Chemical and Physical Stability of Powdered Tagatose as Affected by Temperature and Relative Humidity

by

Lenese D. Grant

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 13, 2010

Keywords: tagatose, prebiotic, deliquescence, stability

Approved by

Leonard N. Bell, Chair, Professor, Professor of Poultry Science
Oladiran Fasina, Associate Professor of Biosystems Engineering
Tung-Shi Huang, Associate Professor of Poultry Science

Abstract

Tagatose is a reduced-calorie monosaccharide that displays prebiotic properties. Water can interact with powdered tagatose to varying extents, depending upon the storage environment. Adsorbed water can impact the physical and chemical stability of tagatose, altering its functionality and usability as an ingredient. Therefore, the objective of this study was to evaluate the physical and chemical stability of bulk tagatose powder as a function of relative humidity (RH) and temperature. Saturated salt solutions were used to create environments having RH values of 33%, 54%, 75% and 85% at 20°C. Tagatose (0.3-0.5 g) was placed in vials and stored in desiccators (i.e., relative humidity chambers) at 20°C, 30°C and 40°C. Duplicate vials were removed at regular time intervals for 12 months. Moisture contents (MC) and physical characteristics were monitored monthly. Samples were dissolved in water and analyzed using HPLC to quantify tagatose degradation. Early stages of browning were measured at 280 nm, whereas brown pigment formation was measured at 420 nm. Critical relative humidity was determined at 20, 30 and 40°C. Using saturated tagatose solutions, the critical RH associated with deliquescence (RH_0) was 85% at 20°C. MC values below RH_0 were all less than 2% (db). The average MC at 85%RH ranged from 53-80% (db), increasing as temperature decreased. At 33%RH/20°C tagatose remained free flowing. As either temperature or RH increased, varying degrees of physical caking occurred. At 85%RH, tagatose deliquesced at all temperatures. Browning occurred in all samples at 40°C.

Despite physical caking and browning, tagatose degradation was only observed in the deliquesced sample at 85%RH/40°C, where a 20% loss occurred during the study. Although RH and temperature must become extreme for tagatose degradation to occur, intermediate RHs and temperatures promote physical caking and deliquescence, which create handling problems during product formulation. Tagatose should be stored in water impermeable packaging at 20°C rather than bulk storage bins in an uncontrolled environment.

Acknowledgments

I would like to thank God for allowing me to advance this far in my education. Sincere gratitude is expressed to my major advisor, Dr. Leonard Bell, for his patience, guidance and expertise. I appreciate the time, direction, and knowledge of my committee members, Drs. Oladiran Fasina and Tung-Shi Huang. Gratitude is extended to the advisors of the Bridge to the Doctorate program, Drs. Overton Jenda and Florence Holland. They continue to encourage me to reach my goals. I would like to thank my immediate family as well as my extended family and friends for their encouragement and motivation. Special thanks to David Grant (father), Fagale Grant (mother), David M. Grant (brother), Loretta Grant (sister), Hurdis Milner-Bozeman, Joyce Hall, Jesse Hall, Justin Vaughner, James Miller, Coretta Collins, Tamisha Jones, Ashley Bryant-Massey, Miniayah DeBruce, Shannon Coleman and Lauren Parker. Appreciation is expressed to my former lab mate Katie Luecke for her kindness and assistance with HPLC. To my uncle (Dr. James Shuford) who introduced me to food science and set an example of academic success, thank you. Although these two persons are no longer with me, they inspired me greatly; thank you Cora Shuford (grandmother) and Charlena Miller (aunt and kindergarten teacher). Lastly, I am grateful for funding from the National Science Foundation and the Alabama Agricultural Experiment Station as well as tagatose from Arla Food Ingredients.

