolysis involves the conversion of sucrose into glucose and fructose, increasing the number of molecules in solution, and thus, increasing the boiling point elevation. Higher hydrolysis in cane sugar meringue cookies than beet sugar meringue cookies could cause higher temperatures during baking, and thus affect drying rates, protein denaturation, and browning. Accurate measurement of temperatures during baking was not possible due to the fragility of the foam structures during baking.

Because sucrose is a non-reducing sugar, it does not participate in Maillard browning reactions. However, the products of sucrose hydrolysis, glucose and fructose, can readily participate in Maillard browning. Sucrose hydrolysis and the thermal decomposition of sucrose can also lead to color formation through caramelization. An example of the observed browning differences between the inside portion of beet and cane sugar meringue cookies which were dried in a desiccator after baking is shown in Figure 3.15. The inside portion of the cane sugar
meringue cookie consistently appeared darker than beet sugar meringue cookie insides. Higher amounts of Maillard browning and caramelization are likely the cause of color differences observed during baking.

Other factor likely associated with differences between beet and cane meringue cookies are protein denaturation and subsequent protein-protein interactions. Because differences were seen in beet and cane sugar meringues only in the later portions of baking and after baking, it is possible that subtle differences in composition between beet and cane sugar affect how egg white proteins denature and interact during heating.

As previously mentioned, beet sugar has a higher sulfite content compared to cane sugar due to the use of sulfur dioxide during processing (Asadi 2007; Godshall 2013). Sulfite has been shown to cleave disulfide bonds in a well understood reaction shown in Equation 3.1 (Clark 1932; Stricks and Koltzoff 1951; Thannhauser and others 1984):

$$RS-SR + SO_3^{2-} \rightarrow RS-O-SO_3^- + RS^-$$

(Equation 3.1)

Ovalbumin, ovotransferin, ovomucoid, and lysozyme all contain disulfide bonds, which could be cleaved by sulfite originating from beet sugar. Ovalbumin, which comprises 54% of egg white proteins by weight, contains 1 disulfide bond and 4 free sulfhydryl groups. During foaming, ovalbumin has been shown to migrate to the gas liquid interface, where it changes conformation and participates in disulfide bonding (Kitabatake and Doi, 1987). However, Doi and others (1989) assert that disulfide bond formation has a limited effect on the foaming properties of ovalbumin (Raikos and others 2007). Additionally, during foam formation in egg white-sucrose mixtures, more ovalbumin is likely to remain in the liquid phase due to hydrogen bonding between ovalbumin and sucrose (Antipova and others 1999; Raikos and others 2007). Both interfacial adsorption during foaming and heating have been shown to increase exposure of sulfhydryl groups in ovalbumin (Kitabatake and Doi 1987; Mine and others 1990). Ovalbumin’s free sulfhydryl groups, the only free sulfhydryl groups in native egg white protein, have been shown to participate in disulfide bonding in solution above 70°C (Mine and others 1990; Lechevalier and others 2003; Raikos and others 2007). In addition to ovalbumin, ovotransferin makes up 12% of egg white proteins and contains 15 disulfide bonds within its native structure.
Ovomucoid and lysozyme, two egg white proteins important in the foaming process, have 9 disulfide bonds and 4 disulfide bonds, respectively (Mine 1995).

Because of the high degree of disulfide bonding within native egg white protein and the limited sulfite content of beet sugar, only a limited portion of the disulfide bonds in egg white proteins are likely to be cleaved in beet sugar meringues. While this cleaving of disulfide bonds in egg white proteins will have an initial destabilizing effect, additional sulfhydryl groups are freed during this process. These sulfhydryl groups are then available to participate in sulfhydryl oxidation or sulfhydryl-disulfide exchange as reported by Turner and others (1959). Thus, the initial breaking of a limited number of disulfide bonds in egg white proteins may lead to a higher degree of inter- and intra-molecular disulfide bonding within the final foam structure. Shimada and Cheftel (1988) showed that inhibition sulfhydryl-disulfide interchange and sulfhydryl oxidation in whey protein isolate decreases water holding capacity. Similarly, Honda and others (1998) suggested that increased disulfide bond formation in heat denatured egg whites was important in increasing water holding capacity at alkaline pH. Based on these finding and differences during and after baking, increased disulfide bonding may increase the water holding capacity of beet meringues, particularly during later portions of baking.

