Characterizing the functional and practical performance of hydrolyzed lactose syrup in selected foods

By

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Dedication

This thesis is in memory of my late father, Lee Budin. Although he is no longer with us, he always believed in me and pushed me to take risks and push myself. You may be gone, but your trust in me has made this journey possible.

Abstract

Greek yogurt acid whey (GAW), a byproduct of Greek yogurt production, is both an expensive waste product and an environmental concern. Previous work has developed a system to convert GAW into sustainable usable products, specifically milk minerals and glucose-galactose syrup (GGS). However, research regarding the applications of GGS is limited. This work aims to fully characterize the physical and functional properties of GGS and characterize the functionality of the syrup in several diverse food applications.

The physical and functional properties of GGS, boiling point elevation, freezing point depression, browning, and rheological behavior, were characterized. Compared to solutions of sucrose and corn syrup, GGS decreased the freezing point and increased the boiling point of water further. At the same solids content, GGS had a lower viscosity than solutions of sucrose and 43 DE corn syrup at freezing and room temperatures. GGS behaved more similarly to corn syrup than to sucrose when undergoing browning reactions.

The ability of GGS to act as a partial replacement, at 40% and 80% molarity, for sucrose in high-ratio cake and as a partial replacement for corn syrup and sucrose in gummies and caramel was determined. In caramels, GGS significantly altered product characteristics: increased browning reactions, decreased hardness and increased stickiness across all formulations. Due to these significant changes, GGS was found to be unsuitable as a direct replacement for corn syrup or sucrose. However, the addition of GGS may be useful for manipulating caramel properties. Gelatin gummies made with 40% and 80% molar replacement of sucrose and corn syrup by GGS showed several significant differences from control formulations. The texture profile of the gummies was highly consistent across all formulations; however, increasing levels of GGS increased the rate of crystallization. GGS was found to be an unrealistic replacement for corn syrup

at 40 and 80% and sucrose at 80% due to the increase in the onset of crystallization, creating an undesirable product. In high ratio cake, GGS increased foamability and yield stress in the batter and significantly affected the baked cake formulations. A significant increase in color occurred in both 40% and 80% molar replacement of sucrose, causing GGS to be an unviable replacement in cake due to production of off flavors and undesirable appearance.

In summary, the functional and physical properties of GGS were measured and compared to those of corn syrup and sucrose. GGS was evaluated in various food applications, demonstrating viability as a partial sucrose replacement in gelatin gummies but proving unsuitable for high-ratio cakes. The effectiveness of GGS in caramels depends on the desired final characteristics of the product.

Acknowledgments

Before I get into the details of my research, there are many people whom I must thank for their help and support throughout my project. I could not have undertaken this journey without my advisors, Drs. Audrey Girard and Richard Hartel, and all their support, advice, and expertise. Additionally, this project would not have been possible without the support of Dr. Scott Rankin, Jarryd Featherman, Dr. Grace Lewis, and the rest of the chemical engineering project team.

I have a tremendous amount of gratitude towards my friends and lab mates. Thank you for all the lunch breaks, laugh sessions, and walks around the building. I am forever grateful for all the encouragement and help throughout my project and personal life.

An extra special thanks to my family, especially my parents and siblings, for supporting me through all my hopes and dreams and keeping me on track during some of the most challenging times.

Graduate school has undoubtedly been a roller-coaster for me through project hurdles and personal issues. The community that Madison has provided me is irreplaceable and unforgettable. After six years in Madison, I am sad to say goodbye but excited for the next steps in my adventures.

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1. Introduction

Greek yogurt acid whey (GAW) is an abundant waste product resulting from the processing of Greek yogurt. GAW is produced when Greek yogurt undergoes centrifugation and straining; the supernatant is the GAW, and the pellet is the desirable yogurt product. During the production of Greek yogurt, 2-3 parts of GAW are produced for every part Greek yogurt (Erickson, 2017). While other whey products can be processed into various valuable products, such as whey protein concentrates and whey protein powders, GAW is most commonly a waste stream due to its low protein content and its high propensity for coagulation during protein powder production (Chandrapala et al., 2015). Disposal methods of GAW include wastewater treatment, mixing into livestock feed, spreading on farmland, and anaerobic digestion (Erikson, 2017). However, these methods are expensive, both economically and environmentally, and therefore, other uses for GAW have been a hot research topic, including the potential to produce glucose galactose syrup (GGS), a liquid sweetener.

While GGS has been produced and studied in the past, it quickly became economically unsustainable (Tsakali et al., 2010). With new findings regarding a unique acid-catalyzed hydrolysis system in which the production of milk minerals and GGS are coproduced, it is now possible for GGS to be an economically sustainable food ingredient (Lindsay et al., 2022). With limitations on past research, there is a significant need to better understand the physical and functional properties of GGS on its own and GGS as a food ingredient, especially as a corn syrup and sucrose replacement. Therefore, the goal of this study is to characterize GGS and its application across a variety of food products.

2. Literature Review

2.1 Greek Yogurt Acid Whey

Greek yogurt acid whey (GAW) is an abundant byproduct produced during the manufacturing of Greek yogurt (Chandrapala et al., 2015). The acid whey is removed from the yogurt through straining or centrifugation, allowing for a thick Greek yogurt. However, this process produces a byproduct, which has been generally viewed as a waste product with no application. It is viewed this way because GAW is high in lactose and minerals and low in protein and pH (Table 2.1).

Table 2.1 Composition of crude acid whey (Román et al., 2010)

	Weight Fraction (%)
Total Solids	6.31- 6.40
Lactose	4.06 - 4.41
Protein	0.83 - 0.89
Fat	0.27 - 0.37

Unlike sweet and acid whey, GAW does not have a high nutrient content and cannot be used directly as a food or food ingredient. While GAW can be used as livestock feed or on farmlands in small amounts,, in large amounts, cows' digestive systems can react poorly, and farmland productivity is likely to decrease significantly (Lievore et al., 2015). In addition, GAW cannot be easily treated in a wastewater facility due to its high biological and chemical oxygen demand. Thus, can overwhelm the capacity of wastewater treatment plants (Lievore et al., 2015). Not only do these streams of disposal have negative environmental impacts, but they are also

costly. Due to the low versatility of GAW, many researchers are looking at different ways to convert this waste product into something both profitable and sustainable.

2.2 Lactose

One of the biggest hurdles in creating a viable product from GAW is that the lactose found in GAW has minimal functionality. Lactose is a disaccharide that is the main carbohydrate found in mammalian milk (Gänzle et al., 2008). The molecule consists of one D-glucose and one D-galactose molecule. An α-1,4-glycosidic linkage connects the aldehyde group of galactose and the C-4 group of glucose, forming a lactose molecule. (Figure 2.1). This disaccharide exists in alpha and beta forms; the difference between these isomers comes down to the configuration of the substituents of carbon 1 of glucose. Glucose can be found in the alpha and beta formations within the lactose molecule, but galactose is always in the beta configuration. In an aqueous solution, lactose undergoes mutarotation, which results in the presence of both the alpha and beta forms. When the solution reaches equilibrium, a ratio of 40:60 for alpha and beta, respectively, is found at room temperature (Zarmpi et al. 2017).

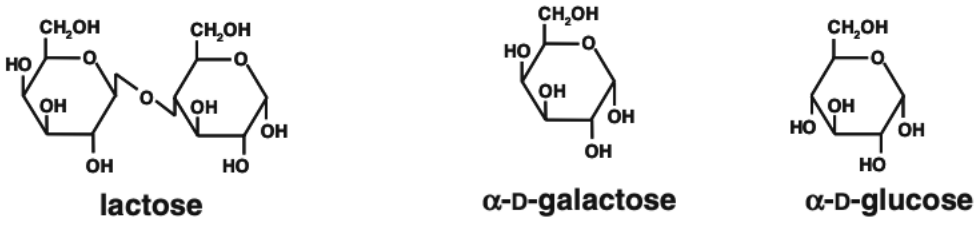

Figure 2.1 Chemical structures of lactose, galactose, and glucose (Fox, 2009)

Each of the mutarotations exhibits unique properties in terms of solubility, melting point, and density (Listiohadi et al., 2009). Beta lactose is more soluble in water and sweeter than alpha lactose. Only alpha lactose can exist as a monohydrate, while beta is always anhydrous. These differences cause a variety of properties, resulting in acutely differences between lactose samples.

Lactose is most abundantly used as a supplement in infant formula but may also be found in confectionery, baking, and more (Paterson, 2022). While lactose can positively affect food products, such as promoting Maillard browning or enhancing mouthfeel, lactose generally has low solubility and sweetness compared to standard sweeteners. Compared to sucrose, lactose is 6.25 times less sweet and five times less soluble (Zadow, 1984). This low solubility leads to limited uses for lactose within sweetened food applications. In most products that contain lactose or in which lactose is vital to mouthfeel and flavor, the lactose is added within dairy products in which enough lactose is inherently present to impart the desired characteristics. When an excess of lactose is found in these added dairy ingredients, negative characteristics may occur, such as crystallization or over-browning (Huppertz & Gazi, 2016).

Lactose can be hydrolyzed to overcome the negative attributes associated with the sugar. Hydrolysis of lactose generally requires an enzyme or a chemical process to promote breakage of the glycosidic linkage (Gänzle et al., 2008). One of the most common methods for hydrolysis of lactose is using enzymes, most referred to as lactase or β-D-galactosidase. Lactase is readily found in plant, yeast, bacterial, and fungal sources and requires no additional equipment in dairy plants (Harju et al., 2012). However, enzymatic hydrolysis depends on many factors, including substrate concentration, pH, temperature, enzyme activity, and contact time. Alternatively, acid hydrolysis has lower production costs and is a more time-efficient process. However, until recently, the full scope of acid hydrolysis of GAW into glucose galactose syrup has not been understood.

Researchers at the University of Wisconsin–Madison in the Chemical and Biological Engineering Department have thoroughly tested different acid hydrolysis techniques, including different catalysts, temperatures, and filtration methods (Lindsay et al., 2020). Through this experimentation, these scientists were able to create a complete reactor system in which an

economical hydrolyzed lactose syrup or glucose-galactose syrup (GGS) is coproduced along with milk minerals, which in return increases the economic feasibility of GGS and creates an efficient and productive way to process GAW. Using this reactor system, both GGS and milk minerals are produced while still maintaining a lower environmental impact than the production of high fructose corn syrup or sucrose (Lindsay et al. 2022).

2.3 Manufacturing and Production of GGS

In the 1980's, there was a large push for industrial applications of hydrolyzed lactose syrups and many studies explored the production of GGS (Gänzle et al., 2008). During this time, the main methodology to producing GGS was based on the utilization of enzymatic hydrolysis, either the "free enzyme" approach or immobilized enzyme reactors. The "free enzyme" method, also known as single-use method, involves the addition of enzymes to the product without the recapture of the enzymes. Immobilized enzyme reactors, or membrane reactors, allow for the effective recapture and reuse of soluble enzymes, but involve greater initial cost (Zadow, 1992). Both methods have been implemented commercially, in which the single-use enzyme systems are more economic for smaller operations and immobilized systems are ideal for daily use larger operations. The immobilized enzyme technology was thought to be the ideal solution to lactose hydrolysis.

The most publicized method was implemented in collaboration between Kroger and Corning, in which enzyme immobilization on sintered glass beads was utilized (Anonymous, 1981). However, even this production was unsuccessful long term due to its unfavorable economic realities and numerous technical difficulties (Gänzle et al., 2008). Other immobilized enzyme commercial processes were explored and implemented, but they are now no longer used to produce

a sweetener syrup and instead are used for the production of lactose free dairy products (Jelen & Tossavainen, 2003).

Since the boom of research in the 1980's, more research has shown the unviable nature of enzymatically-produced GGS. In a study by Bury et al. (2000), the economic feasibility of lactose enzymatic hydrolysis from cheese whey was evaluated and determined to not be economically viable. However, lactose can also be hydrolyzed with the use of acidic conditions and elevated temperatures. This approach further increased interest in lactose hydrolysis in Australia, the Netherlands, and New Zealand, leading to patented processes (Gänzle et al., 2008). These processes, however, never made it to commercialization and were found to have extremely limited industrial application.

Due to the increase in GAW production, due to an increase in Greek yogurt production, the need for an alternate process to produce an economically viable solution has increased dramatically. Work done by Lindsay et al. (2022), indicated that through a new optimized reactor, GGS could become an economically viable solution to the increase in GAW. A unique multi-component system was designed to streamline the production of GGS and allow for continuous processing (Figure 2.2).

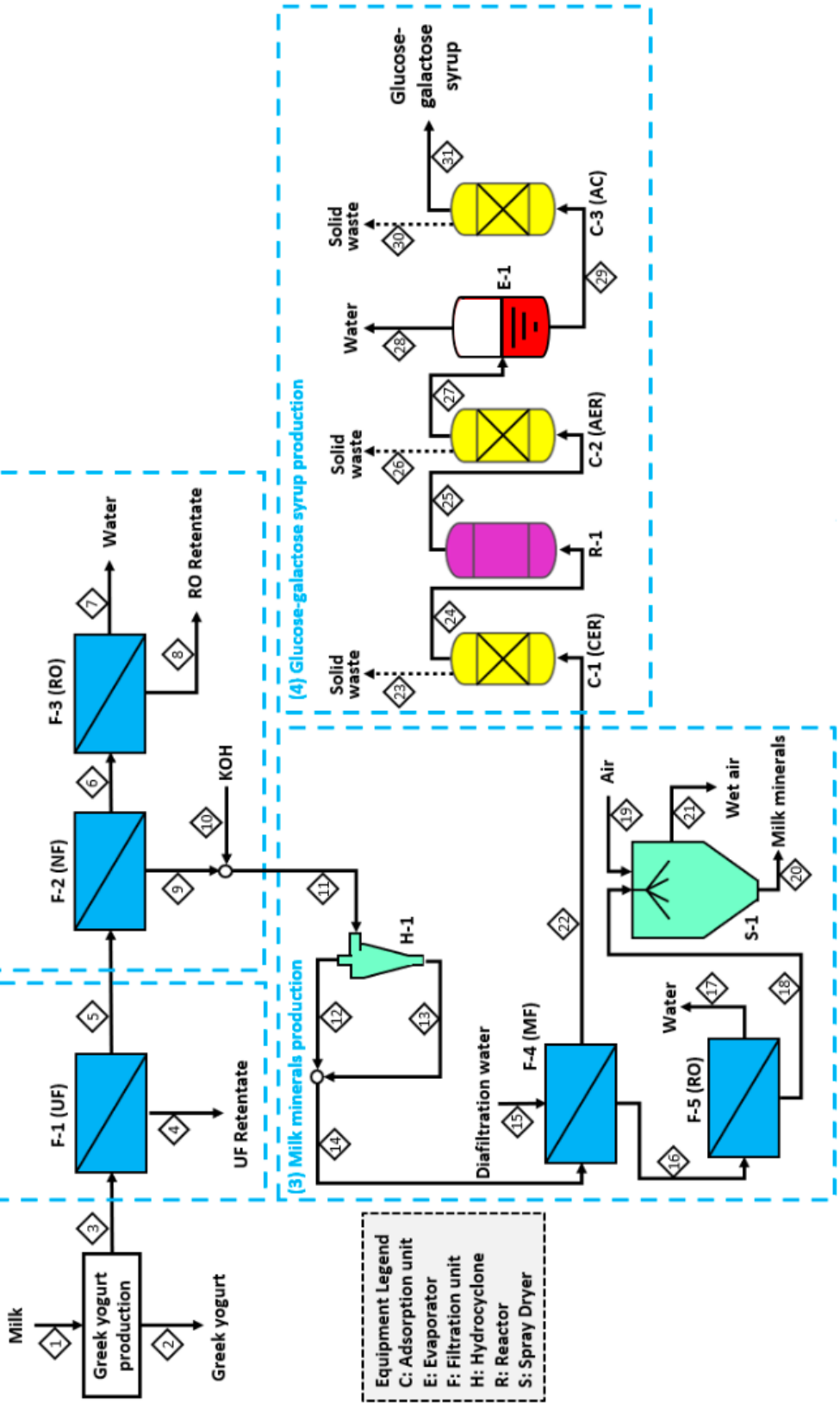

Figure 2.2 Block flow diagram of the University of Wisconsin Greek yogurt acid whey process to produce milk minerals and glucose galactose syrup in tandem (Lindsay et al., 2020)

This reactor produced GGS by first purifying GAW sourced from Greek yogurt producers. The GAW underwent filtrations in the following order: ultrafiltration, nano filtration and reverse osmosis. Ultrafiltration was used to remove protein and fat from the acid whey and then followed by nanofiltration to further remove non-protein nitrogen (NPN). The permeate then was concentrated through reverse osmosis, in which water was removed and the solids content was increased from 1% up to 8%.

The concentrated retentate was then heated and neutralized through the addition of KOH until the pH reached 7.18, and the calcium phosphate precipitated out of solution. A hydrocyclone was then utilized to separate the solution into two phases, in which centrifugal force accelerates the settling of particles and allows for separation according to size, shape and specific gravity (Jankovic, 2022). The precipitated calcium phosphate was discharged through the underflow and the permeate was discharged through the overflow pipe. Any remaining calcium phosphate was separated from the overflow permeate through another round of ultrafiltration, producing more isolate calcium phosphate and a permeate from which GGS would be produced. The calcium phosphate collected by the hydrocyclone, and ultrafiltration processes were then combined and spray-dried resulting in the final product, milk minerals.

As modeled in Figure 2.2, the permeate was passed through a cation exchange column, removing NPN and salt, while acidifying the solution. The acidified permeate was then hydrolyzed within the reactor utilizing an acid catalyst, Amberlyst 70, to hydrolyze the lactose. To optimize the hydrolysis, temperature was held at 140°C and the flowrate was held constant. The reaction product was passed through an anion exchange resin to neutralize the solution and remove organic acids, anions, NPN, and hydroxymethyl furfural (HMF). An evaporator was then used to concentrate the solution to produce a syrup at 71% solids. The syrup was then passed through an

activated carbon column to reduce the off colors produced during hydrolysis as well as further remove any organic acids, ash, HMF, NPN and humins not removed through anion exchange resin.