Table of Contents

Abstract....................ii

Acknowledgments....................iv

List of Tables....................vii

List of Figures....................ix

Chapter 1. Introduction....................1

Chapter 2. Literature Review....................3

Water activity, moisture content and relative humidity....................3
Properties that affect water activity....................5
Chemical stability of food systems....................6
Physical stability of food systems....................8
Methods for measuring the critical relative humidity....................11
Deliquescence....................12
Tagatose properties and applications....................22
Tagatose as a prebiotic....................24
Tolerance of tagatose....................29
Metabolism of tagatose....................32
Tagatose in foods....................34
Stability of tagatose in solution....................37

Chapter 3. Materials and Methods....................41

Sample preparation....................41
Particle size characterization....................41
Preparation of saturated salt solution....................42
Experimental Overview....................43
Physical stability....................44
Moisture content....................45
Photographs....................45
Critical relative humidity determination....................46
Chemical degradation analysis....................46
Browning of tagatose....................48

pH..49

Chapter 4. Results and Discussion..50

Moisture sorption..50
Physical stability..53
Critical relative humidity..56
Tagatose browning..57
Chemical stability..63

Chapter 5. Summary and Conclusion..69

References..70

Appendix A..77

Appendix B..91

Appendix C..93

Appendix D..97

List of Tables

Table 3.1 Average particle size distribution of food grade tagatose as a percentage with standard deviation....42

Table 3.2. Saturated salt solution relative humidity as a function of temperature (Bell and Labuza 2000)....44

Table 4.1. Actual RH values associated with temperatures and the notation that will be used throughout this section....50

Table 4.2. Physical characteristics of tagatose as affected by RH and temperature after 12 months and time at which final physical state was observed....54

Table 4.3. Environmental RH in which tagatose deliquescence was observed and RH_0 of tagatose at 20, 30 and 40°C....57

Table 4.4. Pseudo-zero order rate constants (OD/g/d) with 95% CL times 10^4 for browning of tagatose at 20, 30 and 40°C....60

Table A1. Moisture content, physical appearance, optical density and tagatose remaining of samples stored over drierite at room temperature....78

Table A2. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 33% RH at 20°C....79

Table A3. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 33% RH at 30°C....80

Table A4. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 33% RH at 40°C....81

Table A5. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 54% RH at 20°C....82

Table A6. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 54% RH at 30°C....83

Table A7. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 54% RH at 40°C....84

Table A8. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 75% RH at 20°C..85

Table A9. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 75% RH at 30°C..86

Table A10. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 75% RH at 40°C..87

Table A11. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 85% RH at 20°C..88

Table A12. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 85% RH at 30°C..89

Table A13. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 85% RH at 40°C..90

Table B1. Duplicate pH values for tagatose at time 6, 9 and 12 months.........................92

List of Figures

Figure 2.1. Moisture sorption data of sucrose, citric acid anhydrous and 50/50 mix of both components at 25°C. The RH_0 is determined where the arrows meet. Adapted from Salameh and Taylor (2006b)..12

Figure 2.2. Depiction of deliquescence process. Adapted from Van Campen and others (1983)..13

Figure 4.1. Moisture adsorption profile of samples stored at 85% RH at 20, 30 and 40°C...52

Figure 4.2. Moisture sorption isotherm for tagatose at 20, 30 and 40°C after exposure for 12 months...52

Figure 4.3. Tagatose at 20°C after 12 months, left to right, 33, 54, 75 and 85% RH......54

Figure 4.4. Tagatose at 30°C after 12 months, left to right, 33, 54, 75 and 85% RH......55

Figure 4.5. Tagatose at 40°C after 12 months, left to right, 33, 54, 75 and 85% RH......55

Figure 4.6. Early stages of browning in tagatose at 40°C as affected by RH.................59

Figure 4.7. Brown pigment formation in tagatose at 40°C as affected by RH................60

Figure 4.8. Figure 4.8. The effect of time on browning of tagatose at 40°C, left to right, after 6 months, 9 months and 12 month. Tagatose at 33, 54, 75 and 85% RH top to bottom...61

Figure 4.9. Rate constants for tagatose browning (420 nm) as a function of %RH at 40ºC. 62

Figure 4.10. Chemical degradation of powdered tagatose at 40°C as affected by RH......64

Figure C1. Duplicate pictures of tagatose at time 6...94

Figure C2. Duplicate pictures of tagatose at time 9...95

Figure C3. Duplicate pictures of tagatose at time 12...96

Figure D1. Moisture content (db) vs. time for particles < 250 nm at 85% RH/20°C.....100

Figure D2. Moisture content (db) vs. time for particles > 500 nm at 85% RH/20°C.....100