Similarly, the cleaving action of sulfite could be responsible for the limited increase in cohesiveness seen in beet sugar gels compared to cane sugar gels. Ovalbumin contains one intramolecular disulfide bond, which has little effect on the native conformation of ovalbumin, but increases ovalbumin’s resistance to heat denaturation (Takahashi and others 1991). Cleaving this intramolecular bond leads to the denaturation of ovalbumin at lower temperatures, which could lead to more developed protein networks in beet sugar gels than cane sugar gels. Additionally, sulfhydryl-disulfide interchange has been shown as an important part of the gelation of egg white proteins (Ma and Holme 1982; Shimada and Cheftel 1988; Mine 1995). Cleaving of disulfide bonds leads to an increase in concentration of free sulfhydryl groups, which could lead to increased sulfhydryl-disulfide interchange and sulfhydryl oxidation between protein molecules, as reported by Turner and others (1959).

3.3.7 Storage and Oven Conditions

Notable variation was observed within and between days in meringue cookie texture, moisture content, and water activity. These differences are likely due to variations in
environmental conditions and variations inherent in the baking process, such as piping and location on the baking sheet. Urbanus and others (2014b), however, reported consistent textural differences between beet and cane sugar meringue cookies over multiple days. Discrepancies in variation observed between this study and that of Urbanus’s is likely due to storage conditions. In this study, meringue cookie samples were analyzed immediately after baking. Immediately after baking, the outside of both beet and cane meringue cookies were crispy and the inside texture varied from crispy to soft for both sugar types. Urbanus, on the other hand, stored meringue cookie samples in sealed individual sample cups overnight before evaluation by sensory panelists. Urbanus described beet meringue cookies as marshmallow-like and cane meringue cookies as hard, crunchy, and foam-like. Urbanus observed these differences to be consistent between replications, but reported large variation in R index value (percentage) between replications. Upon replication of the storage methods described by Urbanus and others (2014), the majority of beet meringue cookies were observed to be soft throughout and the majority of cane meringue cookies were observed to be firm and crispy throughout, though there were still significant textural variations within batches. Thus, differences between the consistency of textural observation in this study and that of Urbanus and others (2014b) is likely due to moisture migration between the inner material, outer material, and atmosphere of the sealed container during storages, as well as differences in oven conditions.

Additionally, beet sugar meringues were occasionally observed to have a crispy texture, which was never reported by Urbanus. One possible explanation for this discrepancy could be that Urbanus used a gas convection oven and this study used a non-convection electric oven. Because gas ovens produce water as a byproduct of gas combustion during heating, gas ovens are often more humid than electric ovens (Xue and others 2016). Less humid in oven condition during this study could contribute to higher observation of crispy beet sugar meringues in this study compared to those of Urbanus. In future work, a convection oven is recommended for even heating throughout the oven.

3.3.8 Conclusions

Prior to baking, no meaningful differences in moisture content, water activity, or foam stability were found between beet and cane meringues. After baking, however, significant differences were found in moisture content, water activity, and texture. These differences were
observed to be further accentuated during storage of meringue cookies for 24 hours in sealed containers. Additionally, heat denatured sugar-egg white gels made with beet sugar showed a limited increase in cohesiveness compared to cane sugar gels, suggesting the formation of a stronger protein network.

Differences in moisture content and texture are likely due to increased hydrolysis and browning in cane meringues and the disulfide bond cleaving effect of sulfite, which is in higher concentrations beet sugar than cane sugar. Hydrolysis has been shown to be higher in cane sugar solutions than beet sugar solutions at elevated temperatures and thus could cause increased Maillard browning, caramelization, and boiling point elevation. Browning was observed to be more substantial in the internal portion of cane cookies than beet cookies.