2.4 Glucose Galactose Syrup

The final glucose galactose syrup produced by the UW-Madison reactor is a mixture of glucose, galactose, lactose, humins, hydroxymethyl-5-furfural, and a small amount of residual ash (Table 2.2). Galactose is more abundant than glucose, although lactose hydrolyzes into a 1 to 1 ratio of glucose and galactose. This disproportionate ratio of monosaccharides is due to excess galactose found in the original GAW. Bacteria fermenters can use glucose and lactose as energy sources during yogurt fermentation. However, galactose cannot be utilized and, therefore, can be found in the whey and the yogurt. Subsequently, when the GAW is processed through the reactor, the unfermentable galactose remains in the final syrup.

Table 2.2 Composition of glucose galactose syrup produced by hydrolysis reactor, hydroxymethylfurfural[1]

Component	Weight Fraction (%)
Water	29.0
Oligosaccharides	8.4
Monosaccharides	60.6
Ash	0.1
[1]HMF	0.5
Humins/other	1.4

During the production of GGS, side reactions occur when non-protein nitrogen compounds begin to react with sugars, causing Maillard and browning reactions. These side products include chemicals such as humins and hydroxymethyl furfural. These side reactions limit the degree of hydrolysis possible; in this reactor, the system is optimized to get high conversion of lactose to glucose and galactose while accumulating a minimal concentration of humins and hydroxymethylfurfural (HMF). While there are currently no known adverse side effects associated with these browning reaction compounds, they can lead to off-flavor and colors (Abraham et al., 2011). GGS has thus been optimized to create the best application product as a bulk sweetener.

2.5 Bulk Sweeteners

Sweeteners play a significant role in the food industry, contributing to sweetness and physical properties (Saraiva et al., 2020). Traditional sweeteners consist of carbohydrates characterized by their chemical formula of $C_x(H_2O)_y$ (Garaita & Kennedy, 1997). While seemingly simple, carbohydrate variations, from chain length to branching, can account for numerous combinations, creating a diverse range of characteristics in sugars. Some of the most common groups of sweeteners include monosaccharides, oligosaccharides, and polysaccharides. Monosaccharides and oligosaccharides are used more commonly as sweetening agents due to their increased sweetness and functional properties as compared to polysaccharides (Hartel et al., 2018).

Some common monosaccharides include glucose, fructose, and galactose, while common disaccharides include lactose, sucrose, and maltose. Lactose consists of one glucose and one galactose molecule, sucrose consists of one glucose and one fructose molecule, and maltose consists of two glucose molecules. These monosaccharides are bonded by α-1,4-glycosidic linkages to form disaccharides, and potentially larger oligosaccharides and polysaccharides.

Oligosaccharides and polysaccharides are also commonly found as chains of glucose in the form of glucose syrups, also known as corn syrup.

GGS was produced with the intent of partially replacing ordinary bulk sugar, allowing for optimization of unique properties, while maintaining desired characteristics (Lindsay et al., 2022). In industry, many sweeteners can be utilized in different products. Sweeteners are generally chosen based on the product's functionality and consumer acceptance. Sucrose and corn syrup are the two main common industry sweeteners (Hartel et al., 2018).

2.5.1 Sucrose

Sucrose is a simple disaccharide in which α-D-glucose and β-D-fructose are bonded by a glycosidic linkage between the one-carbon of glucose and the two-carbon of fructose (Queneau et al., 2007). Due to its natural occurrence in fruits and vegetables and its abundant availability, sucrose has become a standard reference for sweetness. Commercially available sucrose is produced from sugar can and sugar beets due to their high concentration of sugar juices (Lu et al., 2017). Both sources result in nearly pure sucrose through complex processing and are virtually indistinguishable. Sucrose is commonly purchased with respect to its physical state. Most sucrose is purchased in its crystalline form, although solubilized sucrose syrups are also available commercially (Hartel et al., 2018). Crystalline sucrose comes in a large variety of granular sizes and shapes, ranging from coarse (>650 μm) to powdered (<25 μm), although fine or coarse granulated (380-635 μm) are most commonly used.

2.5.2 Corn Syrup

Corn syrup, also known as glucose syrup, is a sweet syrup derived from hydrolyzed starch, resulting in polymers of different lengths of glucose (Hull, 2010). Glucose is commonly called dextrose when crystalline; however, both terms refer to the same molecular structure. The exact molecular composition of corn syrup depends on the degree of hydrolysis and the hydrolysis method: acid, enzyme, or both. When selecting corn syrup for a specific application, the main deciding factor is the DE (dextrose equivalent).

DE is a measure of the reducing power of the syrup, calculated in terms of the percentage of glucose (dextrose) equivalents of the total solids. Each saccharide class has its own DE, dependent on the ratio of reducing groups to molecular weight. Therefore, starting with pure glucose, the DE begins at 100, with the DE decreasing as polymer chains increase in molecular weight. By manipulating both acid and enzyme hydrolysis independently and in combination, it is possible to create a wide variety of DE corn syrups with different compositions (Featherstone, 2015). Due to the degree and nature of hydrolysis, the saccharide distribution in corn syrup can differ, leading to different average molecular weights. Generally, as DE increases, the average molecular weight decreases. Functional use and properties change with DE. The most used DE corn syrups are 36, 43, and 64 DEs. Texture, crystallization prevention, viscosity, foam, and emulsion stability decrease with an increase in DE, whereas browning, sweetness, boiling point elevation, and hygroscopicity increase with DE (Hartel et al., 2018). Therefore, depending on the application of corn syrup, different DEs may be desirable.

2.6 Sweetener Characteristics

Sucrose, corn syrup, and other nutritive sweeteners are compared on many characteristics. These characteristics include solubility and crystallization, boiling point elevation and freezing point depression, rheological behavior, glass transition, moisture sorption behaviors, relative sweetness, and reducing ability (Garaita & Kennedy, 1997).

2.6.1 Solubility and Crystallization

The solubility of sugar molecules depends on various vital factors, including molecule size and complexity, number of hydroxy groups, glycosidic bonds and molecular geometry, degree of branching, and molecular charge (Macedo, 2005). Smaller molecules have a greater ability to interact with water molecules and, in turn, generally allow for higher levels of solubilization. However, this interaction with water molecules can be further increased with more available hydroxy groups, increasing solubility. Saccharides with more hydroxy groups can form more hydrogen-bonding interactions. As saccharides polymerize, fewer hydroxy groups are available for hydrogen bonding. In addition, the glycosidic bonds and overall geometry can affect the positioning and availability of hydroxy groups, further altering the solubility. For larger polysaccharides, the degree of branching and molecular charge play a prominent role in the solubility levels (Garaita & Kennedy, 1997). Linear polysaccharides and polysaccharides with anionic groups will be more soluble than highly branched and uncharged polysaccharides. Branching causes steric hindrance, which decreases the molecules' ability to interact with water molecules. Anionic groups can increase solubility due to polar interactions between the anionic groups and polar water molecules.

Sweeteners can have various solubilities in water. However, mono and disaccharides are all considered soluble, and most are accepted as having a relatively high degree of solubility

(Eliasson, 2006). While these solubilities differ amongst sugars, all sugars exhibit increased solubility with an increase in temperature. At room temperature, fructose is the most soluble sugar, followed by sucrose, glucose, galactose and lactose respectively (Gould, 1940; Hartel et al., 2018). However, at elevated temperatures glucose becomes more soluble than sucrose due to changes in anomer solubility; the same phenomenon is seen with lactose as well.

Solubility and crystallization behavior of saccharides are dependent on one another. State diagrams are commonly used to understand the relationship between the saturation of a solution and temperature (Figure 2.3). Crystallization is driven thermodynamically based on degree of supersaturation and occurs in three crucial steps: generation of supersaturation, formation of nuclei, and growth of nuclei until equilibrium is attained (Hartel & Shastry, 1991). Supersaturation is a non-equilibrium state where a solution has more solute than it can dissolve under a given temperature and pressure.

Crystallization, however, does not always occur when a solution is supersaturated (Hartel et al., 2011). While crystallization may be thermodynamically favorable, nucleation must first occur, which is inhibited by an energy barrier. Nucleation is defined as the process by which atoms or molecules come together to form a new phase of structure; in the context of crystallization, small clusters of atoms or molecules come together to form a nucleus (Mullin, 2001). Nucleation can occur by either a primary or secondary mechanism. Primary nucleation occurs in the absence of existing crystals, whereas secondary nucleation occurs when crystals are already present in the solution. In primary nucleation, the nucleus can be formed around a foreign particle (heterogenous nucleation) or the nucleus can be entirely driven by the spontaneous formation of nuclei in pure solutions (homogenous nucleation). Once nuclei reach a critical size, they are stable and can grow into larger crystals. When too small, nuclei can dissolve back into the solution. There are many

parameters that affect nucleation, including supersaturation, temperature, rate of cooling, energy input of agitation rate, suspension density, and the presence of additives and impurities (Hartel & Shastry, 1991).

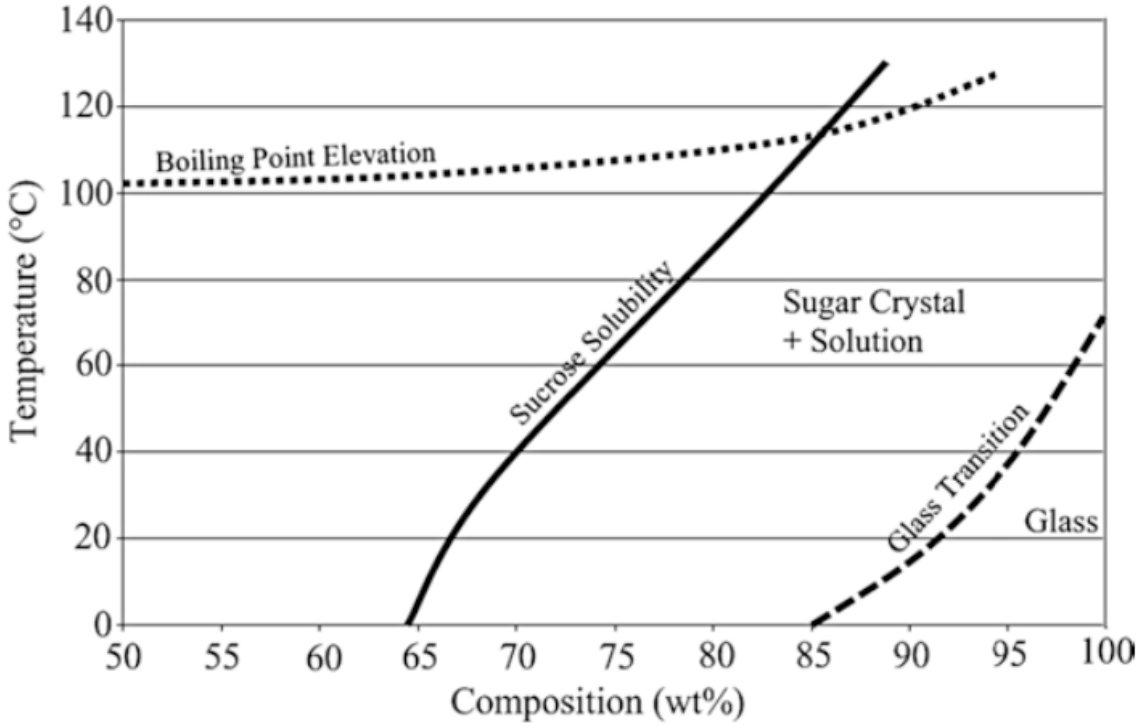

Figure 2.3 State diagram for sucrose showing solubility, boiling point elevation, and glass transition boundaries (Ergun et al., 2010)

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After the formation of the nucleus, growth occurs immediately and continues until there is no longer supersaturation. Sweetener molecules move from the bulk solution into the crystal interface, as governed by molecular weight, concentration, temperature, viscosity, and agitation (Hartel et al., 2018). The rate of crystallization is dependent on the molecular nature of the crystallizing species, supersaturation, temperature, viscosity of solution, agitation rate, pH, source of nuclei, suspension density, electric/magnetic/sonic fields and the presence of additives and impurities. The supersaturation in the aqueous phase is the primary factor affecting the growth rate, in which higher supersaturation values lead to higher rates of crystal growth.

Crystallization in foods can be either undesirable or desirable (Hartel & Shastry, 1991). In products such as grained caramels, frosted cereals, and panned confections, crystallization is a desired characteristic and gives the texture and mouthfeel associated with these products. However, in other products like ungrained caramels, hard candies, ice cream, and sweetened condensed milk, crystallization indicates an undesirable texture and causes a flavor loss and/or a change in appearance.

Prevention of crystallization is crucial in many food and confectionery applications where a smooth, non-grainy texture is desired. Doctoring agents are used to moderate the crystallization of the primary sweetener in confections (Edwards, 2018). The most common doctoring agents

include glucose syrups and invert sugar. Doctoring agents prevent crystallization by changing the solubility concentration and disrupting crystal formation. This is achieved by the increase in viscosity and the engagement in chemical interactions, which impede the mobility of molecules.

Combinations of different sweeteners can be used to manipulate the solubility and crystallization of sugars in products. A variety of doctoring agents are used to control the crystallization in confections. The crystallization rate is also affected by the glass transition; the closer a material is to its glass transition. The more crystallization is impaired due to decreased molecular mobility. Hot candy syrup may be cooled quickly through the crystallization range to quickly reach the glassy state and avoid crystallization (Hartel et al., 2018).

Sweeteners' solubility and crystallization behaviors are crucial factors that significantly influence the texture, stability, and overall quality of food products. The interplay between solubility and crystallization impacts the shelf life and sensory attributes of confections and other food items. Producers can balance crystallization and supersaturation by utilizing doctoring agents and carefully selecting sweetener combinations.

2.6.2 Boiling Point Elevation and Freezing Point Depression

Colligative properties are characteristics of solutions that depend on the number of solute particles relative to the number of solvent particles rather than the chemical nature of the solute (Atkins et al., 2023). Boiling point elevation and freezing point depression are colligative properties that describe how non-volatile solutes in a solution affect its thermal behavior. Specifically, these phenomena refer to the increase in boiling temperature and the decrease in freezing temperature of the solution compared to the pure solvent. These changes occur due to the dissolved solutes interfering with the expected phase transition processes of the solvent molecules.

When sweeteners are added to aqueous solutions in food, sugar molecules influence interactions among water molecules. The influence of sugar molecules depends on the number of molecules and their size, expressed as molality. Changes in boiling point temperatures (ΔT_{bp}) and freezing point temperatures (ΔT_{fp}) can be estimated by combining the Clausius-Clapeyron relation and Raoult's law (Atkins et al., 2023).

Boiling Point Elevation

$$\Delta T_{bp} = K_b * b_{solute} * i$$

$$K_b = ebullioscopic\ constant\ of\ the\ solvent$$

$$K_{b:water} = 0.521°C * \frac{kg}{mol}$$

$$b_{solute} = solute\ molality$$

$$i = van't\ Hoff\ factor$$

Freezing Point Depression

$$\Delta T_{fp} = K_f * b_{solute} * i$$

$$K_{fp} = cyroscopic\ constant\ of\ the\ solvent$$

$$K_{f:water} = 1.853°C * \frac{kg}{mol}$$

$$b_{solute} = solute\ molality$$

$$i = van't\ Hoff\ factor$$

These equations are dependent of the cyroscopic constant of the solvent, the solute molality and the van't Hoff factor. For sugar solutions, the solvent is water and therefore the K_f for water is utilized. The van't Hoff factor is the ratio between the concentration of particles when the

substance is dissolved in water over the concentration of its mass; for sugars, the van't Hoff factor is one, as sugars do not dissociate in water. The molality is calculated using the following equation.

$$Molality: m = \frac{moles\ of\ solute}{kg\ of\ solvent}$$

Molality is a measure of molecular concentration and is not affected by the average molecular size. When comparing sugar solutions of the same molarity, they will have the same impact on colligative properties. However, solutions of the same weight by weight concentration of different sugars will have different molalities (Smith & Bradley, 1983). Sugars with larger average molecular weights will result in a decreased molality compared to solutions with smaller average molecular weights at the same weight concentration. Therefore, on a weight-by-weight basis, smaller sugar molecules have a greater influence on colligative properties than larger molecules.

Boiling point elevation plays a crucial role in candy making, as the sugar concentration directly correlates with cooking temperatures, influencing the candy's final texture (Saska, 2003). As the sugar concentration increases, the boiling point of the solution rises, allowing the sugar syrup to reach higher temperatures. This phenomenon enables candy makers to determine the sugar concentration in a cooking syrup by measuring its temperature. However, boiling point elevation varies with different types of sugars, leading to changes in boiling points as different sugars are introduced. Additionally, cooking temperatures may vary due to changes in atmospheric pressure and altitude, necessitating the determination of final cooking temperatures on a case-by-case basis (Hinkova et al., 2015; Holven, 1936).

Freezing point depression plays a crucial role in the preservation and quality of frozen foods (Smith & Bradley, 1983). The lowered freezing point can help prevent the formation of large ice crystals. Proper manipulation of freezing point depression ensures that frozen foods maintain a desirable texture. Insufficient solute can result in a hard, icy texture, while too much can prevent proper freezing, leading to a product that is too soft. In addition, freezing points that are too low cause decreased frozen water, which in turn increases the effects of heat shock, resulting in increased freezer burn.

Understanding the impact of sugar concentration on food matrices' boiling and freezing points is vital. The choice of sweetener and concentration is essential in controlling the colligative properties of water and, consequently, the quality of foods.

2.6.3 Viscosity

Viscosity is a measure of a fluid's resistance to flow. Much like solubility and crystallization, the viscosity of saccharides in an aqueous solution is highly dependent on both temperature and molecular weight (Chetana et al., 2004). An increase in temperature, and thus an increase of energy, causes a decrease in viscosity due to the rise in molecular mobility. In terms of size, higher molecular weight molecules have a greater resistance to flow and decreased mobility within a solution, increasing viscosity (Longinotti et al., 2014).

In turn, monosaccharide solutions at the same molarity and temperature as disaccharide solutions will have a slightly decreased viscosity due to the high mobility of the smaller molecular structures (Mazurkiewicz & Nowotny-Różańska, 1998). In addition, when mono and disaccharide solutions are cooled, and the overall kinetic energy of the solution is lowered, the viscosity increases. Viscosity changes more dramatically when larger polymers are introduced, such as the glucose polymers found in corn syrup, in which much higher viscosities are exhibited.