Chapter 1: Introduction

The food industry utilizes a large number of food ingredients that come in bulk powdered forms. These ingredients may include flour, powdered supplements, confectionery sugar and many other items. There are many advantages to using dry ingredients, such as a longer shelf life due to a lower inherent water activity than liquid ingredients. However, what happens when the environmental storage conditions become unfavorable for powdered ingredients? This is likely to occur in the confectionery industry and bakeries where thermal processes may cause an increase in the temperature of the environment. These food manufacturing facilities often do not control their environmental conditions due to economical purposes. Therefore, precautions must be taken to prevent or limit the exposure of ingredients which are susceptible to caking and deliquescence (liquification) to extreme environmental conditions, such as elevated temperatures and relative humidities. More importantly, adverse physical changes, such as caking and deliquescence may increase the risk of chemical degradation, causing loss of beneficial properties. One powdered ingredient that may be susceptible to caking and deliquescence and subsequent chemical degradation is tagatose, because sugars are known to deliquesce (Hancock and Shamblin 1998; Salameh and Taylor 2006a)

Tagatose is a monosaccharide that has a structure similar to fructose, with the exception of the hydroxyl group that is inverted on carbon 4 (Levin 2002).
Studies have shown that tagatose displays prebiotic properties (Venema and others 2005; Laerke and others 2000). Prebiotics are important because they facilitate the formation of

beneficial bacteria in the intestines. In addition to its prebiotic properties, tagatose is approximately 92% as sweet as sucrose, but has a lower caloric value (Levin 2002). Therefore, it may have many applications in foods as a nutritional additive or sweetener. However, limited research has been conducted that addresses how bulk powdered tagatose reacts to various environmental conditions that could be encountered in an industrial setting. Due to the versatility and novelty of tagatose, the effect of environmental storage conditions should be investigated. The acceptability of tagatose will be jeopardized if environmental storage conditions affect its storage stability. Therefore, the purpose of the research was to investigate how environmental conditions affect tagatose physical and chemical stability.

Chapter 2: Literature Review

Water plays a critical role in foods that can affect their palatability, shelf life, processing and storage conditions. Water activity, moisture content and relative humidity are related, as will be explained later. After extensive research, it has been established that water activity, in particular, significantly affects the microbial, physical and chemical stability of various foods. This section will focus primarily on the food's physical and chemical attributes.

Water Activity, Moisture Content and Relative Humidity

Water activity (a_w) may be defined in terms of vapor pressure, chemical potential and availability. One definition of water activity is "the ratio of the equilibrium partial vapor pressure of water in the system (p_w) to the equilibrium partial pressure ($p^o{}_w$) of pure liquid water at the same temperature" (Reid 2007). Water activity is also a thermodynamic property, based on the chemical potential of water in a food system. At equilibrium, the chemical potential of water in the food is equal to the chemical potential of the surrounding water in the atmosphere. Consequently, the vapor pressure of water in a food is equal to the vapor pressure of water vapor in the surroundings (Roos 2007). Due to its thermodynamic properties, a_w determines the direction of moisture movement until equilibrium is obtained; however, the equilibration rate is unknown (Bell 2007). Water activity may also be described as the energy status of water and its availability to

behave as a solvent and reactant, causing changes in chemical and physical stability (Chirife and Fontana 2007).

Water activity and relative humidity (RH) are quite similar terms, where %RH is equal to the product of a_w and 100. When the water in food has equilibrated with the surrounding environment, the percent relative humidity is termed the equilibrium relative humidity (ERH). The a_w can be determined by using the following equation: a_w = %ERH/100 (Bell and Labuza 2000).

Moisture content also has a dramatic effect on the stability of food. Moisture content is usually expressed on a dry basis as a percentage or *x* grams of water/ 100 g solids (food). Dry basis moisture content may be plotted against water activity creating a moisture sorption isotherm. A moisture sorption isotherm is an illustration that shows "the steady state amount of water held by the food solids as a function of water activity or storage % relative humidity at constant temperatures" (Labuza and Alrunakar 2007). Moisture adsorption isotherms are critical to understanding the stability of foods because they allow predictions to be made about reactions that will occur at specified moistures. Moreover, isotherms also allow modifications of a_w to be made with the selection of appropriate ingredients (Labuza and Alrunakar 2007). Although moisture content is important, it may be difficult to make predictions regarding stability since it encompasses all water, not just water that is available for the reaction. Therefore, a_w provides means for a more suitable prediction of chemical and physical stability as it relates to water behaving as a solvent and reactant (Bell 2007).