Due to the complex and synergistic activity of egg white proteins during foaming and gelation, the exact effect of sulfite on protein functionality in beet sugar meringues could not be determined during this study. However, lack of meaningful difference in moisture content, water activity, and foam stability prior to baking, and moisture content in the early portion of baking suggests that sulfite does not have a noticeable effect on the egg white proteins’ foaming capability. Moisture content differences later in the baking process, as well as moisture content, water activity, and textural differences in final meringue cookies, suggest that sulfite’s effect on egg white foams is greater during later portions of baking, as proteins denature due to increased temperature and participate in sulfhydryl oxidation and sulfhydryl-disulfide exchange. Higher moisture content and water activity values in beet meringue cookies after baking suggests that a stronger protein network with a higher water holding capacity developed during baking. This is additionally supported by texture results from heat denatured sugar-egg white gels. Gels made with beet sugar showed a limited increase in cohesiveness compared to cane sugar gels, suggesting the formation of a stronger protein network in beet sugar gels compared to cane sugar gels.
3.4 Tables

Table 3.1: Moisture content (% wet basis) and water activity mean and standard deviation values before and after baking. Mean values within a row with the same superscript letter indicate no significant differences (P < 0.05) between sugar sources.

<table>
<thead>
<tr>
<th>Timing</th>
<th>Analysis</th>
<th>C&amp;H (Cane)</th>
<th>Pioneer (Beet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Baking</td>
<td>Moisture Content (%)</td>
<td>45.14±0.34</td>
<td>44.97±0.33</td>
</tr>
<tr>
<td></td>
<td>Water Activity</td>
<td>0.920±0.0033</td>
<td>0.922±0.0034</td>
</tr>
<tr>
<td>After Baking</td>
<td>Moisture Content (%)</td>
<td>2.75±1.34</td>
<td>4.43±1.14</td>
</tr>
<tr>
<td></td>
<td>Water Activity</td>
<td>0.288±0.1372</td>
<td>0.410±0.1174</td>
</tr>
</tbody>
</table>

Table 3.2: Mean and standard deviation values for texture analysis data. Mean values within a row with the same superscript letter indicate no significant differences (P < 0.05) between sugar sources.

<table>
<thead>
<tr>
<th>Analysis Method</th>
<th>Parameter</th>
<th>C&amp;H (Cane)</th>
<th>Pioneer (Beet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture Profile</td>
<td>Hardness (g)</td>
<td>3815.5±1375.45</td>
<td>3130.2±1027.15</td>
</tr>
<tr>
<td></td>
<td>Cohesiveness (%)</td>
<td>4.1±1.84</td>
<td>5.3±3.33</td>
</tr>
<tr>
<td>Three-Point Break</td>
<td>Force to break (g)</td>
<td>981.5±223.09</td>
<td>853.2±203.35</td>
</tr>
<tr>
<td></td>
<td>Final probe distance (mm)</td>
<td>10.2±1.52</td>
<td>10.2±1.72</td>
</tr>
<tr>
<td></td>
<td>Distance to break (%)</td>
<td>36.9±8.60</td>
<td>40.2±17.3</td>
</tr>
</tbody>
</table>

Table 3.3: Mean and standard deviation values form differential scanning calorimetry of beet and cane meringue cookies after 24 hours storage in individual sample cups.

<table>
<thead>
<tr>
<th>Event</th>
<th>Analysis</th>
<th>Pioneer Beet</th>
<th>C&amp;H Cane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Transition</td>
<td>Onset (°C)</td>
<td>2.9±10.08</td>
<td>11.0±4.84</td>
</tr>
<tr>
<td></td>
<td>Midpoint (°C)</td>
<td>10.0±8.9419219</td>
<td>17.2±5.22</td>
</tr>
<tr>
<td></td>
<td>Endpoint (°C)</td>
<td>14.1±8.96</td>
<td>21.1±4.64</td>
</tr>
<tr>
<td></td>
<td>ΔCp (J/(g °C))</td>
<td>0.740±0.0555</td>
<td>0.777±0.0436</td>
</tr>
</tbody>
</table>
Table 3.4: Mean and standard deviation values from differential scanning calorimetry of dried beet and cane meringue cookies.