The viscosity of bulk sweeteners is essential when looking at processing and food matrices. Sweeteners with higher viscosities may hinder mixing action and increase the difficulty of pumping (Metcalfe & Lester, 2009). However, higher viscosities can exhibit more desirable textural attributes in food, such as improved mouthfeel and the ability to form more stable structures (Wagoner et al., 2018). Low-viscosity bulk sweeteners are easier to handle, incorporate into systems more efficiently, and require less energy to pump. However, low viscosity may lead to more crystallization or cause undesirable effects on the product's viscosity. The desired viscosity of a bulk sweetener ultimately depends on the specific application and ideal product characteristics.

2.6.4 Glass Transition

Glass transition temperature (T_g) is the temperature range at which amorphous regions transition from a glassy, brittle state to a rubbery, flexible state or vice versa (Mohamed et al., 2016). Regarding the glass transition of sugars, T_g is the range of temperatures in which a sugar glass rapidly loses its solid-like characteristics and begins to flow, and the midpoint of this zone is referred to as the T_g (Nowakowski & Hartel, 2002). The T_g of bulk sweeteners is a crucial parameter; T_g affects storage, moisture migration, and sensory attributes. When products are stored at temperatures below their T_g, they are more stable and less prone to changes in characteristics. When T_g is near room temperature, confections will become sticky, lose flavor, and are more highly susceptible to graining.

T_g is highly dependent on the average molecular weight of a material; however, other factors, which are less well understood, also have an effect (Gabarra and Hartel 1998). As molecular weight increases and, in turn, the DE of maltodextrin corn syrup decreases, the Tg increases (Roos & Karel, 1991). Monosaccharides have relatively low glass transition

temperatures, and in general, T_g increases for disaccharides. Of saccharides, fructose has the lowest Tg of 7.8 -8.6°C and is the only saccharide with a Tg below room temperature (Seo et al., 2012). Glucose and galactose have very similar T_g's ranging from 32.7 – 36.0°C and 34.5 – 37.8°C, respectively. Sucrose and maltose also have similar T_g's ranging from 68.9 – 71.9°C and 72.6 – 75.9°C, respectively. When different saccharides are combined, T_g's generally fall between the T_g of each component, therefore, can be controlled through the strategic choice of sweetener ratios in final products.

2.6.5 Moisture Sorption Behaviors

Moisture sorption isotherms graphically represent the relationship between water activity and moisture content at a constant temperature (Fontana & Carter, 2020). These curves are crucial for understanding how foods interact with environmental moisture, which can affect their texture, stability, and shelf life. Sugars are highly hygroscopic materials, meaning that they readily absorb moisture from the environment, and therefore highly affect moisture sorption isotherms (Hartel et al., 2018). The hygroscopicity of sugars is influenced by their physiochemical characteristics. Highly soluble sugars tend to be more hygroscopic, which can lead to changes in texture and stability of food products (Gichau et al., 2020).

The hygroscopic nature of sugars can lead to undesirable textural changes in products. The uptake of moisture can cause clumping and caking in powdered products, stickiness in hard candies, and sogginess in dehydrated fruits (Bronlund & Paterson, 2004). By analyzing moisture sorption isotherms, food developers can optimize formulations and packaging to maintain product quality; for example, desiccant packets may be added to a product's package in order to absorb moisture more readily than the food product (Dak et al., 2014). However, not all water adsorption

in food is bad; the hygroscopicity of sugars in baked goods help maintain moisture and desirable textural attributes across shelf life (Ding & Yang, 2021).

2.6.6 Reducing Ability and Browning

There are two main types of browning in food: enzymatic and non-enzymatic. Polyphenol oxidases primarily catalyze enzymatic browning and are not directly dependent on sweeteners (Corzo-Martínez et al., 2012). Non-enzymatic browning, however, is significantly influenced by the presence and type of sweeteners and can be divided into two subcategories: Maillard browning and caramelization. Maillard browning and caramelization play crucial roles in flavor and color development in various food products.

Maillard browning is caused by the reaction between reducing sugars and an amine source (Wedzicha & Leong, 2005). Reducing sugars are sugar molecules that contain an aldehyde group or can form one. All monosaccharides are reducing sugars as are lactose and maltose. Sucrose is not a reducing sugar because the glycosidic linkage between glucose and fructose involves their reducing groups, preventing them from being free to participate in reactions. Other sugars may only participate in Maillard browning after hydrolysis occurs and a reducing sugar is formed. Amines are organic functional groups that contain a basic nitrogen atoms with a lone pair of valence electrons; proteins are the most common source of amines in foods (Tamanna & Mahmood, 2015).

The Maillard browning reaction consists of three distinct phases in which multiple reactions occur (Lund & Ray, 2017; Wedzicha & Leong, 2005) . The first phase consists of sugar-amine condensation and Amadori rearrangement. In the second phase, sugar dehydration and fragmentation occur, followed by amino acid degradation by the Strecker reaction. In the final

step, heterocyclic nitrogen-containing polymers and copolymers are formed. Through the Maillard reaction, various compounds are produced that impart vital flavor, aroma, and color characteristics.

A variety of factors influence the rate and extent of the Maillard reaction, as well as influencing the specific compounds formed. These factors include the reactant concentrations, sugar type, amine source, temperature, pH, moisture content, and minerals and metals. An increase in reactant concentration, temperature, and pH (up to 9) increases the rate and level of browning (Lund & Ray, 2017). The reaction rate of different sugars is related to the availability of open-chain forms of sugars; ketoses have been found to be more reactive than aldoses (Brands & van Boekel, 2001). Like sugar sources, amine groups influence the reaction rate, and different amino acids are more or less reactive (Hemmler et al., 2018). Moisture content has a complex relationship with Maillard browning. While water is necessary for Maillard browning, excessively high water content dilutes reactants and decreases the reaction rate. However, if the moisture content increases too far, viscosity increases, leading to decreased molecular mobility and reaction rate (Tamanna & Mahmood, 2015). Minerals and metals may act as catalysts in Maillard browning and stabilize intermediates; minerals and metals may be added through hard water and milk ingredients.

The other type of non-enzymatic browning, caramelization, occurs when reducing sugars are heated in the absence of protein (Seo et al., 2012). Caramelization begins with the reducing sugars undergoing dehydration, fragmentation, and polymerization. For sucrose to undergo caramelization, it must first be hydrolyzed, which can be catalyzed by adding acid and increasing temperatures. The reaction pathway of caramelization is generally very similar to Maillard browning after the initial amine reaction. Therefore, the Maillard browning factors, except for the amine group, also affect caramelization.

Maillard browning and caramelization play crucial roles in flavor and color development in various food products. While they follow similar pathways, their end compounds differ due to the presence of nitrogen in Maillard browning products. Both reactions can coincide in complex food systems containing sugars and proteins.

2.6.7 Relative Sweetness of Sugars

Sugars have different levels of perceived sweetness dependent on their chemical structures and interactions with taste receptors. The mechanism behind sweetness is complex and is still being actively researched. However, in simple terms, sweetness can be understood as the recognition of sweet-tasting molecules by G-protein-couple sweet receptors found on the tongue's surface of tastebuds (Starkey et al., 2022), after which biochemical cascades occur involving protein messengers and neurological signals conveying intensity and quality of sweetness to the brain.

While there are different methods for quantifying the sweetness of different sweeteners, relative sweetness is one of the most common methods (Stone & Oliver, 1969). The relative sweetness scale is based on the sweetness of a 10% sucrose solution, which is assigned a value of 1 or 100. Other sweeteners are then compared at the same concentration and given a value compared to sucrose. Sweetness can be influenced by concentration and temperature. As concentration and temperature increase, perceived sweetness also increases.

Sweeteners can also synergistically affect other sweeteners (Das & Chakraborty, 2017). Combined sweetness can be greater than the sum of the individual sweeteners. Such combinations can help overcome the plateau effect in sweetness, where concentration does not proportionally increase sweetening.

Understanding the relative sweetness of different sweeteners is crucial for formulating products. While some sugars may have more advantageous physical properties, products must reach the expected sweetness and flavor profile.

2.7 Applications of GGS

Due to the inherent newness of GGS, limited research currently exists surrounding the utilization of this sweetener syrup. So far, GGS has been explored as a sweetener in dairy applications but has yet to be applied to other major food categories. The investigation of GGS as a bulk sweetener continues to be evaluated in various applications, focusing on dairy and sweetened goods, such as confections and bakery products.

2.7.1 Dairy Applications

GGS has been evaluated in limited applications, including whole yogurt, sweetened fruit yogurt, milk caramel spread, and frozen dairy desserts. Across the studies, different sources of GGS were utilized.

A recent study evaluated the physicochemical and sensory properties of whole yogurt sweetened with GGS (Mosquera-Martínez et al., 2023). The GGS was produced through the enzymatic hydrolysis of sweet whey solutions, in which filtration was used to remove fat and protein. Composition of the GGS can be found in Table 2.3. The study found that GGS did not affect the yogurt's stability over a 28-day refrigerated storage period. At up to 25% (w/w) sucrose replacement, GGS-sweetened yogurt maintained similar sweetness and overall acceptability compared to the control. However, 50% (w/w) sucrose replacement samples could not maintain these sensory attributes.

Vargas-Díaz et al. (2019) investigated the sensory and stability properties of milk caramel spread, a dairy product obtained by the evaporation of milk with sucrose until 70% total solids was

obtained, sweetened with the same GGS produced in the Mosquera-Martínez et al. (2023) study. The study examined the effects of 10%, 20%, and 30% GGS addition and found that GGS increased Maillard browning, which increased the production of brown pigments. There was no relationship found between replacement levels and the final texture of the product, although sucrose replacement did not exceed 30% (w/w). This study provides valuable insights into the functionality of GGS components despite the differences in the sugar ratios. This GGS had 3.6 parts monosaccharides to 1 part disaccharide, whereas the GGS produced by the UW-Madison reactor contains 7.2 parts monosaccharides to 1-part disaccharides.

Table 2.3 Composition of glucose galactose syrup (GGS) used in yogurt production (Mosquera-Martínez et al., 2023)

Parameters	Syrup (GGS)
Lactose (gL^{-1})	193 ± 5
Glucose (gL^{-1})	393 ± 11
Galactose (gL^{-1})	297 ± 12
GOS-3 (gL^{-1})	77.4 ± 1.3
GOS-4 (gL^{-1})	8.8 ± 0.3
Soluble solids (°Bx)	72.5 ± 2
Density ($g\ mL^{-1}$)	1.37 ± 0.01
Moisture ($g\ 100\ g^{-1}$)	24 ± 3
Water activity (Aw)	0.73 ± 0.03
Acidity (g lactic acid $100\ g^{-1}$)	1.25 ± 0.09
pH	4.85 ± 0.07
Protein ($g\ 100\ g^{-1}$)	< 2.5
Calcium ($g\ 100\ g^{-1}$)	0.27 ± 0.05
Phosphorus ($g\ 100\ g^{-1}$)	0.368 ± 0.04
Magnesium ($g\ 100\ g^{-1}$)	0.082 ± 0.008
Potassium ($g\ 100\ g^{-1}$)	0.9 ± 0.1
Ash ($g\ 100\ g^{-1}$)	3.4 ± 0.3
Color:	
L^*	35 ± 2
a^*	−3.35 ± 0.95
b^*	17 ± 2
Sweetening power	0.5

Somov et al. (2015) evaluated a version of GGS as a sucrose replacement in sweetened fruit yogurt, replacing 25% of the sucrose by weight. GGS was produced from cheese whey using the baromembrane method. This method consisted of the same general steps as done in the Mosquera-Martínez et al. (2023) study, in which the lactose was hydrolyzed enzymatically. GGS was produced with two different hydrolysis levels and the sugar composition can be found in Table 2.4. Their findings indicated that the sweetness and aftertaste of the GGS-sweetened yogurt were not significantly different from the control samples. However, the study did not explore other attributes of the yogurt, such as texture, viscosity, or microbial stability. In addition, the GGS used in this study had lower levels of hydrolysis and a higher ratio of glucose to galactose than the GGS produced by UW-Madison.

Table 2.4 GGS sugar composition (Somov et al., 2015)

Syrup Type	Sample #	Glucose %	Galactose %	Lactose %
1	1	21.1	17.0	13.7
	2	20.8	15.2	14.7
2	1	8.9	5.9	20.6
	2	8.1	5.2	22.4

Evdokimov et al. (2020) explored the use of GGS as a sweetener in ice cream and frozen desserts. Due to patent issues, the study provided limited methodological details. Nevertheless, it was found that GGS performed optimally at 50% (w/w) sucrose replacement in frozen dairy desserts. Other specific findings from this study were not detailed, limiting the conclusions that can be drawn from this research.

The studies reviewed indicate that GGS can be a viable partial replacement for sucrose in various dairy products, maintaining sweetness and acceptability at certain replacement levels. Further research is needed to comprehensively understand the effects of GGS on a broader range of products, as well as a more thorough investigation of the effects of GGS on the properties of the applications. In addition, these studies were conducted with GGS with higher levels of glucose than galactose, due to the whey source, which is not representative of GGS made from GAW, in which galactose is much more abundant than glucose. These GGS's also had lower levels of hydrolysis with higher disaccharide content in the final product.

2.7.2 Other Applications

Bulk sweeteners are most often the main component of confections and play a significant role in physical and functional attributes and flavor. Corn syrup and sucrose are commonly combined in confections to obtain desired characterizations.

2.7.2.1 Caramel

Caramels are a popular confection in which bulk sweeteners are crucial in determining the final product characteristics (Hartel et al., 2018). Many different products exist within the classification of caramels, from smooth caramel sauces to chewy, grainy caramels and hard candy-type caramels. These characteristics are primarily determined by their moisture content, sweetener composition, and processing conditions.

The most common sweeteners in caramels include sucrose and 43 DE corn syrup. Higher DE corn syrups may be utilized to create a softer caramel or increase crystal size control in grained caramels. Depending on the sucrose and corn syrup ratio, caramels may be partially grained with a short texture or ungrained with a stretchy, chewy structure (Steiner et al., 2003). As sweeteners

with a higher tendency to grain increase and doctoring agents decrease, more graining and higher crystal content will occur.

Bulk sweeteners are not the only sugars in caramels; lactose is also present when milk ingredients are added (Atapattu, 1997). If lactose content is too high, the caramel becomes more prone to graining due to lactose's low solubility.

Browning of the caramels also depends on the sweetener content and the cooking conditions (Corzo-Martínez et al., 2012). Maillard browning and caramelization occur during cooking as amine groups are present due to the added dairy ingredients. Browning depends on the temperature and concentration of reducing sugars; increasing either will enhance browning.

In previous studies, a decrease in sweetener average molecular weight decreased hardness and lowered resistance to flow (Miller & Hartel, 2015). This study also found that higher DE corn syrups lead to increased browning due to an increase in reducing sugars. In another study, it was found that hardness had a positive correlation to stickiness, which may relate to the consumers' experience when biting through caramel (Wang & Hartel, 2021).

2.7.2.2 Gelatin Gummies

Gummy candy is one of the most popular types of confections and has an extensive global market (Tarahi et al., 2023). Gummies can be made with various gelling agents, although gelatin is most commonly used (Ge et al., 2021). Other than the gelling agents, gummies most commonly consist of sucrose, corn syrup, water, organic acids, flavors, and colors.

Sucrose and corn syrup provide sweetness and body to gummies and are the bulk of most candy (Hartel et al., 2018). Due to the nature of higher water content and high glucose content in gummies, graining or crystallization is usually not a concern, as the sucrose can remain solubilized entirely. The sweeteners in gelatin gummies are known to raise the melting point of gelatin and

impede gel formation. Glucose has been found to be less effective at increasing the melting temperature of the gummy than sucrose. Gelatin gels are better stabilized by sucrose than galactose; however, galactose increases stability better than glucose (Naftalin & Symons, 1974).

As in agreement with caramels, increased DE corn syrup produces a softer product; however, in gummies, this also decreases the chewiness (Puch et al., 2019). Another study also found that an increase in lower molecular weight sugars negatively impacted gelation, causing the gels to have diminished mechanical strength (Wang et al., 2024).

2.7.2.3 High Ratio Cake

High ratio cakes contain a higher ratio of sugar to flour and are commonly used in the bakery industry (Rosenthal, 1995). These cakes are well known for their excellent eating quality and long shelf life.

Cakes are sweetened solely by sucrose, providing both bulk and water-holding properties. Sucrose is vital in starch gelatinization and protein denaturation temperatures (Wrigley & Morris, 1996). In addition, sucrose promotes fat crystal aggregates, increases batter viscosity, and increases foam stability (Hicsasmaz et al., 2003).

A study by Miller et al. (2017) found that cake volume was significantly increased by increasing the replacement of sucrose with fructose, but the texture was negatively impacted, causing a gummy mass during chewing. This study indicates that a decrease in sugar average molecular weight leads to an increase in volume. Other studies have also found that sucrose substitutes in high ratio cake significantly affect the timings of starch and protein biopolymer transitions during baking (Godefroidt et al., 2023). These changes caused cakes to have undesirable crumb texture, decreasing crumb cohesiveness and softness.

Sweeteners also affect the color formation in cakes during baking. Maillard and caramelization browning occur during baking due to amines in the flour. Evaporation occurs during the baking of cakes, first on the outer surface and then continuing towards the core, generating the crust (Purlis, 2010). Increased reducing sugar content will, in turn, increase the browning of the crust of baked good products.

2.7.3 Potential Applications of GGS

Sweeteners play a large role in a variety of products as seen in section 2.7.2; however, the application of GGS as a sweetener in these products has not been evaluated. Due to the unique formulation of GGS produced with an acid hydrolysis reactor and the lack of diversity in the applications of GGS, further research must be conducted. To fully understand the functionality of GGS, it should be further researched for its properties in and out of applications.

3. Materials & Methods

The materials and methods section is divided into two parts, corresponding to the two major phases of this research. Section 3.1 discusses the materials and methods utilized to characterize glucose galactose syrup's functional and physical properties (GGS). In section 3.2, the materials and methods employed for the application of GGS in confectionary and bakery applications are outlined.

3.1 Characterization and Functionality

This section outlines the materials and methods utilized in creating and characterizing GGS. Due to unforeseen difficulties in acquiring the lactose hydrolysis reactor, GGS was formulated using its components rather than obtaining the syrup directly from the chemical engineering team. The GGS was then optimized for the greatest solids content and simplified to only consist of the major components: water, glucose, galactose, and lactose (Table 3.1). The syrup was then characterized for its functional and physical, namely rheological properties, freezing point depression, boiling point elevation, and browning properties, utilizing 43 DE corn syrup and bakers' sugar as comparative controls.