Properties that Affect Water Activity

The properties that will be discussed have a lowering effect on a_w. They include the capillary effects, colligative properties, and surface interactions. Foods, including powders, may contain capillaries, which are small pores and channels where water may exist. The curved liquid meniscus in the pores causes a lowering effect in the vapor pressure above this area compared to that of pure water. This is due to changes in hydrogen bonding directly related to the surface curvature. Increased water molecules are able to interact with each other on the curved capillary surface, which depresses the water activity. Foods generally contain pores ranging in radius from 0.1 to 300 µm, with most foods having pores between 10 µm and 300 µm (Farkas and Singh 1990; Xiong and others 1991; Hicsasmaz and Clayton 1992). Generally, larger capillaries do not have a pronounced effect on a_w; however, smaller capillaries (less than 0.1 µm) have a greater effect on lowering a_w (Bell and Labuza 2000).

The second factor that plays a role in a_w lowering is surface interaction. Surface interaction affects water activity because water interacts with other chemical molecules through hydrogen bonding, dipolar-ionic interactions, dipole-dipole forces, ionic bonding, and van der Waals interactions. Because of the interaction between water and other molecules, water molecules need additional energy to go from the liquid to vapor phase. Consequently, the water molecules are less able to move into the vapor phase, causing a depressed a_w. Another concept that is associated with surface interaction is the monolayer value. The monolayer value is the moisture content at which the food's surface contains a single layer of water molecules. The monolayer is significant because

it often correlates with optimum chemical stability for low moisture products (Bell and Labuza 2000).

The last property that affects water activity is the colligative property of vapor pressure lowering. Colligative properties include freezing point depression, boiling point elevation, vapor pressure lowering and osmotic pressure lowering. This section will focus primarily on vapor pressure lowering. As mentioned earlier, water activity is defined as the partial vapor pressure of water in the food divided by the vapor pressure of pure water at constant temperature. Therefore, vapor pressure is directly related to water activity. When a solute interacts with water through ionic, dipolar or hydrogen bonding, the escaping tendency of water as well as the chemical potential of water is reduced, resulting in decreased water activity. The water/solute ratio, molecular weight and degree of interaction also play a role in a_w lowering by solutes (Bell and Labuza 2000).

Chemical Stability of Food Systems

Stability is characterized by the "ability of a substance to resist change over a specific period of time" (Bell 2007). The length of storage time a product remains acceptable is referred to as the storage stability or shelf life of the product. Various environmental conditions, such as relative humidity and temperature, affect the chemical and physical stability of food products. Chemical instability may alter the nutritional value, color, and flavor of foods. In addition, changes in chemical stability may alter the pH or cause degradation of food products (Bell 2007).

Bell (2007) mentions a number of chemical reactions that alter the chemical stability of food products causing deterioration. Hydrolysis or cleavage with the addition of water is one example. This phenomenon occurs in diet carbonated beverages; due to

the acidic environment, aspartame hydrolysis occurs causing a loss of sweetness. Oxidation is another chemical change that may affect oils and vitamins causing changes in sensory characteristics, appearance and nutritional value. Chemical browning may occur due to enzymatic reactions, nonenzymatic reactions, or the Maillard reaction (Bell 2007). Browning primarily increases with increasing a_w. One browning reaction that is common in the food industry is caramelization. Caramelization is a nonenzymatic reaction that involves the breakdown of monosaccharides by heat, generating a caramel–like flavor and a brown pigment (Troller and Christian 1978). Sometimes these reactions may occur simultaneously resulting in increased deterioration of food quality and shelf life.

One factor that plays a large role in chemical stability is temperature. Chemical reactions may accelerate when temperatures are increased and decelerate when temperatures decrease (Bell 2007). For example, refrigeration is often used to prevent certain chemical reactions from occurring. Temperature has one of the most pronounced effects on chemical stability. The effect of temperature on chemical stability can be modeled by the Arrhenius equation or shelf life plots (Bell 2007).