<table>
<thead>
<tr>
<th>Event</th>
<th>Analysis</th>
<th>Pioneer (Beet)</th>
<th>C&amp;H (Cane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Transition</td>
<td>Onset temperature (°C)</td>
<td>50.3b ± 1.97</td>
<td>52.1a ± 1.32</td>
</tr>
<tr>
<td></td>
<td>Midpoint (°C)</td>
<td>55.9a ± 2.36</td>
<td>57.4a ± 1.32</td>
</tr>
<tr>
<td></td>
<td>Endpoint (°C)</td>
<td>60.6a ± 2.05</td>
<td>63.3b ± 1.42</td>
</tr>
<tr>
<td></td>
<td>$\Delta C_p$ (J/(g °C))</td>
<td>0.612a ± 0.0062</td>
<td>0.614a ± 0.0102</td>
</tr>
<tr>
<td>Cold Crystallization and Crystalline melting</td>
<td>Onset of cold crystallization (°C)</td>
<td>118.9b ± 3.45</td>
<td>121.0a ± 3.54</td>
</tr>
<tr>
<td></td>
<td>Combined integration of cold crystallization and crystalline melting (J/g)</td>
<td>1.04 ± 0.695</td>
<td>1.47 ± 0.647</td>
</tr>
</tbody>
</table>

Table 3.5 pH, conductivity ash, and total sulfite content of Pioneer beet sugar and C&H cane sugar (Lu 2016).

<table>
<thead>
<tr>
<th>Sugar Source</th>
<th>pH</th>
<th>Conductivity Ash (ppm)</th>
<th>Total Sulfite (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pioneer (Beet)</td>
<td>7.02 ± 0.07</td>
<td>72.1 ± 0.8</td>
<td>10.16 ± 3.51</td>
</tr>
<tr>
<td>C&amp;H (Cane)</td>
<td>5.65 ± 0.11</td>
<td>120.4 ± 11.6</td>
<td>&lt;DL*</td>
</tr>
</tbody>
</table>

*Detection Limits (DL) = 5.28 ppm

Table 3.6: Means and standard deviations of texture profile on heat denatured sugar-egg white gels.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Pioneer (Beet)</th>
<th>C&amp;H (Cane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (g)</td>
<td>1579.1a±146.01</td>
<td>1588.3a±108.96</td>
</tr>
<tr>
<td>Cohesiveness (%)</td>
<td>71.2a±0.91</td>
<td>69.8b±0.91</td>
</tr>
</tbody>
</table>
Table 3.7: Mean and standard deviation values for extended baking experiment.

<table>
<thead>
<tr>
<th></th>
<th>C&amp;H Cane</th>
<th>Pioneer Beet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baking Time (min)</strong></td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td><strong>Moisture Content (%)</strong></td>
<td>2.0 ± 0.57</td>
<td>4.6 ± 0.99</td>
</tr>
<tr>
<td><strong>Water Activity</strong></td>
<td>0.168 ± 0.0492</td>
<td>0.365 ± 0.0324</td>
</tr>
</tbody>
</table>
3.5 Figures

Figure 3.1: Example Pioneer beet and C&H cane meringue cookies after baking.

![Example Pioneer beet and C&H cane meringue cookies after baking.](image)

Figure 3.2: Beet and cane sugar meringues in water activity analysis cups.

![Beet and cane sugar meringues in water activity analysis cups.](image)
Figure 3.3: Inside of a beet sugar meringue cookie prior to analysis

Figure 3.4: Example thermograms of fresh beet and cane meringue cookies.
Figure 3.5: DSC thermogram overlay of a Pioneer beet and a C&H Cane meringue cookie from each day of testing stored for 24 hours in individual sample cups.
Figure 3.6: Example DSC thermogram of dried Pioneer beet and C&H Cane meringue cookies.

Figure 3.7: Example of linear integration for a dried pioneer beet meringue cookie.
Figure 3.8: Mean liquid drainage from meringues over time for Pioneer beet meringues and C&H cane meringues.
Figure 3.9: Foam stability apparatus.
Figure 3.10: Simulated baking moisture content versus time plot for beet and cane meringues using the TrueDry moisture analyzer.
Figure 3.11: Drying rate over time from simulated baking in the TrueDry moisture analyzer. The vertical reference line at 5 minutes indicates a transition from the rising rate period to the transitional rate period. The vertical reference line at 8 minutes indicates a transition from the transitional rate period to the falling rate period.
Figure 3.12: Simulated baking plot of section A with fitted non parametric curve and 95% confidence intervals for beet and cane meringues.
Figure 3.13: Simulated baking plot of section B with linear trend line and 95% confidence intervals for beet and cane meringues.
Figure 3.14: Simulated baking plot of section C with fitted non parametric curve and 95% confidence intervals for beet and cane meringues. Horizontal lines represent the average moisture content of beet and cane meringue cookies after baking.
Figure 3.15: Example of browning differences seen between C&H cane and Pioneer beet meringue cookies during baking. Powders in the image are the crushed inside portion of 4 dried meringue cookies.
3.6 References