Table 3.1 Composition of simulated glucose galactose syrup, optimized for the greatest solids content.

	Weight Fraction (%)
Galactose	30.17
Glucose	20.83
Lactose	4.00
Water	45.00

3.1.1 Materials

Dextrose (Clintose A) was sourced from Archer Daniels Midland (Decatur, IL). Lactose was sourced from Leprino Foods (Denver, CO). D- Galactose, 98% HPLC grade, was purchased from VWR International LLC (Randor, PA). Distilled water was purchased from Nestle Pure Life (Vevey, Switzerland). Bakers granulated sugar was donated by American Sugar Refining (West Palm Beach, FL). Forty-three DE corn syrup was purchased from Sweetener Supply Corporation (Brookfield, IL). Hydroxymethal-5-furfural, KH_2PO_4, and $Na_2HPO_4 \bullet 2H_2O$ were all purchased from Sigma-Aldrich Inc. (Saint Louis, MO).

3.1.2 Glucose Galactose Syrup Production

GGS was made in 2 kg batches. Glucose, galactose, and lactose were weighed into a 2L beaker in which heated distilled water was added. The beaker was then placed on a heat plate set to 80°C and stirred by hand with a stir rod until a stir bar could be utilized. Once the stir bar was added to the beaker, the syrup continued to be heated while covered with aluminum foil and stirred at 650 rpm for approximately 45min until the solution was completely transparent and all sugars had dissolved. The syrup was then allowed to cool to room temperature before being transferred into 1-gallon jugs for storage. The syrup was made consistently throughout this research to prevent recrystallization.

3.1.3 Rheology

Controls of 43 DE corn syrup and sucrose solutions were produced by combining the sweeteners with distilled water to produce 55% solids (w/w) solutions. The rheological behavior of GGS and controls were measured by a Discovery DHR-2 hybrid rheometer (TA Instruments, New Castle, DE, USA) with cup and bob geometry. Samples were tested under two conditions to mimic their behaviors at freezing and room temperatures, -5°C and 25°C, respectively. Sweetener

solutions were loaded into the temperature-controlled cell and equilibrated to the desired temperature for 5min. A flow sweep was then conducted from 100 to $10s^{-1}$ shear rate.

Flow curves were then modeled using the Power Law model to determine the consistency coefficients and flow behavior indexes according to the following equation. The apparent viscosity was measured at a shear rate of $10s^{-1}$. The flow sweep test was carried out in triplicate for each sweetener for both temperatures.

3.1.4 Freezing Point Depression

Samples of 43 DE corn syrup, sucrose, and GGS were diluted at 2.5, 5, 7.5, 10, 12.5, and 15% solids (v/v). The freezing point of each solution was determined using an osmometer model 3250 (Advanced Instruments, Norwood, MA, USA), in which a sample tube was loaded with 25µl of the sample. The osmolarity of the sample was measured by the instrument in duplicate for each sample. The osmolarity was then calculated using a standard curve of a known sample's freezing point to the osmolarity. Freezing point depression curves were then modeled by comparing the solids content against the freezing point. The freezing point curve was carried out in duplicate.

3.1.5 Boiling Point Elevation

Simulated GGS syrup, 55% solids, was added to a stainless-steel pot on top of a hot plate. A digital thermometer was attached to the side and submerged into the syrup without touching the bottom of the pot. The hot plate was then set to the highest temperature, 500°C and the syrup was subsequently boiled. Aliquots of syrup were then pulled at various boiling temperatures, from 107.2 to 121.1°C at 2.8°C increments. These aliquots were directly submersed into liquid nitrogen in metal canisters labeled for each temperature. This aggressive temperature drop allowed for the immediate cooling of the samples, preventing further evaporation.

Frozen syrups were then immediately weighed out in 1.5 to 3.0g aliquots into 50ml glass tubes with twist-on screw tops. The syrup samples were immediately capped to prevent excess moisture from entering. Samples were then dissolved and diluted into solvent at a ratio of 1 part sample to 6.25 parts solvent. The solvent medium consisted of methanol (Fischer Chemical, Fair Lawn, NJ) and formamide (Fischer Chemical, Fair Lawn, NJ) at a 1:1 ratio. The samples were immediately capped after the solvent was added, and the tubes were placed into a 60°C water bath. Samples were then intermittently vortexed until the samples were completely dissolved.

While samples dissolved, more solvent was made at 4:3 methanol to formamide. Using an automatic Karl Fischer Titrator (Metrohm 795 KFT Titrino), approximately 60 ml of solvent was added to the jacketed vessel, set to a temperature of 55°C. The titrator was equipped with Hydranal-Composite 5 (Sigma Aldrich, St. Louis, MO) as the titrator solution. Before testing experimental samples, approximately 0.07g of sodium tartrate dihydrate standard (Sigma Aldrich, St. Louis, MO) with a moisture content of 15.66% and approximately 0.4g samples of blank 1:1 solvent were measured in triplicate. These values were then used during data analysis as a correction factor for normal error and variation within the titrator and any moisture in the solvent before the sample was added. Samples were then automatically titrated in triplicate, utilizing samples of approximately 0.4g, capping samples in between runs, ensuring as little water contamination as possible. Water contents were then calculated based on the titrator's dilution factor, correction factors, and moisture content reading. Boiling point elevation was carried out in triplicate.

3.1.6 Browning Properties

Simulated GGS, corn syrup, and sucrose were diluted in pH five phosphate buffer, prepared by combining potassium dihydrogen phosphate and sodium phosphate dibasic dihydrate solutions. Three replicates of 40ml each sweetener were placed in 50ml plastic centrifuge tubes and placed

in an incubator set to 60°C. Samples were subsequently pulled from the oven and tested once a week for 8 weeks. Prior to analysis, the samples were vortexed for 5s to ensure homogeneity. After vortexing, the samples were analyzed using UV-vis spectrometry, 1ml aliquots were deposited into quartz cuvettes and placed into the spectrophotometer previously calibrated with the blank pH 5 buffer solution. Samples were measured in duplicate, for their absorbance at 284 and 420nm, for concentration of HMF and relative brown intensity, respectively. Once samples were tested, the solution was added back to the sample tube and the tubes were all placed back into the oven.

Solutions of HMF in pH 5 buffer were also tested at 284nm in the spectrophotometer and a standard curve was created. These solutions were made in serial dilutions starting with a 100mM solution which was then diluted to be 1 mM and then that solution was used to make dilutions of 0.2, 0.4, 0.6, 0.8mM. This standard curve was then utilized to convert measurements from absorbance at 284nm to concentration of HMF. Sweetener solutions were graphed for their HMF concentrations and relative brown intensities.

3.2 Food Applications

3.2.1 Caramel

3.2.1.1 Ingredients

Dextrose (Clintose A) was sourced from Archer Daniels Midland (Decatur, IL). Lactose was sourced from Leprino Foods (Denver, CO). D- Galactose, 98% HPLC grade, was purchased from VWR International LLC (Randor, PA). Distilled water was purchased from Nestle Pure Life (Vevey, Switzerland). Bakers granulated sugar was donated by American Sugar Refining (West Palm Beach, FL). Forty-three DE corn syrup was purchased from Sweetener Supply Corporation (Brookfield, IL). Sweetened condensed skim milk was provided by Galloway Company (Neenah, WI). Partially hydrogenated palm kernel oil was supplied by Stratas Foods (Memphis, TN). Soy

lecithin, Clearate B-60, was supplied by WA Clearly Product Inc. (Somerset, NJ). Flour fine salt was donated by Food Ingredients Inc. (Waukesha, WI). All ingredients were from the same lot.

Upon receiving the sweetened condensed skim milk (SCSM), it was immediately separated into proportioned 300g plastic bags (Ziplock, S.C. Johnson Inc., Racine, WI). Air was expelled from bags, which were placed into a -18°C freezer. Bags were taken from the freezer and brought to room temperature immediately before utilization in caramel production

3.2.1.2 Formulations

Caramel formulations were based on representative industry formulations for cut and wrap caramels (Hofberger, 2009; Jackson, 1999). All caramels were formulated for a final moisture content of 10.0%, sugar content of 65.7%, fat content of 11.3%, and protein content of 2.0%. The premixes all had an initial moisture content of 24.0%. The control formulation had a ratio of 2:3 sucrose to corn syrup solids. In the replacement formulations, either sucrose or corn syrup were replaced at 40% and 80% on a molar basis.

Molar replacements were calculated on a dry basis with the following equations for corn syrup and sucrose replacements respectively.

$$m_{ggs}\ for\ corn\ syrup\ replacement=$$

$$m_{cs} * \frac{79\ g_{cs\ solids}}{100\ g_{cs}} * \ replacement\ level * \frac{1\ mol_{cs}}{430\ g_{cs}} * \frac{191.95\ g_{ggs}}{1\ mol_{ggs}} * \frac{100\ g_{ggs}}{55\ g_{ggs\ solids}}$$

$$m_{cs}\ for\ corn\ syrup\ replacement=$$

$$m_{cs} * (1 - \ replacement\ level)$$

$$m_{ggs}\ for\ sucrose\ replacement=$$

$$m_{ggs} = m_{suc} * \ replacement\ level * \frac{1\ mol_{suc}}{342.3\ g_{suc}} * \frac{191.95\ g_{ggs}}{1\ mol_{ggs}} * \frac{100\ g_{ggs}}{55\ g_{ggs\ solids}}$$

$$m_{suc}\ for\ sucrose\ replacement=$$

$$m_{suc} * (1 - \ replacement\ level)$$

The calculated masses were used to determine the ratios of sucrose, corn syrup, and GGS masses based on a given replacement condition. The water content and sugar content contributed by each sweetener was calculated based on their known concentrations. The Solver function of Microsoft Excel was then used to determine the mass of sucrose, corn syrup, and GGS necessary to create a combination of water, sucrose, corn syrup, and GGS that totaled the same total sugar content while keeping the ratios between each sweetener constant. The amount of added water was then decreased to have the same total moisture content between the sweeteners and water. These final calculations allowed for formulations to have the same macro nutrient profile and water content. Initial water contents were kept consistent across formulas so that cook times were similar amongst formulations, allowing for less confounding variables on Maillard browning occurring during cooking.

The formulations of the premixes are shown in Table 3.2 and the initial and final macro nutrient profiles are shown in Table 3.3. The experiment consisted of two different phases, one phase for corn syrup replacement and one for sucrose replacement. Within each phase, 40 and 80% molar replacement of the highlighted sweetener was replaced with GGS. Each phase consisted of a complete randomized four-block design in which two replicates of a control, 40% replacement and an 80% replacement formulation were produced and tested per block.

Table 3.2 Premix Caramel Formulation [1]43 DE Corn Syrup, [2] Glucose Galactose Syrup, [3] Palm Kernel Oil, [4] Sweetened Condensed Skim Milk

	Ingredient (%)							
Formulation	**Water**	**Sucrose**	**CS[1]**	**GGS[2]**	**PKO[3]**	**SCSM[4]**	**Salt**	**Lecithin**
Control	9.75	20.68	37.02	0.00	10.52	21.26	0.59	0.18
40% Corn Syrup Replacement	6.94	23.83	25.60	11.08	10.52	21.26	0.59	0.18
80% Corn Syrup Replacement	3.11	28.12	10.07	26.15	10.52	21.26	0.59	0.18
40% Sucrose Replacement	5.13	13.36	39.87	9.08	10.52	21.26	0.59	0.18
80% Sucrose Replacement	0.00	5.57	42.51	19.37	10.52	21.26	0.59	0.18

Table 3.3 The initial and final macro nutrient composition for caramel formulations

	Composition (%)				
	Water	**Sugar**	**Lipid**	**Protein**	**Ash**
Initial	23.96	62.57	10.73	1.93	0.80
Final	10.00	74.05	12.71	2.29	0.95

3.2.1.3 Cooking Procedure

Each batch of caramel was prepared in batches of 750g, following the previously outlined block design. Each block began with weighing all the ingredients into 1L beakers. Once the batches were all weighed out for the block, all the beakers were covered in polyvinylidene chloride (PVC) film and placed in a 60°C Fisherbrand Isotemp GPD 10 water bath (Fisher Scientific, Waltham,

MA). The samples were placed in the water bath to melt the shortening, begin to dissolve the sugars, and decrease viscosity allowing for proper homogenization. After 60min, the solutions were each stirred and homogenized at 8000rpm for 1min (Omni Macro Homogenizer, Omni International, Kennesaw, GA).

After the first homogenization, the beakers were recovered with PVC film and held at room temperature until 30min before that replicate would be further processed and cooked. The next batch to be processed would be placed back into the same 60°C water bath and left for 30min.

Once the formulation had been in the water bath for the second time, the formulation was then homogenized, with the same specifications, for a second time. Then, 700g of the homogenized solution were measured into a stainless-steel pot. The pot was then heated on a 300°C Isotemp hot plate (Fisher Scientific, Waltham, MA) and stirred with a rubber spatula. The batch was incrementally weighed until the desired amount of water was boiled off, 90.0g, allowing for consistent final water content across samples, regardless of boiling point elevation. At this time, the pot was removed from the hot plate and the caramel was immediately molded. The test samples were made by pouring the hot caramel mixture into 25.4mm diameter silicone molds. The molds were overfilled allowing for a precision leveling once cooled. Immediately after pouring, the caramel was covered with PVC film to minimize water evaporation. The samples were left at room temperature overnight to completely cool and equilibrate.

3.2.1.4 Analytical Testing

To prepare samples for analytical testing, a sharp chef's knife was utilized to cut off the top of the caramels and level them, making them flush to the top edge of the mold. The samples were then rewrapped in PVC film to avoid moisture loss throughout the testing process.

3.2.1.4.1 Moisture Content

Moisture content of the final caramel samples was measured to ensure consistency between replicates and acted as a controlled variable. All caramels were cooked to have a final moisture content of 10.0% ± 0.5% to ensure they were equally comparable. This range was determined to be acceptable by utilizing prediction profiling statistical analysis on preliminary data, indicating that the difference in that moisture range did not significantly affect any other properties. If batches of caramel did not fall into the acceptable water content range, the block was completely removed and redone.

Karl Fischer titration was used to measure the moisture content of the caramels due to its ability to analyze products with high sugar content accurately. The moisture content of the samples were measured volumetrically by a 795 Titrino Karl Fischer titration unit (Metrohm Ltd., Herisau, Switzerland). The titrator was set up before each use with a 4:3 mixture of methanol and formamide in the jacket heated testing chamber and the heated jacket was set to 50°C to help increase reaction rate. The titrator was calibrated with sodium titrate dehydrate to calculate the calibration value for subsequent measurements. The calibration value was equal to the ideal value, 15.66, divided by the average of 3 measured values.

To prepare samples for titration, 1.5-3.0g samples were weighed into glass test tubes and dissolved into 1:1 methanol formamide solution at a ratio of 1 part sample to 6.25 parts solvent. The tubes were then capped and placed into a 60°C water bath, with intermittent vortexing, for approximately 1h or until the samples were completely dissolved. Aliquots of sample solutions were then added to the titrator and automatically titrated with Hydranal Composite 5, the titer automatically added titrant until the potentiometric endpoint was determined and the moisture content was calculated. However, due the dilution of the samples, the result provided by the titrator

needed to be further manipulated to take the solvent's water content, the dilution factor and the calibration value into account. The following equation was used to calculate the water content.

$$\%\, H_2O = \; y * d * c - x * (d - 1) * \; c$$

$$y = measured\ water\ content$$
$$d = dilution\ coefficent$$
$$x = average\ blank\ water\ content$$
$$c = calibration\ value$$

To ensure an accurate measurement, for each batch of caramels, samples were prepared in triplicate, a new molded caramel per test tube, and each tube was tested in duplicate.

3.2.1.4.2 Water Activity

Using an Aqualab 3TE water activity meter (Decagon, Pullman, WA), the water activity of the caramels was measured. The water activity meter functions by measuring the equilibrium relative humidity of the samples within the measurement chamber and then converting that measurement into water activity. To most accurately measure the water activity of the caramel samples, caramel was removed from the silicone mold one at a time immediately before testing. The caramel sample was cut in half with a sharp knife and placed into a sample cup with the cut side facing up. After cutting the sample, the cup was placed into the instrument for reading. Six samples were tested for each batch due to the high variance in lower water activity levels.

3.2.1.4.3 Texture Analysis

A TA.XT2 texture analyzer (Texture Technologies, Hamilton, MA) in collaboration with Exponent software was utilized for measuring the texture of all samples. The texture analyzer was calibrated for height and force before testing any samples. A TA-3 25.4mm acrylic probe (Texture Technologies) was equipped with a TA-71 converter plug and calibrated 20mm above the TA-90 heavy duty platform with an aluminum plate. Caramel samples were placed directly from the molds onto the platform, aligning with the innermost circle, with the cut side up. Once the caramel

was appropriately placed, a texture profile analysis (TPA) test was initiated. The probe descended at a rate of 2mm/s until a trigger force of 5g was detected, at which point the probe then increased to a rate of 5mm/s, compressed the sample 10mm, and then returned to the height of the trigger force. After 5s elapsed, the probe descended 10mm again at a rate of 5mm/s before travelling back to the pretest height at a rate of 5mm/s.

The peak positive force was recorded as the hardness, the adhesiveness was measured as the negative area between the first and second compressions, the peak negative force was recorded as the stickiness, the ratio between the total areas under the two compressions was recorded as the cohesiveness, the distance to the second peak fore was recorded as the springiness, and the hardness times the cohesiveness times the springiness was recorded as the chewiness. Four caramel samples were tested per formulation replicate.

3.2.1.4.4 Colorimetry

The color of the caramel samples was measured with a Minolta Chroma Meter CR-300 (Minolta Corporation, Ramsey, NJ). The meter measured the color on the CIELAB color scale system. The CIELAB color scale system gives the values L*, a* and b*, in which L* measures the luminosity with a high value indicating high lightness and a value approaching 0 indicating darkness. Both a* and b* measure color from negative to positive, for a* red versus green is measured with the prior being positive values and the latter negative and for b* the same for yellow versus blue. Before using the colorimeter, it was first calibrated with a white and black standard from Minolta. Samples were then demolded and flattened to cover the lens of the colorimeter. The sample was placed over the lens and a cup was used on top to ensure that samples were measured consistently. Triplicates of each formulation were tested, and the meter automatically provided the average for each formulation.