Water activity and moisture content may also contribute to the chemical stability of foods. Water is required for the dissolving, diffusing and reacting of chemicals in foods. Moisture affects the viscosity of the reaction medium as well as reactant mobility; for example, a less viscous medium may increase degradation by reactants becoming more mobile. When water activity and moisture content continue to increase above the amount necessary for complete reactant dissolution, reactants become diluted, causing the reaction rate to decrease. Consequently, chemical reactivity and a_w typically have a

proportional relationship, increasing until maximum reactivity occurs, followed by a decrease in reactivity (Bell 2007). As mentioned previously, most reactions cease at the monolayer due to low reactant dissolution and restricted reactant mobility.

Physical Stability of Food Powders

Like chemical stability, physical stability is also impacted by water activity, moisture content, temperature and time. The glass transition temperature is one factor that impacts physical stability. The glass transition is defined as "a relaxation process occurring in food solids during transformation of noncrystalline solids to a more liquid-like supercooled state" (Roos 2007). More simply stated, glass transition is the change of an amorphous material from a glassy state to a rubbery state. Water can behave as a plasticizer, which allows for better movement of components in the material (Roudaut 2007). Plasticization can cause amorphous glassy foods to convert into the amorphous rubbery state. A significant decrease in viscosity and increase in molecular mobility also occurs (Bell 2007). These phase conversions are important in understanding the physical stability of foods because changes in temperature or water activity can change the physical state of the product, thus altering its physical stability.

For powders, physical stability at the macroscopic level may be described in terms of caking, stickiness, collapse and deliquescence (liquification of a solid due to exposure at very high RH). While there are other occurrences, such as fat melting, surface crystals solubilization and electrostatic attraction that may contribute to caking, this literature review will focus primarily on moisture-induced caking. Caking occurs when individual particles of a free flowing powder stick to each other causing a larger mass to be formed (Roudaut 2007). Moreover, caking may also be described as the

conversion of free flowing powder to a substance with aggregates and lumps, causing difficulty in mechanical handling (Salameh and Taylor 2006a). Stickiness is defined in terms of the sticky point of the substance, which is "the temperature at which the power needed to stir the powder in a tube increases sharply" (Roudaut 2007). Maintaining a free flowing product is important for numerous reasons including, functionality, storage, formulation and mixing (Adhikari 2001).

Caking occurs through a variety of mechanisms including the following: 1) formation of liquid bridges, which may form solid bridges, 2) electrostatic or van der Waals forces, 3) mechanical shape interlocking, and 4) particle surface sintering (Adhikari and others 2001; Salameh and Taylor 2006a). Foods that typically experience caking and structural collapse are powdered products with elevated amounts of minerals, soluble sugars or protein hydrolysates. The extent of caking varies from easily breakable aggregates to extremely hard lumps. The amount of caking also varies from a small lump to the entire powder being caked. As a result of physical changes in powders, physical properties such as flowability, water dispersibility, handling properties, appearance and solubility are adversely affected (Roudaut 2007).

Moisture-induced caking may occur as a result of drying or storage. Powders may become hydrated through accidental wetting, water sorption, or moisture condensation, which causes the particle surface to become plasticized allowing contact with neighboring particles through interparticle liquid bridging. Water may contact particle surfaces through capillary condensation or adsorbed mono/multilayers (Adhikari and others 2001). Caking occurs in several stages causing a free flowing powder to eventually turn into a solid mass. The beginning stages of caking occur when fine

particles develop into larger particles through agglomeration. The initial stages of caking may be desirable because handling and wetability may improve. As the powder becomes more hydrated, the particle bridges thicken and porosity decreases, causing the powder to become compact and collapse. Caking may be quantified by using a caking index, which corresponds to the percentage of sample retained by a specified mesh (Roudaut 2007). The caking kinetics of fish hydrolysates were investigated by storing the sample at humidities of 0.75, 0.63, 0.53 and 0.44. The higher the humidity or temperature was, the higher the caking index (Aguilera and others 1995).