An Overview of Texture Profile Analysis (TPA) [Internet]. Hamilton, MA: Texture


Urbanus BL, Schmidt SJ, Lee S. 2014c. Does Information about Sugar Source Influence


Appendix A: Exploratory Experiments

A.1: Crystal Growth Preliminary Experiment

To investigate if crystallization is affected by sucrose source in model rock candy systems, supersaturated solutions of beet and cane sugar were produced by heating mixture of 75% sugar (either C&H cane or Pioneer Beet) and 25% deionized water while stirring until all sugar was completely dissolved. These solutions were transferred to glass containers. Plastic stir straws, either coated in sugar crystals or uncoated, were placed in half of the glass containers. Containers with coated stir straws were considered seeded, containers with uncoated stir straw were considered unseeded. After 1 month, no differences were observed between Pioneer beet and C&H cane crystallization in either the seeded or unseeded containers. Pictures of glass containers with supersaturated solution and crystals after one month are shown in Figure A.1.

Figure A.1: Containers with supersaturated sucrose solutions and crystals after 1 month. (A) Pioneer beet seeded, (B) C&H cane seeded, (C) Pioneer beet unseeded, (D) C&H cane unseeded.
A.2: Crème brûlée

Differences in browning behavior between crème brûlées made with beet and cane sugars have been widely reported in media sources (Morgan 1999, Urbanus 2014). An example of reported differences is shown in Figure A.2. Additionally, Lu and others (2015) reported thermal differences between beet and cane sugar, which could lead to differences in browning behavior between beet crystalline sucrose in heated products, such as crème brûlée. To explore these possible thermal differences, 1.2 grams of sugar (either Pioneer beet or C&H cane) was spread evenly across a glass microscope slide. Slides were then browned for approximately 20 seconds using a kitchen blowtorch. Equipment and results are shown in Figure A.3. However, no noticeable differences were observed between beet and cane sugars.

Figure A.2: Beet and cane sugar crème brûlée published in SFGate (Morgan 1999).
Figure A.3: (A) Equipment and results from crème brûlée exploratory experiment. “Beet” refers to Pioneer beet sugar, “Cane” refers to C&H cane sugar. (B) Enlarged photo of glass slides with browned sugar.

A.3: Sample cup storage

Meringue cookies were produced as described in section 3.2.2.1.2. After production and cooling for 5 minutes, samples were placed in individual 3 oz sample cups, as shown in Figure A.4. After 24 hours of storage at ambient temperatures (approximately 23°C), moisture content, water activity, Texture profile analysis (TPA) hardness, and TPA cohesiveness were obtained as described in sections 3.2.2.2, 3.2.2.3, and 3.2.2.4. Two batches of meringue cookies were produced on 2 different days. Analysis was carried out on 4 samples for water activity and moisture content. Analysis was carried out on 20 samples per batch for texture profile analysis.

Mean and standard deviation values for moisture content, water activity, TPA hardness, and TPA cohesiveness are presented in Table A.1. No significant differences in moisture content
and water activity values of stored beet and cane meringue cookies were observed. Moisture content and water activity values after storage were similar to moisture content and water activity values immediately after baking, which were discussed in section 3.3.1. Hardness and cohesiveness of beet and cane meringue cookies was not significantly different after storage. Notably high variation in textural attributes was observed after storage, with some meringue cookies being brittle, some hard and tacky, and some very soft. This was observed in both sugar types and both batches. While hardness means and standard deviations were similar before and after storage, cohesiveness means and standard deviation were significantly higher after storage than before storage.

Table A.1: Mean and standard deviation values for texture analysis data. Mean values within a row with the same superscript letter indicate no significant differences (P < 0.05) between sugar sources.