3.2.2 Gelatin Gummies

3.2.2.1 Ingredients

Dextrose (Clintose A) was sourced from Archer Daniels Midland (Decatur, IL). Lactose was sourced from Leprino Foods (Denver, CO). D- Galactose 98% HPLC grade was purchased from VWR International LLC (Randor, PA). Distilled water was purchased from Nestle Pure Life (Vevey, Switzerland). Bakers granulated sugar was donated by American Sugar Refining (West Palm Beach, FL). Forty-three DE corn syrup was purchased from Sweetener Supply Corporation (Brookfield, IL). Gelatin (250 bloom 20 mesh bovine skin) was provided by Nutra Food Ingredients (Kentwood, MI). Citric acid was provided by ADM (Chicago, IL).

3.2.2.2 Formulations

Gummies were formulated to resemble a typical gelatin gummy in the consumer market. The gummies were formulated for a final moisture content of 21.5%, sugar content of 68.5%, fat content of less than 0.1% and protein content of 5.0%. The premixes all had an initial moisture content of 36.9%. The control formulation had a ratio of 3:2 sucrose to corn syrup solids. In the replacement formulations, either sucrose or corn syrup were replaced at 40% and 80% on a molar basis. To control the confounding effects of other ingredients, the total sugar and water content was kept consistent across all formulas. These calculations were completed in the same fashion as outlined in section 3.1.1.2. The formulations of the gummies premix are shown in Table 3.4 as well as their initial and final macro nutrient profiles in Table 3.5. The experiment was split into two different phases, one phase for corn syrup replacement and one for sucrose replacement. Within each phase, 40% and 80% molar replacement of the highlighted sweetener was replaced with GGS. Each phase consisted of a complete randomized four-block design in which two

replicates of a control, 40% replacement and an 80% replacement formulation were produced and tested per block.

Table 3.4 Premix Gummy Formulation [1]43 DE Corn Syrup, [2] Glucose Galactose Syrup, [3]Water added to sugars in the first cook step, [4]Water used to hydrate gelatin

	Ingredient (%)						
Formulation	**Sucrose**	**CS[1]**	**GGS[2]**	**Water (Sucrose)[3]**	**Gelatin**	**Water (Gelatin)[4]**	**Citric Acid (50%)**
Control	29.39	35.75	0.00	18.43	4.93	9.86	14.79
40% Corn Syrup Replacement	33.00	24.08	10.42	16.07	4.93	9.86	14.79
80% Corn Syrup Replacement	37.61	9.15	23.76	13.06	4.93	9.86	14.79
40% Sucrose Replacement	19.36	39.24	13.16	11.81	4.93	9.86	14.79
80% Sucrose Replacement	7.15	43.49	29.17	3.76	4.93	9.86	14.79

Table 3.5 The initial and final macro nutrient composition for gummy formulations

	Composition (%)				
	Water	**Sugar**	**Lipid**	**Protein**	**Ash**
Initial	36.90	57.99	0.00	4.22	0.89
Final	21.50	72.17	0.00	5.25	1.10

3.2.2.3 Cooking Procedure

Before the production of the gummies, the beginning of each block began with the blooming of the gelatin for all six replicates being produced that day. Gelatin was mixed with warm water at 1 part gelatin to 2 parts deionized water, enough stock gelatin solution was made to complete all replicates being made during that block, 175g gelatin and 350g water. The gelatin mixture was then stirred and hydrated in an incubator at 55°C for 4h.

The remaining gummy ingredients, excluding the citric acid solution, were combined in a 600mL beaker. The sugar mixture was then placed on a hot plate and boiled, stirring incrementally, until the syrup mixture lost 93.1g, allowing consistent water content across samples, regardless of boiling point elevation. The sugar syrup was then allowed to cool for about 10min, until there were no noticeable changes in water loss. Once the syrup weight stabilized, the pre-bloomed gelatin was removed from the incubator and added to the syrup and citric acid. The mixture was stirred well for 1 minute with a glass stir rod. Once the solution was homogenous, the beaker was covered with PVC film and placed in a 69°C sonicator bath (Branson 2800 Ultrasonic Cleaner, Fisher Scientific, Waltham, MA) and was left to degas for 8min. The beaker was removed from the sonicator, and the PVC film was removed. The top layer of the gummy solution was then skimmed with a spoon and discarded to remove excess bubbles. The gummy mixture was then immediately poured into 25.4mm diameter silicon molds and a stir rod was used to level off the tops of the gummies, ensuring consistent heights. The molded gummies were left too cool uncovered for 2h, after which they were covered in PVC film and allowed to set overnight fully. Allowing the gummies to cool unwrapped initially ensured that the PVC film could peel off the gummies the next day.

3.2.2.4 Analytical Testing

Samples were left in the covered molds and removed one at a time immediately before analytical testing to ensure significant moisture loss did not occur between replicates and tests.

3.2.2.4.1 Moisture Content

Moisture content of the final gummy samples was measured to ensure consistency between replicates and acted as a controlled variable. All gummies were cooked to a moisture content of 21.5% ± 0.5% to ensure they were comparable. This range was determined to be acceptable by utilizing prediction profiling statistical analysis on preliminary data, indicating that the difference in that moisture range did not significantly affect any other properties. If batches of gummies did not fall into the acceptable water content range, the block was removed and redone.

The moisture content of the samples were measured volumetrically by a 795 Titrino Karl Fischer titration unit (Metrohm Ltd., Herisau, Switzerland) in the same fashion as previously outlined. To ensure an accurate measurement, samples were prepared in triplicate, each tube prepared for one molded gummy, and were tested in duplicate for each batch of gummies.

3.2.2.4.2 Water Activity

Using an Aqualab 3TE water activity meter (Decagon, Pullman, WA), the water activity of the gummies was measured. To most accurately measure the water activity of the gummy samples, one gummy was removed from the silicone mold immediately before testing. The gummy sample was cut in half with a sharp knife and placed into a sample cup with the cut side facing up. Directly after cutting the sample, the cup was placed into the instrument for reading. For each batch, gummies were tested in triplicate.

3.2.2.4.3 Texture Analysis

A TA.XT2 texture analyzer (Texture Technologies, Hamilton, MA) in collaboration with Exponent software was utilized for measuring the texture of all samples. The texture of the gummies utilized the same setup, method and variables as outlined in the caramel section. Four gummy samples were tested per formulation replicate.

3.2.2.4.4 Susceptibility to Graining

After all other analyses were completed, six remaining gummies were placed into a sandwich sized plastic bag (Ziplock), the excess air was removed from the bag and sealed. The samples were then monitored every few days, visually examining the gummies for evidence of crystallization. Preliminary experiments indicated that crystallization was evident by the naked eye; therefore, microscope imaging was unnecessary to identify crystallization. When crystallization occurred on any of the gummies from that replicate, the date and time between production and crystallization were recorded.

3.2.3 High Ratio Cake

3.2.3.1 Ingredients

Dextrose Clintose A was sourced from Archer Daniels Midland (Decatur, IL). Lactose was sourced from Leprino Foods (Denver, CO). D- Galactose 98% HPLC grade, was purchased from VWR International LLC (Randor, PA). Distilled water was purchased from Nestle Pure Life (Vevey, Switzerland). Bakers granulated sugar was donated by American Sugar Refining (West Palm Beach, FL). Partially hydrogenated palm kernel oil was supplied by Stratas Foods (Memphis, TN). Flour fine salt and low heat nonfat dried milk (NFDM) were donated by Food Ingredients Inc. (Waukesha, WI). Cake flour was donated by King Arthur Baking Company (White River

Junction, VT). Baking powder was purchased from Metcalfe Sentry Grocery Store and was produced at Clabber Girl Corporation (Terre Haute, IN).

3.2.3.2 Formulations

Cake formulations were based on combining two different AACC methods, 10.90 White Layer Cakes and 10.52 Angel Food Cake (AACC, 1976, 1982). These two formulas and methods were combined to optimize the effects of a sweetener on a cake while allowing for the cake to produce in a convection oven. In the replacement formulations, sucrose was replaced at 40% and 80% on a molar basis. To control the confounding effects of other ingredients, the total sugar and water content was kept consistent across all formulas. These calculations were completed in the same fashion as outlined in section 3.1.1.2.

The formulations of the cake batters are shown in Table 3.6 and the macro nutrient profile is found in Table 3.7. In this experiment there was a total of four blocks and within each block duplicates of the control, 40% and the 80% were produced and tested.

This experiment was single phased as only sucrose underwent partial replacement at 40 and 80% molar replacement with GGS. The experiment consisted of a complete randomized three-block design in which two replicates of a control, 40% replacement and an 80% replacement formulation were produced and tested per block.

Table 3.6 Cake batter formulation, [1]Glucose Galactose Syrup, [2] Palm Kernel Oil [3] Low Heat Nonfat Dry Milk

Ingredient (%)									
Formulation	**Water**	**Sucrose**	**GGS[1]**	**Cake Flour**	**PKO[2]**	**NFDM[3]**	**Egg White Powder**	**Salt**	**Baking Powder**
Control	23.65	32.79	0.00	23.42	11.71	2.81	4.22	0.70	0.70
40% Sucrose Replacement	16.25	23.74	16.45	23.42	11.71	2.81	4.22	0.70	0.70
80% Sucrose Replacement	4.98	9.98	41.49	23.42	11.71	2.81	4.22	0.70	0.70

Table 3.7 Cake batter macro nutrient profile

Composition (%)					
Water	**Carbohydrate**	**Added Sugar**	**Lipid**	**Protein**	**Ash**
27.44	53.74	35.06	12.19	4.77	0.72

3.2.3.3 Baking Procedure

Cakes were made in 500 g batches and the baking procedure was based on the combination of two different AACC methods, 10.90 White Layer Cakes and 10.52 Angel Food Cake (AACC, 1976, 1982). These methods were combined and optimized to contain an egg white foam while maintaining structure when cooked in a convection oven. Water was added in two steps. The water added in the first step was the total water minus 24.9g and the second addition was the remaining 24.9g. This amount was the total water added to the 80% replacement formulation and therefore was the amount used in the second water addition for all formulations. For this reason, no water was added in the first step for the 80% replacement, as the GGS sufficiently replaced the water needed to create the egg sugar foam. Before the batter was produced, two muffin pans, with 12

mini muffins each, were brushed with a mixture of 1:1 shortening and flour to facilitate the removal of the cakes after baking.

To produce the cakes, the first portion of water, GGS, sucrose and egg white powder were added to a 4.2L Kitchen Aid Stainless Steel Mixing Bowl (Whirlpool Cooperation, Benton Harbor, MI). The bowl was placed into the stand mixer and was fitted with the whisk attachment. The mixture was stirred on speed 4 for 30s and then for 10min at full speed, speed 10. After the whip time, the edges of the bowl were wiped down with a spatula. Melted shortening was then added as the foam was stirred on speed 4. Once all the shortening was added, the mixer was turned to speed 8 for 10s. The dry ingredients, cake flour, salt, baking soda and NFDM, were added to the foam and were combined on speed 6 for 30s. After incorporating the dry ingredients, the bowl was scraped with a spatula. Batter was then mixed on speed 6 as the final water portion was added and then whipped on speed 10 for 10s. The whisk was then removed from the batter and a spatula was used to fold the batter 10 times, ensuring that all ingredients were fully incorporated without deflating the batter.

At this point a portion of the batter was removed for analytical testing and the rest was deposited and 15g weighed directly into muffin tins. The mini cupcakes were then baked for 10min at 149°C, both trays of cupcakes were placed on the same shelf and baked in unison. After baking, the cupcake trays were placed on a wire and cooled for 15min. The mini cupcakes were removed from the trays and cooled entirely on the wire racks for 30min.

3.2.3.4 Analytical Testing

The analytical properties of both the cake batter and the final baked products were evaluated. Batter assessments were conducted immediately following the mixing process and cake assessments were conducted at multiple points after cakes were cooled.

3.2.3.4.1 Specific Gravity of Batter

Specific gravity is a standardized test used to evaluate the amount of air incorporated into the batter during mixing. The specific gravity of the cake batter was determined following the AACC Internation Method 10-90.01 (AACC, 1976). Immediately after mixing was completed, a 59.1ml cup was filled with batter and leveled off with a spatula held at a 45° angle, ensuring consistency. The weight of the batter-filled cup was recorded, and the specific gravity was calculated according to the equation below.

$$Specific\ Gravity = \frac{weight\ of\ batter}{weight\ of\ equal\ volume\ of\ water}$$

3.2.3.4.2 Yield Stress

The rheological properties of the batter were assessed using a Brookfield DV-III Ultra Programmable Viscometer (Brookfield, Middleboro, MA), allowing for measurements of the batter's yield stress and torque. To prepare for each test, a stainless-steel cup was filled with 177ml of batter, using a #16 scoop for consistency. This amount was sufficient to reach the second immersion mark on the size 71 vane spindle (Brookfield, Middleboro, MA), ensuring optimal contact between the batter and the measuring apparatus.

Prior to each measurement, the viscometer was leveled and zeroed per the manuals protocol. The measurement process, controlled by EZ-Yield software (Brookfield, Middleboro, MA), consisted of three distinct phases. Initially, the batter underwent a pre-shear step, during which it was subjected to a constant speed of 10rpm for 5min. This step served to standardize the batter's condition before the main measurement and allowed for the dynamic yield stress to be measured. Following this, a zero operation was performed, where the spindle rotated backwards until the torque percentage reached zero, establishing a baseline for the subsequent measurement.

The final step was the actual yield measurement, in which the spindle rotated at a speed of 0.1rpm, allowing the viscometer to determine the percentage of torque at the yield point and calculate the yield stress.

Given the time-sensitive nature of the batter's properties, as well as the irreversible changes induced by the testing procedure, each formulation replicate underwent a single evaluation immediately following its preparation.

3.2.3.4.3 Cake Volume Index, Symmetry and Uniformity

The volume index was measured by an adjusted method based on the AACC method 10-90 in which cakes are evaluated for their volume and shrinkage (AACC, 2000). The volume was measured 1h, and 24h post bake. The mini cupcakes were cut in half and three height measurements were taken, one at each edge and one in the middle. These measurements were evaluated for symmetry and uniformity. The volume of the cupcake was estimated using the mathematical model of a truncated cone. Three measurements were taken for each sample using a ruler with 0.1mm precision, peak height (h) from base to the highest point, half of bottom diameter (r), and half of maximum width (R) (at the base of the dome). The volume and shrinkage was calculated using the following equations.

$$Volume = \left({}^{1}/_{3}\right) * P * h * (r^2 + r * R + R^2)$$

$$Shrinkage\ \% = \frac{V_i - V_f}{V_i} * 100\%$$

All size measurements were taken in duplicate using two randomly selected cupcakes from each replicate. The same cupcakes were used for initial measurements and for final measurements.

3.2.3.4.4 Colorimetry

The color of the outside and the inside of cake samples were measured with a Minolta Chroma Meter CR-300 (Minolta Corporation, Ramsey, NJ. The outside color of the samples was evaluated first by covering the lens with the base of the cupcake and a cup was used on top to ensure that samples were measured consistently. After the outside color was measured, the cupcakes were cut in half and the same method was repeated but the cut side of the cupcake faced the lens. Triplicates of each formulation were tested for the outside and the inside, and the meter automatically provided the average for each formulation.

3.2.3.4.5 Texture Analysis

The texture of the cakes was tested throughout a one-week shelf life. Cakes were tested on bake day, day 0, and then three more times on days 1, 4 and 7, allowing for the evaluation of changes in staling overtime with different sugar sources.

A TA.XT2 texture analyzer (Texture Technologies, Hamilton, MA) in collaboration with Exponent software was utilized for measuring the texture of all samples. The texture analyzer was calibrated for height and force before testing any samples. A TA-70 acrylic probe (Texture Technologies) was equipped and calibrated to be 35mm above the TA-90 heavy duty platform with an aluminum plate. Cake was tested by placing a mini cupcake directly onto the platform, aligning with the innermost circle, with the dome of the cupcake facing down. Once the mini cupcake was appropriately placed, a texture profile analysis (TPA) test was initiated. The probe descended at a rate of 2mm/s until a trigger force of 5g was detected, at which time the probe then increased to a rate of 5mm/s, compressed the sample 15mm, and then returned to the height of the trigger force. After 5s elapsed, the probe descended 15mm again at a rate of 5mm/s before travelling back to the pretest height at a rate of 5mm/s. The peak positive force was recorded as

the hardness, the ratio between the total areas under the two compressions was recorded as the cohesiveness, the distance to the second peak fore was recorded as the springiness, and the hardness times the cohesiveness times the springiness was recorded as the chewiness. Four mini cupcakes were tested per formulation replicate.

3.3 Statistical Analysis

All statistical analyses were performed using JMP Pro 11 (SAS Institute Inc., Cary, NC). The functional and physical property datasets were subjected to one-way analysis of variance (ANOVA), and a Tukey test with $\alpha < 0.05$ was utilized for comparison. For the application data, a randomized complete block design (RCBD) ANOVA was employed. This approach accounted for variability due to treatments, blocks, and residual error. The RCBD ANOVA allowed for the control of potential confounding factors introduced by the blocking variable. Subsequently, Tukey's HSD test was conducted to discern specific differences among treatments, with the significance threshold maintained at $\alpha < 0.05$.

4. Results and Discussion

4.1 Characterization and Functionality

Glucose galactose syrup (GGS) was characterized for its physical and functional properties. These properties include rheology, freezing point depression, boiling point elevation and browning properties.

4.1.1 Rheology

Control and glucose galactose samples were analyzed for the main rheological properties as shown in Table 4.1. A one-way ANOVA indicated there were significant differences across properties and temperatures. Both temperature and sweetener type significantly impacted the consistency indexes and the apparent viscosities.

Consistency index and apparent viscosity were inversely correlated with the temperature, indicating that lower temperatures lead to higher viscosity due to the decreased mobility of molecules at lower temperatures. Molecular size is directly correlated to consistency index and apparent viscosity, as larger molecules exhibit lower mobility.