A fundamental property that influences caking is glass transition, which is the temperature at which change from a glassy phase to a rubbery phase takes place. Lowering the glass transition temperature below ambient temperatures by plasticization of amorphous food powders produces optimum conditions for caking and sticking (Adhikari and others 2001). Although sugars appear to be in crystalline form at room temperature, they are usually labeled amorphous due to the effect of size reduction operations (Kelley and others 1974). In the anhydrous state, amorphous sugars have glass transition temperatures between 5-100 °C, depending on the type, that may be further lowered due to water plasticization. Sugars with low molecular weight are more susceptible to caking and stickiness because the glass transition temperature decreases with decreasing molecular weight. Moreover, if the glass transition temperature of a powder is below the storage temperature, caking, stickiness and agglomeration are sure to occur (Adhikari and others 2001).

Particle size also has a dramatic effect on caking mechanisms. Powders with a particle size greater than 200 µm are generally free flowing, while smaller particles may

experience cohesion and their flowability significantly affected. Cohesion is an internal property of a powder and a measure of the force binding two particles together. Smaller particles (less than or equal to 1 μm) are subject to electrostatic or molecular forces which may significantly deform the particles creating a larger contact area (Adhikari and others 2001). The larger surface area promotes faster moisture adsorption, which may lead to extensive caking and deliquescence.

Methods for Measuring the Critical Relative Humidity

In order to understand deliquescence or a solid liquefying at elevated RH levels, the concept of critical relative humidity must first be addressed. The critical relative humidity, RH_0, is the humidity at which deliquescence begins. Critical relative humidity is material specific and often times temperature sensitive. Two methods are generally used to determine the critical relative humidity (RH_0) of a material. The first method involves measuring the water activity of a saturated solution of the material using a water activity meter. In theory, when deliquescence takes place, the solid particles are surrounded by a saturated solution at the critical relative humidity. When this method is used, the equilibrium water activity of the saturated solution may be referred to as RH_0. The other method that may be used to determine RH_0 involves moisture sorption data. A series of saturated salt solutions, with increasing a_w, are used to get a representative number of moisture sorption data points at constant temperature. The samples are weighed at various time points to ensure equilibration and monitor weight gain (or moisture uptake). Following equilibration, a graph is generated with moisture content (db) or change in weight as a function of water activity. The steep increase in weight change is where the deliquescence occurs (Mauer and Taylor 2010). The linear portions

before and after the deliquescence are extrapolated to obtain the RH_0 at the intersection. Figure 2.1 shows moisture sorption data used to obtain RH_0 (Salameh and Taylor 2006b). When obtaining RH_0, these two methods usually agree (Salameh and Taylor 2006b).