<table>
<thead>
<tr>
<th>Test</th>
<th>Pioneer Beet</th>
<th>C&amp;H Cane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content (%)</td>
<td>6.03³ ± 1.081</td>
<td>5.29³ ± 1.252</td>
</tr>
<tr>
<td>Water Activity</td>
<td>0.339³ ± 0.0493</td>
<td>0.324³ ± 0.0415</td>
</tr>
<tr>
<td>Texture Profile Analysis</td>
<td>Hardness (g)</td>
<td>3138.37³ ± 1580.14</td>
</tr>
<tr>
<td></td>
<td>Cohesiveness (%)</td>
<td>17.9³ ± 12.61</td>
</tr>
</tbody>
</table>
A.4 Angel Food Cake

Media sources have reported textural differences between angel food cakes made with beet and cane sugar. To investigate these textural differences, angel food cakes were produced using the formula in Table A.2, using either C&H cane or Pioneer beet sugar. Egg white powder, water, and sugar were combined and whipped as described in section 3.2.2.1.1, producing a meringue. Swans Down cake flour (Reily Foods Company, New Orleans, LA) was then sifted and folded into the meringue using a rubber spatula. Approximately 40 folding strokes were used. The resulting batter was baked in rectangular loaf pans and as small round cookies (approximately 4 centimeters in diameter). Slight differences in external texture and color were observed, with beet sugar angel food cakes having a smoother top layer and less browning in angel food cakes baked as cookies and in loaf pans. Internal structure of beet and cane meringues had similar appearances and textures in angel food cake cookies and loaves. Examples of angel food cakes are shown in Figure A.5 through Figure A.8.
Table A.2: Formula used for angel food cake production, compiled through examination of numerous recipes from online sources and recipe books.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar</td>
<td>41.9</td>
</tr>
<tr>
<td>Flour</td>
<td>16.3</td>
</tr>
<tr>
<td>Egg white powder</td>
<td>4.2</td>
</tr>
<tr>
<td>Water</td>
<td>37.7</td>
</tr>
</tbody>
</table>
Figure A.5: Angel food cakes made with Pioneer beet sugar baked in a loaf pan.

Figure A.6: Angel food cakes made with C&H cane sugar baked in a loaf pan.
A.5: Frozen Fresh Meringue Cookie Differential Scanning Calorimetry

Fresh meringue cookies were frozen using liquid nitrogen and analyzed using Differential Scanning Calorimetry (DSC) to investigate the thermal properties of meringue cookies after baking. Samples were frozen with liquid nitrogen to mitigate sample preparation problems caused by stickiness in fresh meringue cookies.

Meringue cookies were made as described in section 3.2.2.1.3. After cooling, meringue cookies were submerged in liquid nitrogen for approximately 30 seconds. A small portion of the inside of each cookie was extracted using forceps and this small portion was then submerged in liquid for approximately 5 seconds. A TA Tzero aluminum DSC pan was also submerged in
liquid nitrogen for 5 seconds. Approximately 10 g of the frozen inner portion was inserted into the DSC pan. This was done quickly to ensure samples remained frozen during the sample preparation process. Pans were then sealed using an inner and hermetic outer lid. DSC measurements were completed using a Q2000 DSC and analysis was completed using TA Universal Analysis version 4.5A (TA instruments, New Castle, DE). Samples were equilibrated at 50°C, then -60°C, then ramped from -60°C to 100°C at a rate of 20°C/min.

Results obtained from meringue cookies frozen in liquid nitrogen were similar to results obtained from unfrozen fresh meringue cookies. Example beet and cane thermograms are presented in Figure A.9. All C&H cane samples exhibited Tg midpoint glass temperatures ranging from 10 to 43°C and ΔCp ranging from 0.67 to 0.74 J/(g °C). High variation between samples was observed and is likely due to variation in moisture content between samples. Most pioneer beet samples did not exhibit a notable thermal event prior to 80°C. One sample exhibited a long sloping transition from -40 to -10°C. Above 80°C, most pioneer beet thermograms exhibited a large exothermic event, likely caused by the pan becoming unsealed due to pressure. Based on results from this experiment, the glass transition of beet meringue cookies maybe occurring over a very wide temperature range or maybe occurring at very low temperatures, below the DSC temperature range used herein. Freezing of fresh meringue cookies using liquid nitrogen drastically improved the ease and quality of sample preparation and is recommended for use in future experimentation involving sticky samples.
Figure A.9: Example thermograms of frozen fresh beet and cane meringue cookies.

A.6 References
Morgan M. 1999. SUGAR, SUGAR / Cane and beet share the same chemistry but act differently in the kitchen. SF Gate.