Flow behavior for all conditions were very close to 1, indicating near-Newtonian behavior. There were no significant differences in flow behavior between different sweeteners; however, temperature had a significant effect on flow behavior of GGS and sucrose, but not corn syrup. GGS and sucrose exhibited flow behavior indexes slightly above 1, suggesting a minor dilatant tendency. However, these values are so close to 1 that they are likely not practically significant deviations from Newtonian behavior. The decrease in flow behavior index with a decrease in temperature is likely due to increased intermolecular interactions, causing the solutions to exhibit increased pseudoplastic behavior.

At room temperature, these sweeteners can be treated as essentially Newtonian fluids, while at lower temperatures, they exhibit mild pseudoplastic behavior. GGS is more like sucrose solutions than corn syrup solutions, though GGS has lower consistency indexes and apparent viscosities than sucrose solutions.

Table 4.1 Consistency index, flow behavior index and apparent viscosity at $10s^{-1}$of the power law model for samples of glucose galactose syrup (GGS), sucrose and corn syrup normalized to the same solids content. Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Temperature	Sweetener	Consistency Index (mPa*s)	Flow Behavior Index	Apparent Viscosity at 10 s^{-1} (mPa*s)
25°C	GGS	$11\pm 0^{A,a}$	$1.01\pm 0.00^{A,a}$	$11 \pm 0^{A,a}$
	Sucrose	$18\pm 1.0^{B,a}$	$1.02\pm 0.03^{A,a}$	$20 \pm 2^{B,a}$
	Corn Syrup	$27\pm 3^{C,a}$	$0.98\pm 0.02^{A,a}$	$24 \pm 1^{C,a}$
-5°C	GGS	$68\pm 3^{A,b}$	$0.956\pm 0.008^{A,a}$	$56 \pm 2^{A,b}$
	Sucrose	$121\pm 5^{B,b}$	$0.959\pm 0.004^{A,a}$	$100 \pm 3^{B,b}$
	Corn Syrup	$147\pm 6^{C,b}$	$0.959\pm 0.005^{A,b}$	$122 \pm 7^{C,b}$

[1] Values in the same column with the same letter are not significantly different at $\alpha<0.05$
[A, B, C] Indicate differences amongst samples with the same temperature treatment
[a, b, c] Indicate differences amongst samples with the same sweetener

4.1.2 Freezing Point Depression

The freezing point of GGS was measured in comparison to sucrose and corn syrup to characterize its behavior in frozen food applications. It was found that the freezing point of GGS was lower than both corn syrup and sucrose at all solids content and that, as concentration increased, the freezing point increased more than the other sweetener solutions (Figure 4.1). Freezing point is a colligative property of water and can be decreased as solute concentration increases. All these sweeteners have the same disassociation factor of 1 and therefore the only factor affecting the freezing point is the molecular size. GGS has the lowest average molecular weight, 191.3g/mol, followed by sucrose, 342.3g/mol, and corn syrup, 430.0g/mol. The average

molecular weight of GGS is approximately 2.5 times smaller than that of 43 DE corn syrup and 1.6 times smaller than that of sucrose. Therefore, GGS decreased the freezing point the most at the same weight by weight concentration.

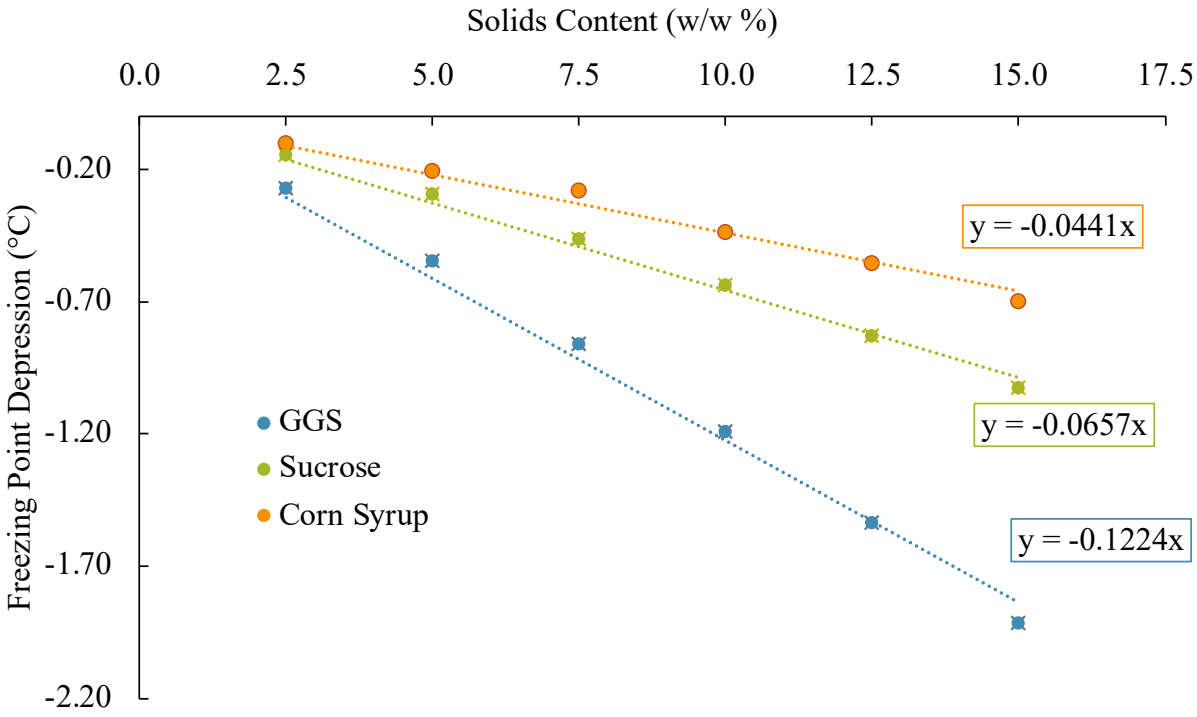

Figure 4.1 The average freezing point depression of water with glucose galactose syrup (GGS), sucrose, and corn syrup at various concentrations. (n=2)

4.1.3 Boiling Point Elevation

Moisture content of solution is directly related to the effects of sugar on boiling point elevation. The size of sugars greatly alters the boiling point elevation at the same moisture content. To test the effect of GGS on boiling point temperature and consequently the effect on moisture content, GGS was boiled to different temperatures and water content was measured. In addition, the predicted solids content of GGS, sucrose, and corn syrup were calculated at the test temperatures.

Boiling point elevation (T_{bp}) is directly correlated to the nature of the solute and its concentration. T_{bp} can be calculated by combining Clausius-Clapeyron relation and Raoult's law, when a non-volatile solute is assumed (Atkins et al., 2023).

$$\Delta T_{bp} = K_b * b_{solute} * i$$
$$K_b = ebullioscopic\ constant\ of\ the\ solvent$$
$$K_{b:water} = 0.521°C * \frac{kg}{mol}$$
$$b_{solute} = solute\ molality$$
$$i = van't\ Hoff\ factor$$

$$Molality: m = \frac{mol\ solute}{kg\ solvent}$$

In the case of these sugar mixtures, for a set change in temperature, the same molality is calculated due to the same dissociation factor. Molality is directly correlated to average molecular weight, where molality is equal to the mols of solute over the mass of solvent. For the same molality, the same number of mols are required, which in turn is a larger mass for higher molecular weight sugars.

Experimental results for the moisture content of GGS were compared to calculated values for GGS, sucrose, and corn syrup (Figure 4.2). Moisture content was consistently higher for experimental data than calculated data; however, the same trend and general ranges were followed. This difference in boiling point is likely due to measurement error in which excess evaporation is occurring at the time of sampling. In addition, the calculated value is based on ideal conditions, which in turn differs from experimental results due to non-ideal environmental conditions. GGS required significantly less solids content to reach higher temperatures when compared to sucrose and corn syrup.

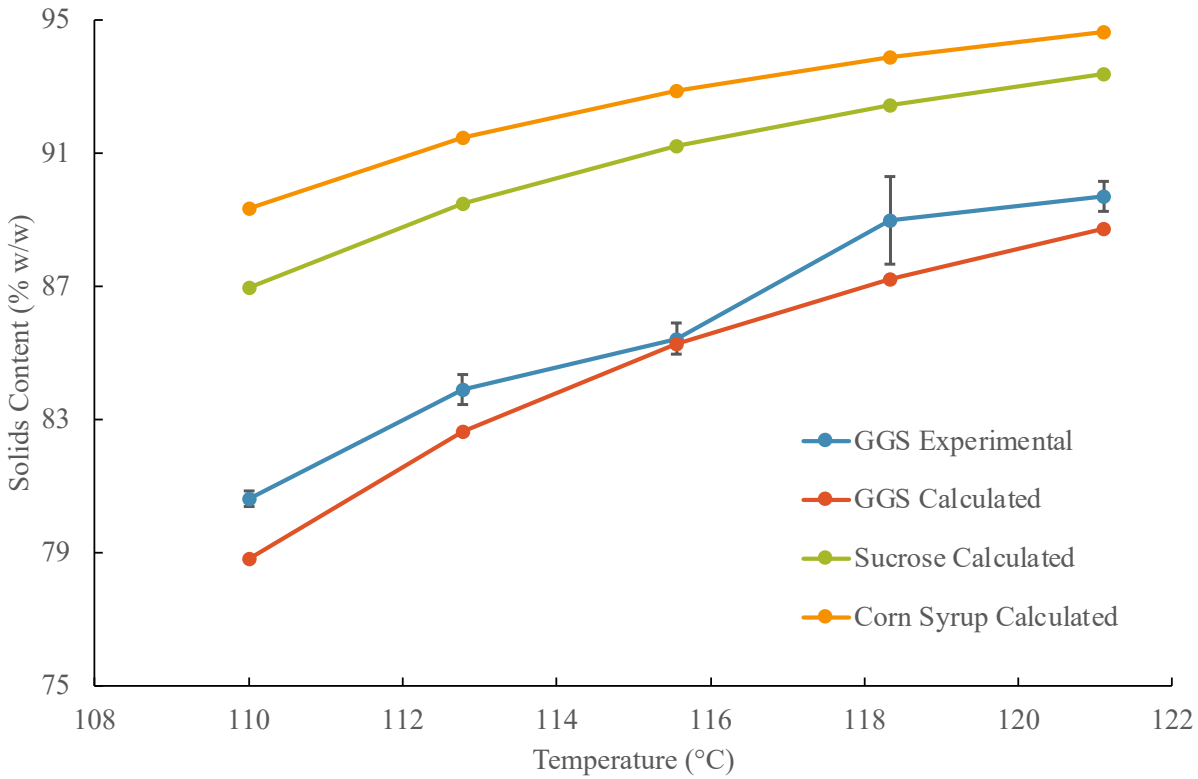

Figure 4.2 Comparison of calculated boiling point elevation data of glucose galactose syrup (GGS), sucrose, and corn syrup; to boiling point elevation mean data of GGS with error bars representing standard error values

4.1.4 Browning Properties

The browning properties of GGS were measured alongside sucrose and corn syrup. The sweeteners were standardized at 0.27M and at pH 5. Samples were measured for their relative brown intensity and their 5-hydroxymethylfurfural (HMF) concentration over the course of eight weeks at 60°C (Figure 4.3 and 4.4).

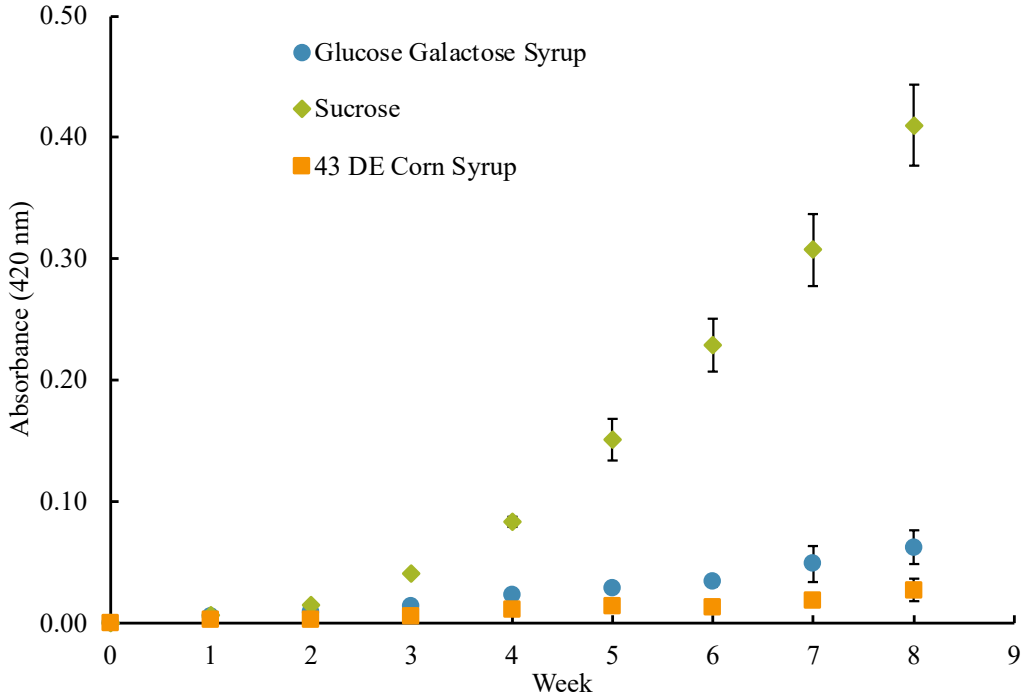

Figure 4.3 Average brown index absorbance at 420nm of 0.27 M sweetener solutions, glucose galactose syrup, sucrose and corn syrup over the course of 8 weeks at 60°C (n=3), with error bars representing the standard errors

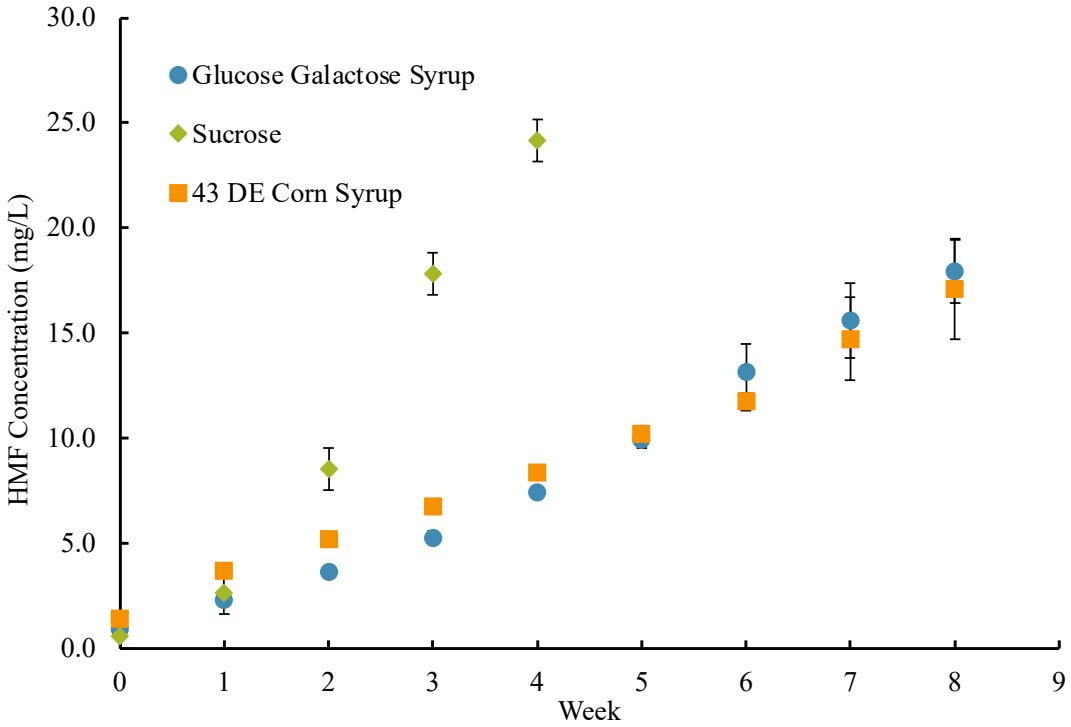

Figure 4.4 Average 5-hydroxymethylfurfural (HMF) concentration of 0.27 M sweetener solutions, glucose galactose syrup, sucrose and corn syrup over the course of 8 weeks 60°C (n=3), with error bars representing standard errors

Sucrose had the greatest and fastest production of brown pigments and HMF with about 25 mg/L at week 4 compared to corn syrup and GGS of which less than 10 mg/L were measured. These results are in agreement with previously literature in which, at the same pH, sucrose browned at a much higher rate than glucose (Bostan & Boyacioğlu, 1997). This browning might be assumed to be caramelization, due to the lack of amines; however, this cannot be caramelization because caramelization requires much higher temperatures than what was reached in this experiment. Therefore, this browning must be Maillard browning due to the low temperatures, and the amines were accidently introduced into the system most likely through impure sugar solutions.

Other studies have found that the addition of phosphate ions, present in the buffering solution, act as a catalytic species and enhance the degradation of Amadori compounds (Davidek et al., 2002). When phosphate-based buffer was compared to citric acid-based buffer, only the phosphate based buffer browned, therefore further proving the influence phosphate has on the Maillard reaction (Bell, 1997). The phosphate ions also enhance the browning and fragmentation of sucrose during storage (Oh et al., 2006). Solutions containing only deionized water and sucrose did not brown whereas solutions of sucrose in phosphate buffer did brown.

The sucrose solution browned the most rapidly, therefore hydrolysis of sucrose into fructose and glucose must have occurred. The acidic conditions and prolonged heating increased the rate of hydrolysis and produced significant amounts of fructose and glucose. Within the sucrose solution, the fructose browned at an increased rate compared to glucose, which agrees with what was found in the Bostan & Boyacioğlu (1997) study. Additionally, in the Oh et al. (2006) study, sucrose and fructose browned at similar rates, but glucose browned at a significantly decreased rate in acidic conditions.

It can be concluded that in the presence of phosphate ions, sucrose undergoes a faster rate of non-enzymatic browning than corn syrup or GGS. In addition, galactose did not brown at a significantly higher rate than glucose, as seen by the similar browning rates of corn syrup, in which only glucose and glucose polymers are present, and GGS.

4.2 Food Applications

Glucose galactose syrup (GGS) was used as a partial molar replacement for sucrose in caramels, gelatin gummies and cake, as well as corn syrup in caramels and gelatin gummies. The molar replacement of sucrose and corn syrup was not a perfect molar replacement due to the need to keep the total composition consistent. The formulations were calculated as molar replacement initially. If GGS is used to truly replace sucrose and corn syrup on a molar basis, the total sugar content would significantly decrease because GGS has a lower average molecular weight than sucrose and corn syrup. To keep the sugar content consistent across all formulations, the molar replacement values of sucrose, corn syrup and GGS were all increased by the same factor. In turn, formulations with replacement have slightly higher molarity and molality than the control formulas. The goal of the food application was to investigate how GGS would function in food applications as a viable replacement for current bulk sweeteners. So, this small deviance was considered acceptable.

4.2.1 Caramel

This experiment examined the viability of GGS as a partial replacement for corn syrup and sucrose in caramels. The experiment was spilt into two different phases, one phase for corn syrup replacement and one for sucrose replacement. Within each phase, 40 and 80% molar replacement of the highlighted sweetener was replaced with GGS. Each phase consisted of a four-block design in which two replicates of a control, 40% replacement and an 80% replacement formulation were

produced and tested. Caramels were compared within each phase in which the block effect was considered.

All caramels were cooked to ensure 10.0%±0.5% moisture content. A one-way ANOVA showed no significant differences in moisture content of the caramels with either corn syrup replacement (p=0.9532) or sucrose replacement caramels (p=0.3912). Each caramel was formulated to have the same final macro nutrient profile of 72.7% carbohydrates, 13.9% protein, 2.5% lipids and 1.0% ash. The molarity of each sugar, and the total sugar molarity can be found in Table 4.2.

Table 4.2 Sugar Molarity of Caramels [1]43 DE Corn Syrup, [2] Glucose Galactose Syrup, [3]Sweetened Condensed Skim Milk

	Molarity (mol/L)				
Formulation	**Sucrose**	**[1]Corn Syrup**	**[2]GGS**	**[3]SCSM**	**Total**
Control	0.604	0.689	0.000	0.172	1.465
40% Corn Syrup Replacement	0.696	0.476	0.317	0.172	1.662
80% Corn Syrup Replacement	0.821	0.187	0.749	0.172	1.930
40% Sucrose Replacement	0.390	0.742	0.260	0.172	1.565
80% Sucrose Replacement	0.163	0.791	0.555	0.172	1.681

4.2.1.1 Water Activity

The replacement of corn syrup with GGS had no practical impact on the water activity of the caramels when the water content was held constant (Table 4.3). Although statistical analysis indicated statistically significant differences in measured values, these differences were not of large enough magnitude to meaningfully affect the properties and behavior of the caramels. Further, these differences are due to the increased molarity of the samples. Similarly, replacement

of sucrose with GGS had no significant impact on the water activity, likely due to the too small of a change in molarity (Table 4.4).

Table 4.3 Water content and water activity of caramels with partial molar replacement of corn syrup by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Water Content (w/w %)	Water Activity
Control	9.8 ± 0.3	0.53 ± 0.02[a]
40% Corn Syrup Replacement	9.8 ± 0.3	0.52 ± 0.03[b]
80% Corn Syrup Replacement	9.8 ± 0.4	0.50 ± 0.04[c]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

Table 4.4 Water content and water activity of caramels with partial molar replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Water Content (w/w %)	Water Activity
Control	9.9 ± 0.4	0.52 ± 0.02[a]
40% Sucrose Replacement	10.0 ± 0.3	0.52 ± 0.02[a]
80% Sucrose Replacement	10.1 ± 0.4	0.52 ± 0.02[a]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

The main driving factors in the water activity of caramels are the presence of low molecular weight molecules and the water content (Ergun et al., 2010). Water content and salt concentrations were kept constant to minimize their effects on the water activity. There would be no difference in the water activity in a perfect molar replacement, as the water activity is directly dependent on the molality of the material. If water content and sugar molarity were held constant, molality would remain the same and therefore there would be no different in water activity.

4.2.1.2 Texture Profile Analysis

The texture of the caramels was evaluated by performing a texture profile analysis (TPA) test in which the hardness, adhesiveness, springiness, cohesiveness, and resiliency were measured. Hardness and adhesiveness are direct measures of the compression of the samples, whereas springiness, cohesiveness, and resiliency are relative ratios of the direct measurements. Springiness measures the ability of a product to recover its original shape after the deforming force is removed. Cohesiveness represents the strength of internal bonds within the product and its ability to maintain its structure. Resiliency measures how well a product recovers its original height immediately after the first compression

The results of the TPA test for corn syrup replacement and sucrose replacement are shown in Tables 4.5 and 4.6 respectively. Corn syrup replacement by GGS in caramels had significant effects on all textural attributes whereas sucrose replacement caramels only had significantly different hardness and cohesiveness values.

In both corn syrup and sucrose replacement formulations, hardness was significantly decreased, compared to the control, at 40% replacement and 80% replacement, with 80% replacement being significantly less hard than 40% replacement. Hardness is decreased due to the decrease in viscosity; the molecular components of GGS are smaller and have less intramolecular interactions than sucrose or corn syrup, thus decreasing the force required for flow. Adhesiveness was significantly increased in corn syrup replacement caramels, where the perceived stickiness increased with increased corn syrup replacement. There was no significant effect of sucrose replacement on adhesiveness. Stickiness is highly affected by the T_g of the product (Hartel et al., 2018). Both molecular nature and water content can affect a product's T_g. In this instance, water content was held constant, and molecular nature was not, with the average molecular size

decreasing as GGS levels increased. As the average molecular size increased with increased GGS levels, the T_g was decreased, and the surface became stickier. The difference in average molecular size between sucrose and GGS was not sufficient to significantly change the T_g to cause stickiness.

Table 4.5 Texture profile analysis of caramels with partial molar replacement of corn syrup by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Hardness (g)	Adhesiveness (g*sec)	Springiness	Cohesiveness	Resiliency
Control	8700 ± 900[a]	290 ± 130[a]	0.4 ± 0.0[a]	0.3 ± 0.0[a]	0.053 ± 0.002[a]
40% Corn Syrup Replacement	4700 ± 900[b]	460 ± 190[b]	0.6 ± 0.1[b]	0.4 ± 0.1[a]	0.043 ± 0.003[b]
80% Corn Syrup Replacement	1400 ± 200[c]	590 ± 200[c]	0.9 ± 0.1[c]	0.9 ± 0.2[b]	0.037 ± 0.005[c]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

Table 4.6 Texture profile analysis of caramels with partial molar replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Hardness (g)	Adhesiveness (g*sec)	Springiness	Cohesiveness	Resiliency
Control	10000 ± 2100[a]	290 ± 190[a]	0.4 ± 0.1[a]	0.29 ± 0.03[a]	0.051 ± 0.004[a]
40% Sucrose Replacement	6800 ± 1700[b]	320 ± 160[a]	0.4 ± 0.1[a]	0.33 ± 0.05[ab]	0.044 ± 0.003[a]
80% Sucrose Replacement	4300 ± 900[c]	340 ± 180[a]	0.5 ± 0.1[a]	0.37 ± 0.06[b]	0.055 ±0.006[a]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

For the relative measures, increased corn syrup replacement significantly increased springiness and cohesiveness but significantly decreased resiliency. GGS decreased T_g which is known to decrease the brittleness of caramel. This decreased brittleness increased the cohesiveness and springiness of the caramel. However, these same characteristics will lead to a softer, less resilient caramel. Increased sucrose replacement did not have a significant effect on springiness or resiliency and only had a significant effect on increased cohesiveness at 80%

replacement. This was likely due to the T_g of sucrose and GGS not being different enough, and therefore only high levels of replacement showed significant differences in some attributes.

In summary, caramels with GGS exhibit significantly different textures than those made without. The texture of caramels with sucrose replacement better resembled the controls than caramels with corn syrup. Lower levels of replacement of both sweeteners better retained their textural characteristics than those made with higher levels of replacement.

4.2.1.3 Colorimetry

When caramels are produced, Maillard browning occurs, in which reducing sugars react with free amino groups to produce dark pigments and flavor compounds. The caramels were measured using a colorimeter where higher L* values indicate lighter color and more positive a* values indicate higher redness compared to blueness. Therefore, higher L* and less positive a* values would indicate less brown pigment formation and less Maillard reaction. The color of the caramels with corn syrup and sucrose replacement are found in Tables 4.7 and 4.8, respectively.

Table 4.7 Color parameters (L*, a*) of caramels with partial molar replacement of corn syrup by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	L*	a*
Control	60 ± 5^{a}	14 ± 2^{a}
40% Corn Syrup Replacement	53 ± 3^{b}	16 ± 1^{b}
80% Corn Syrup Replacement	49 ± 4^{c}	17 ± 1^{b}

[1] Values with the same letter are not significantly different at $\alpha<0.05$

Table 4.8 Color parameters (L*, a*) of caramels with partial molar replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	L*	a*
Control	59 ± 1[a]	14.7 ± 0.6[a]
40% Sucrose Replacement	55 ± 2[b]	15.8 ± 0.7[b]
80% Sucrose Replacement	51 ± 1[c]	16.2 ± 0.5[b]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

Replacement of corn syrup and sucrose by GGS caused a significant decrease in L* and an increase in a*. The amount of reducing sugars was increased by GGS replacement, which led to increased Maillard browning and the formation of dark brown pigments. All sugars present in GGS are reducing sugars (lactose, glucose and galactose). In addition to having a high ratio of reducing groups relative to its molecular weight. Corn syrup is made up of glucose molecules, which also contain reducing groups; however, when formed into polymers the ratio of reducing groups to average molecular weight is decreased significantly, causing a decreased availability for Maillard reaction (Wedzicha & Leong, 2005). Sucrose, on the other hand, is a non-reducing sugar and therefore needs to hydrolyze into glucose and fructose to expose reducing groups. While corn syrup and sucrose have different mechanisms that limit their participation in the Maillard reaction, the result is similar: both have a decreased ability to undergo the reaction compared to GGS.

The increased Maillard browning with GGS was due to its composition of entirely reducing sugars and its favorable molecular weight to reducing group ratio, which makes it more reactive in this context than either corn syrup or sucrose.

4.2.1.4 Summary

Caramels in which GGS replaced corn syrup had decreased hardness, resiliency and luminosity and increased adhesiveness, springiness, cohesiveness and relative redness. Caramels in which GGS replaced sucrose had decreased hardness and luminosity and increased cohesiveness and relative redness. Corn syrup and sucrose replacement with GGS had no significant effect on the water activity, and sucrose replacement had no significant effect on adhesiveness, springiness or resiliency of the caramels.

The increased molecular mobility and decreased average molecular size of GGS caused the changes in the textural properties, leading to softer caramels for both corn syrup and sucrose replacement and higher resiliency in corn syrup replacement. The decreased T_g of GGS led to increased adhesiveness, springiness and cohesiveness in caramels with corn syrup replacement and only the cohesiveness in sucrose replacement. The increased number of reducing sugars and increased ratio of average molecular weight to reducing groups of GGS causes increased Maillard reaction in all replacements leading to darker more brown caramels.

Overall, the use of GGS to replace either sucrose or corn syrup caused significant differences compared to the controls. While GGS does not function as a direct replacement for corn syrup or sucrose, it can be used to manipulate the characteristics of a caramel to create different profiles for different applications. For example, GGS may be favorable in caramel sauces in which replacing corn syrup with GGS would allow for decreased water activity and lower water content while maintaining the desired flow and color characteristics.

4.2.2 Gummies

This experiment examined the viability of GGS as a partial replacement for corn syrup and sucrose in gelatin gummies. The experiment was spilt into two different phases, one phase for corn syrup replacement and one for sucrose replacement. Within each phase, 40 and 80% molar replacement of the highlighted sweetener was replaced with GGS. Each phase consisted of a four-block design in which two replicates of a control, 40% replacement and an 80% replacement formulation were produced and tested. Gummies were compared within each phase in which the block effect was considered.

The molarity of each sugar, and the total sugar molarity can be found in Table 4.9. All gummies were produced to have a final moisture content of 21.5%±0.5 (Tables 4.10 and 4.11). A one-way ANOVA showed no significant differences in moisture content of the gummies with corn syrup replacement ($p=0.5378$) as well as in sucrose replacement gummies ($p=0.9174$). Each gummy was formulated to have the same final macro nutrient profile, 68.3% carbohydrates, 5.0% protein, and 1.0% ash.

Table 4.9 Sugar Molarity of Gelatin Gummies [1]43 DE Corn Syrup, [2] Glucose Galactose Syrup,

	Molarity			
Formulation	**Sucrose**	**[1]Corn Syrup**	**[2]GGS**	**Total**
Control	0.859	0.665	0.000	1.524
40% Corn Syrup Replacement	0.964	0.448	0.299	1.711
80% Corn Syrup Replacement	1.099	0.170	0.681	1.950
40% Sucrose Replacement	0.566	0.730	0.377	1.673
80% Sucrose Replacement	0.209	0.809	0.836	1.854

4.2.2.1 Water Activity

The replacement of corn syrup and sucrose with GGS had no practical impact on the water activity of the gummies when the water content was held constant (Tables 4.10 and 4.11). Although statistical analysis indicated significant differences in measured values, these differences were not of large enough magnitude to meaningfully affect the properties and behavior of the gummies. Therefore, from a practical standpoint, these differences are considered negligible.

While the water activity of the gummies were statistically different, these differences would not be meaningful in application. These slight statistical differences were caused by the slightly increased molarity, as seen in Table 4.9.

Table 4.10 Water content and activity of gummies with partial molar replacement of corn syrup by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Water Content (w/w %)	Water Activity
Control	21.1 ± 0.5	0.76 ± 0.01[a]
40% Corn Syrup Replacement	21.3 ± 0.4	0.75 ± 0.01[b]
80% Corn Syrup Replacement	21.7 ± 0. 5	0.73 ± 0.01[c]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

Table 4.11 Water content and water activity of gummies with partial molar replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Water Content (w/w %)	Water Activity
Control	21.6 ± 0.4	0.76 ± 0.02[a]
40% Sucrose Replacement	21.5 ± 0.3	0.75 ± 0.01[ab]
80% Sucrose Replacement	21.6 ± 0.6	0.74 ± 0.01[b]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

4.2.2.2 Texture Profile Analysis

The texture of the gummies were evaluated by performing a texture profile analysis (TPA) test in which the hardness, adhesiveness, springiness, cohesiveness, and resiliency were measured. The results of the TPA test for corn syrup replacement and sucrose replacement are shown in Tables 4.12 and 4.13 respectively.

The texture profile of gummies with corn syrup replacement only exhibited significantly different attributes for the adhesiveness and resiliency; hardness, springiness and cohesiveness were not affected by the corn syrup replacement. The adhesiveness was significantly decreased in the 80% corn syrup replacement samples, which is contradictory to what was expected to occur and what happened in section 4.2.1.2 with the caramels where GGS caused increased adhesiveness. This difference is likely due to the difficult nature of measuring the adhesiveness of food matrices and is likely not an accurate representation of the overall stickiness of the gummy. In addition, when gummies are produced commercially, they are coated in wax or oil, therefore making any differences in adhesiveness negligible. The resiliency was also increased significantly in the corn syrup replacement gummies and is likely due to the interactions between the smaller sugar molecule and the gelatin matrix and in turn creating a more resilient gummy.

Table 4.12 Texture profile analysis of gummies with partial molar replacement of corn syrup by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Hardness (g)	Adhesiveness (g*sec)	Springiness	Cohesiveness	Resiliency
Control	8700 ± 900^{a}	300 ± 100^{a}	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}	0.053 ± 0.002^{a}
40% Corn Syrup Replacement	4700 ± 900^{b}	500 ± 200^{b}	0.6 ± 0.1^{b}	0.4 ± 0.1^{a}	0.043 ± 0.003^{b}
80% Corn Syrup Replacement	1400 ± 200^{c}	600 ± 200^{c}	0.9 ± 0.1^{c}	0.9 ± 0.2^{b}	0.037 ± 0.005^{c}

[1] Values with the same letter are not significantly different at $\alpha<0.05$

Table 4.13 Texture profile analysis of gummies with partial molar replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Hardness (g)	Adhesiveness (g*sec)	Springiness	Cohesiveness	Resiliency
Control	1300 ± 200[a]	250 ± 50[a]	0.96 ± 0.009[a]	0.99 ± 0.01[a]	0.61 ± 0.02[a]
40% Sucrose Replacement	1200 ± 200[a]	250 ± 40[a]	0.96 ± 0.007[a]	0.99 ± 0.01[ab]	0.61 ± 0.02[a]
80% Sucrose Replacement	1300 ± 200[a]	240 ± 60[a]	0.96 ± 0.009[a]	0.98 ± 0.01[b]	0.62 ± 0.01[a]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

Sucrose replacement gummies had no significant textural differences compared to the control outside of cohesiveness, which significantly decreased with 80% sucrose replacement. This could be due to the interruption of the gel matrix by smaller sweetener molecules restricting the cohesive properties of the gel.

In summary, gummies with GGS as a corn syrup and sucrose partial replacement exhibited very similar textural characteristics. The differences that did exist in the textural properties are either disrupted by normal oiling of the market gummies or are too small to have a detectable difference.

4.2.2.3 Crystallization

The formation of sugar crystals in gummy candies is an undesirable phenomenon that causes changes in textural characteristics and can indicate the end of shelf life. Crystallization is dependent on many factors including the supersaturation, the propensity for specific molecules to crystalize, and the presence of inhibitors (Hartel et al., 2018). Doctoring agents are commonly used to prevent crystallization; common doctoring agents include corn syrups and invert sugar. Doctoring agents work mainly by limiting the mobility of sugar molecules and by physio-chemical interactions with crystallizing molecules. In standard formulations in which crystallization occurs, it is commonly sucrose that is crystallizing (Hartel et al., 2018). During preliminary studies, it was

found that GGS commonly crystallized after just a couple of weeks whereas sucrose solutions at the same concentration did not, thus GGS had a higher propensity for crystallization. Therefore, it is likely the crystallization occurring in the following gummies is attributed to the GGS, with galactose or lactose crystallizing due to their lower solubilities than glucose. Studies show that at hydrolysis levels of 70% and lower, lactose crystallizes, whereas higher hydrolysis levels led to the crystallization of galactose (Zadow, 1992). Identification of the crystals as lactose or galactose would require chromatography, which was not in the scope of this research (Shah & Nickerson, 1978).

The partial replacement of corn syrup by GGS at 40% and 80% and the replacement of sucrose by GGS at 80%, led to a significant decrease in the shelf life, or days until crystallization occurred, as seen for corn syrup and sucrose replacement gummies, respectively (Table 4.14 and 4.15).

Table 4.14 Period of days until crystallization occurred in gummies with partial replacement of corn syrup by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Days to Crystallization
Control	>150[a]
40% Corn Syrup Replacement	103 ± 21[b]
80% Corn Syrup Replacement	41 ± 7[c]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

After 150 days of storage, gummies with 40% and 80% corn syrup replacement and 80% sucrose replacement had undergone crystallization. Gummies made with corn syrup replacement all crystallized, which is likely due to the decrease in doctoring agents and the increased molecular mobility. As the replacement of corn syrup was increased, the gummies crystallized faster due to

the removal of the kinetic barrier. In addition, the increased galactose and lactose concentration increased the supersaturation, the driving force for crystallization.

Table 4.15 Period of days until crystallization occurred in gummies with partial replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=8)

Formulation	Days to Crystallization
Control	>150[a]
40% Sucrose Replacement	>150[a]
80% Sucrose Replacement	60 ± 9[b]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

It was unexpected for gummies with sucrose replacement to crystallize as there was still a higher level of doctoring agents present in the product; however, this crystallization was likely driven by the inherent propensity for GGS to crystallize. In the case of 80% sucrose replacement with GGS, the kinetic barriers created by doctoring agents were not enough to prevent the galactose or lactose molecules from crystallizing due to high level of supersaturation of galactose and lactose.

4.2.2.4 Summary

In conclusion, gummies made with partial replacement of corn syrup and sucrose by GGS showed several important differences from control formulas in which no GGS was used. While the texture profiles and water activity of the gummies were not meaningfully affected, crystallization was greatly influenced by GGS.

While upon initial examination of the initial attributes of the gummies before storage, GGS stood as an excellent replacement for both corn syrup and sucrose, the decreased shelf life due to premature crystallization makes GGS an unrealistic replacement for corn syrup at 40 and 80% and

sucrose at 80%. However, at 40% replacement, GGS performs nearly identically to the control with no significant differences in water activity, texture or crystallization.

4.2.3 High Ratio Cake

This experiment examined the viability of GGS to behave as a partial replacement for sucrose in a high ratio cake. Within the experiment, two different levels of replacement were evaluated, 40 and 80% molar replacement of sucrose with GGS. The experiment consisted of a four-block design in which two replicates of a control, 40% replacement and an 80% replacement formulation were produced and tested. Cakes were compared with the consideration of the block effect.

All cakes were formulated to have an initial batter with the same macro nutrient composition and were baked in the same oven for the same amount of time to ensure continuity between samples. The initial batters contained 27.4% water, 53.7% carbohydrates, 35.1% added sugar, 12.2% lipids, 4.8% protein and 1.9% ash, with the sweeteners and water adjusted proportionally while keeping the other components consistent.

4.2.3.1 Batter Properties

Directly after the batter was made, samples were taken to measure the specific gravity and yield stress. Specific gravity is the ratio of specified volume of batter to the weight of the same volume of water and provides insight into how much air has been incorporated into a batter (Yazici & Ozer, 2021). In a high ratio cake, where egg whites are initially whipped in the presence of sugars, a low specific gravity is favorable where more air has been incorporated to create a lighter, airier final cake. Yield stress and other rheological properties have been found to correlate with the volume of the final cake (Christaki et al., 2017). The batter properties of the cakes can be found below in Table 4.16.

Table 4.16 Batter properties, specific gravity, and yield stress of high ratio cake with partial molar replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=6)

Formulation	Specific Gravity	Yield Stress (Pa)
Control	0.67 ± 0.03^{a}	19 ± 6^{a}
40% Sucrose Replacement	0.65 ± 0.03^{a}	19 ± 4^{a}
80% Sucrose Replacement	0.56 ± 0.04^{b}	32 ± 3^{b}

[1] Values in the same column with the same letter are not significantly different at $\alpha<0.05$

The partial replacement of sucrose by GGS led to a significant decrease in specific gravity of the cake batter. When sugars are added to egg whites, they increase the viscosity of the continuous phase. As found in section 4.1.1, sucrose increases viscosity more significantly than GGS. An increase in viscosity will allow for the egg white foam to form smaller more stable air bubbles (Lekjing et al., 2022). In addition, the increase of sugars decrease the ability for rapid diffusion of protein molecules at the gas-liquid interface, and therefore decreases the foam capacity (Sun et al., 2022). When GGS is increased, the viscosity is subsequently decreased, allowing for the formation of larger and more numerous air bubbles. Thus, consequently the specific gravity decreases as more large air bubbles are formed.

The incorporation of air and viscoelastic properties of egg white foam are dependent on the rapid diffusion and unfolding of proteins at the interface of ovalbumin (Yang & Foegeding, 2010). However, due to hydrogen bonding between the proteins and sugar moles, ovalbumin molecules remain in the bulk phase rather than supporting the interface. Therefore, the protein air interface is better maintained and supported when less protein molecules are interacting with sugar molecules through hydrogen bonding. GGS has a lower hydrogen bond density than sucrose, which leads to lower rates of hydrogen bonding with the proteins (Woodbury et al., 2023). At

higher sucrose replacement, a higher proportion of proteins can support the foam interface and incorporate more air. The increase of proteins at the foam interface in turn increases the surface concentration leading to viscoelastic behavior (Lexis & Willenbacher, 2014). The increase of interfacial elasticity has been found to increase yield stress, which in turn explains why batter with higher levels of replacement exhibit higher yield stresses.

4.2.3.2 Cake Properties

The cake batter formulations were portioned and baked into mini cupcakes. The mini cupcakes were then evaluated for their textural properties, color profile and volume.

4.2.3.2.1 Textural Properties

The texture of the baked mini cupcakes was evaluated by performing a texture profile analysis (TPA) test in which the hardness, springiness, cohesiveness, and resiliency were measured. TPA tests were conducted on the day of bake and then on days 1, 4 and 7 to document the changes in properties across a one-week shelf life. The textural attributes of the formulations can be found below in Table 4.17.

Sucrose influences the timing of starch gelatinization and protein denaturation during baking, which are critical for setting the cake's structure (Godefroidt et al., 2023). Increased replacement of sucrose causes a change in starch gelatinization and protein denaturation timing, therefore affecting the final textural properties of the cakes.

For all cakes, hardness at day 0 was significantly lower than day 1 and days 1, 4 and 7 had no significant differences in hardness. The hardness of the cupcakes decreased significantly at the high level of sucrose replacement, but no significant difference was detectable at the low level of replacement. This trend held true across all days of storage. Decreased hardness is likely a result of disruption of starch gelatinization and protein denaturation with higher sucrose replacement.

Table 4.17 Hardness, resilience, springiness and cohesiveness of high ratio mini cupcakes with partial replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=6)

	Formulation	Day 0	Day 1	Day 4	Day 7
Hardness (g)	**Control**	5400 ± 1300^{Aa}	7700 ± 1600^{Ab}	7600 ± 1000^{Ab}	8900 ± 1900^{Ab}
	40% Sucrose Replacement	4400 ± 1100^{Aba}	6400 ± 1000^{Ab}	7400 ± 1300^{Ab}	7900 ± 800^{Ab}
	80% Sucrose Replacement	3200 ± 900^{Ba}	4100 ± 1000^{Bab}	5200 ± 1100^{Bb}	5000 ± 900^{Bb}
Resilience	**Control**	0.21 ± 0.02^{Aa}	0.21 ± 0.01^{Aab}	0.19 ± 0.01^{Aab}	0.19 ± 0.01^{Ab}
	40% Sucrose Replacement	0.23 ± 0.02^{Aa}	0.21 ± 0.01^{Aab}	0.19 ± 0.01^{Ab}	0.19 ± 0.01^{Ab}
	80% Sucrose Replacement	0.24 ± 0.01^{Aa}	0.22 ± 0.01^{Ba}	0.19 ± 0.01^{Ab}	0.18 ± 0.01^{Ab}
Springiness	**Control**	0.79 ± 0.02^{Aa}	0.76 ± 0.02^{Ab}	0.75 ± 0.02^{Ab}	0.76 ± 0.01^{Ab}
	40% Sucrose Replacement	0.82 ± 0.02^{Ba}	0.79 ± 0.02^{Bb}	0.76 ± 0.02^{Bc}	0.76 ± 0.02^{Ac}
	80% Sucrose Replacement	0.85 ± 0.02^{Ca}	0.84 ± 0.01^{Ca}	0.81 ± 0.02^{Bb}	0.80 ± 0.02^{Bb}
Cohesiveness	**Control**	0.58 ± 0.04^{Aa}	0.56 ± 0.03^{Aab}	0.54 ± 0.02^{Abc}	0.52 ± 0.04^{Ac}
	40% Sucrose Replacement	0.60 ± 0.02^{Aa}	0.57 ± 0.01^{Aa}	0.52 ± 0.01^{Ab}	0.52 ± 0.02^{Ab}
	80% Sucrose Replacement	0.60 ± 0.02^{Aa}	0.57 ± 0.02^{Aa}	0.49 ± 0.02^{Bb}	0.48 ± 0.02^{Bb}

[1] Values in the same column with the same letter are not significantly different at $\alpha<0.05$
[A, B, C] Indicate differences within a textural attribute and day of samples with different sweeteners
[a, b, c] Indicate differences within a textural attribute and sweetener level of samples across different days

The resilience of samples was not different between replacement levels on any day other than 80% replacement on day 1 in which the formulation exhibited higher resilience. Across the shelf life, the control was able to retain its resilience the best and only significantly decreased on day 7 whereas both replacement levels had a significant decrease starting on day 4. The higher

resilience of the 80% replacement on day 1 might be due to increased hygroscopicity of the GGS, temporarily maintaining moisture. However, the significant decrease in resilience for replacement cakes from day 4 suggests that the GGS is unable to maintain long-term structural stability. GGS caused changes in starch gelatinization and protein denaturation timing, which disrupted formation of the cake structure.

Springiness was significantly higher for replacement samples on all days, except for day 7 on which only the 80% replacement had higher springiness. Within the same treatment, springiness decreased significantly first for the control and 40% replacement on day 1 and not until day 4 for 80% replacement. Overall, increased replacement of sucrose lead to increased springiness and better retention of springiness over time. This retention of springiness is likely due to higher protein denaturation occurring in the presence of GGS. Cakes undergo the highest rate of protein denaturation at baking, but over time protein denaturation continues at a much slower rate (Singh et al., 2019). The molecules of GGS disrupt protein denaturation less than those of sucrose. As a result, the protein structure in cakes were better preserved over time, leading to improved retention of springiness.

Cohesiveness was not significantly different between replacement levels up to day 4. On and after day 4, 80% replacement had significantly lower cohesiveness than the control and 40% replacement. All formulations significantly decreased in cohesiveness on day 4 and only the control significantly decreased again on day 7. Overall, the control decreased in cohesiveness more gradually than either replacement level, and had an overall significantly smaller decrease in cohesiveness than the 80% replacement formulation. This is likely also due to the improper setting of the cake texture due to changes in timing of starch and protein biopolymer transitions during baking.

In summary, cakes with higher levels of sucrose replacement exhibited lower levels of hardness and no difference in resiliency across the whole shelf life. Cakes with increased sucrose replacement exhibited increased springiness across the entire shelf life and decreased cohesiveness after 4 days of storage. Increasing the levels of sucrose replacement with GGS led to differences in the timing of starch gelatinization and protein denaturation during the baking process. As a result starch gelatinization and protein denaturation occur at different moments during the baking process resulting in differences in cake structure and crumb texture (Godefroidt et al., 2023).

4.2.3.2.2 Colorimetry

When the cakes are baked, Maillard browning occurs, in which reducing sugars react with free amino groups to product dark pigments and flavor compounds. While water is required for the Maillard reaction, too much water retards the reaction and therefore the crusts of baked goods experience increased Maillard browning due to the decreased water concentration and the increased exposure to heat (Vanin et al., 2009). The color of the cakes were measured using a colorimeter where higher L* values indicate lighter color and more positive a* values indicate higher redness compared to blueness. Therefore, higher L* and less positive a* values would indicate less brown pigment formation and less Maillard reaction. The color of the cake crusts and interiors are found in Table 4.18.

Replacement of sucrose by GGS caused a significant decrease in L* and an increase in a* in both the outside crust and the inside of the cakes. The amount of reducing sugars was increased by GGS and therefore led to an increase in Maillard browning and the formation of dark brown pigments.

Table 4.18 Color parameters (L*, a*) of cake crust and cake interior produced with sucrose replacement by glucose galactose syrup (GGS) 1h post bake. Average values of measurements are reported with standard deviations (±) among replicates (n=6)

	Outside Crust		Inside	
Formulation	**L***	**a***	**L***	**a***
Control	70 ± 3[a]	12 ± 3[a]	87 ± 1[a]	0.2 ± 0.2[a]
40% Sucrose Replacement	54 ± 3[b]	16 ± 1[b]	83 ± 2.[b]	3.7 ± 0.6[b]
80% Sucrose Replacement	54 ± 3[b]	17 ± 1[b]	78 ± 3[c]	4.8 ± 0.5[c]

[1] Values with the same letter are not significantly different at $\alpha<0.05$

The increased Maillard browning with GGS is due to its composition of entirely reducing sugars. While some Maillard browning can be desired, excessive browning can lead to undesirable off flavors and appearance, as well as increased production of toxic compounds such as acrylamide (Vanin et al., 2009).

4.2.3.2.3 Volumetric Properties

The volumetric properties of the mini cupcakes were measured 1h and 24h post bake; these measurements were then utilized to estimate the volume of the cupcakes through use of the truncated cone volume equation. The volumes were used to calculate the amount of shrinkage that occurred over the 24h. The volumetric properties of the cakes can be found in Table 4.19.

At high levels of sucrose replacement, cakes had increased volumes at 1h and 24h post bake. This is caused by the increased air in the batter, as seen by the lower specific gravity (Table 4.16), which expands during baking and allows for a greater rise in the cakes. There was no

significant difference found in the shrinkage between the cake formulations. This is mainly due to high variation in the shrinkage within each formulation.

Table 4.19 Volumes at 1h and 24h post bake, and relative shrinkage of cakes produced with molar replacement of sucrose by glucose galactose syrup (GGS). Average values of measurements are reported with standard deviations (±) among replicates (n=6)

Formulation	Volume 1 hour Post Bake (cm^3)	Volume 24 hours Post Bake (cm^3)	Shrinkage (%)
Control	29.3 ± 0.9^{a}	25 ± 1^{a}	15 ± 4^{a}
40% Sucrose Replacement	32 ± 2^{a}	26 ± 3^{ab}	20 ± 7^{a}
80% Sucrose Replacement	37 ± 3^{b}	29 ± 5^{b}	22 ± 7^{a}

[1] Values with the same letter are not significantly different at $\alpha<0.05$

4.2.3.3 Summary

In conclusion, high ratio cakes made with partial replacement of sucrose by GGS showed several important differences from control formulas in which no GGS was used. The addition of GGS increased the foamability of the egg white foam by increasing the protein concentration at the liquid/air interface, which in turn, decreased the specific gravity and increased the yield stress of the batter and the volume of the cakes. Cake texture was significantly influenced by GGS, decreasing hardness and cohesiveness, and increasing springiness. The color of the cakes was significantly increased in both the outside and inside of cakes upon the addition of GGS.

5. Conclusions and Recommendations

Glucose galactose syrup (GGS) was evaluated for its physical and functional properties. The study found that GGS behaved as a Newtonian fluid and behaved more closely to sucrose solutions than corn syrup solutions, although GGS had lower apparent viscosities. In addition, it was found that at pH 5, GGS underwent browning at similar rates to corn syrup and significantly decreased rates compared to sucrose. Both freezing point depression and boiling point elevation were more greatly influenced by GGS than corn syrup or sucrose. GGS has a larger impact on colligative properties due to having a higher concentration by weight at the same molality as sucrose and corn syrup. The characterization of GGS allowed for better understanding of the basic functions of the syrup.

GGS was also evaluated for its use as a partial replacement for sucrose in caramels, gelatin gummies and cake, and corn syrup replacement in caramels and gelatin gummies. The study investigated how GGS functioned in food applications as a replacement for bulk sweeteners.

In caramels, there were significant differences observed between the control formulations and formulations with 40% and 80% molar replacement of sucrose and corn syrup. GGS was unable to perform as a viable replacement in caramels; however, the addition of GGS may be desirable to manipulate the characteristics of a caramel to create new and ideal products.

Gelatin gummies made with partial replacement of corn syrup and sucrose by GGS showed several differences from the controls. The texture profile and water activity of the gummies was highly consistent across all formulations; however, the rates of crystallization were significantly impacted. The study found that GGS was an unrealistic replacement for corn syrup at 40 and 80% and sucrose at 80% due to the increase in the onset of crystallization. However, at 40% replacement of sucrose, GGS performed as a viable partial replacement for sucrose in gelatin gummies.

In high ratio cake, the study found that GGS increased foamability and yield stress in the batter and significantly affected the baked cake formulations. A significant increase in color occurred in both 40% and 80% formulations, causing GGS to be a non-viable direct replacement, however further evaluation of the cakes through sensory evaluation would be necessary to draw further conclusions on the consumer acceptability.

During this research it was discovered that there is a unique relationship between glucose, galactose and lactose solubility. Future research should explore this interaction and the mechanisms behind the high rates of crystallization. This research would allow for the optimization of GGS and allow for greater application of the syrup. If further research is done to decrease the water content and increase the doctoring ability of GGS, the applications of the syrup would be greatly improved, both applications tested and in new applications.

Glucose galactose syrup should also be further investigated within different applications than those explored in this study. In applications such as sorbet and sodas, GGS may stand as a more viable replacement. Water content would be much higher in these products; therefore, crystallization would not be a concern and the initially high water content of the syrup would not have a large impact on the product.

GGS produced by the UW- Madison catalytic reactor should be evaluated for its physical and functional properties as done in this study because of differences in composition. Additional characterization of the sensory and volatile attributes should be investigated. The reactor GGS should be verified as a viable replacement in the applications study here and in additional applications. Due to certain constraints, simulated GGS was utilized in this research; however, this syrup still requires further characterization and testing to fully understand its properties and potential uses.

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