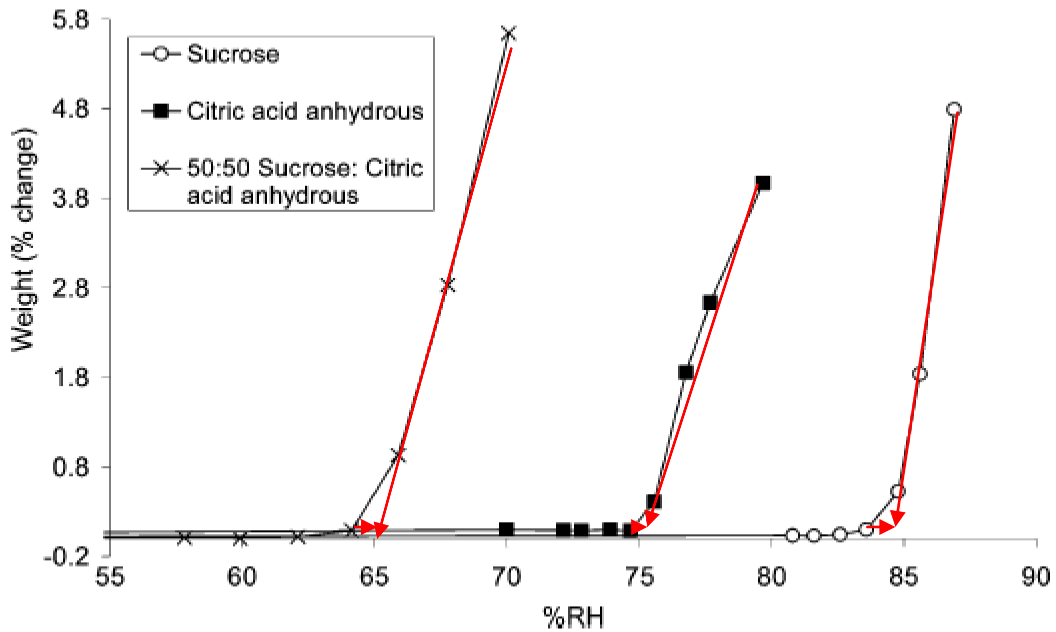


Figure 2.1. Moisture sorption data of sucrose, citric acid anhydrous and 50/50 mix of both components at 25°C. The RH_0 is determined where the arrows meet. Adapted from Salameh and Taylor (2006b).

<u>Deliquescence</u>

Although extensive amounts of work have been conducted on various aspects of water activity, few studies have correlated water activity or caking with the phenomenon of deliquescence. Deliquescence is a "water solid interaction that is known to cause vapor condensation in highly water-soluble and highly crystalline solids at water activities less than 1 or relative humidities less than 100%" (Salameh and Taylor 2006a). In other

words, deliquescence is the dissolution of a material due to water adsorption at high water activities. Figure 2.2 shows a schematic of the deliquescence process. When the environmental RH is less than RH_0, the powder remains in solid form. As the environmental RH increases, water condenses around the solid. When the RH_0 exceeds the environmental RH, complete dissolution or deliquescence of the solid occurs. Caked powders that continue to absorb water may eventually undergo deliquescence.

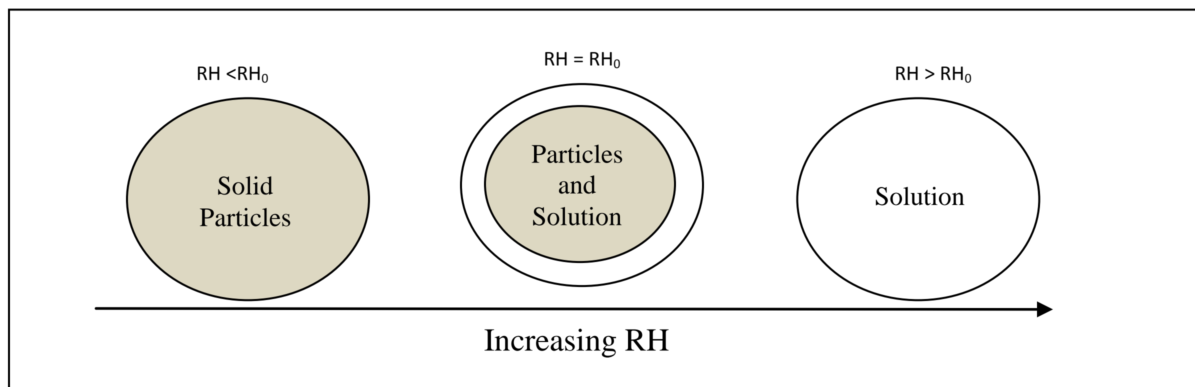


Figure 2.2. Depiction of deliquescence process. Adapted from Van Campen and others (1983).

Liquid bridges contribute to deliquescence and may be created by two mechanisms. Liquid bridges are formed when neighboring particles interact with another surface that has come into contact with water. In the first mechanism, elevated RH values allow increased water vapor sorption, causing increased surface wetting and plasticization of the solid regions of the powder. The second mechanism is also caused by a high RH, which allows deliquescence at the surface of the solid and development of a concentrated thin film of solution at the particle surface (Salameh and Taylor 2006b).

Even though certain powders are deliquescent, when they are held at low RHs, they absorb small amount of water through hydrogen bonding. However, when the critical RH (RH_0) is exceeded, the solid dissolves in the condensate film to produce a saturated solution (Salameh and Taylor 2006a). The RH_0 is material and temperature specific and is the RH at which deliquescence is initially observed (Hancock and Shamblin 1998). As the RH continues to increase, more solid dissolution occurs due to vapor condensation, ultimately leading to complete deliquescence (Salameh and Taylor 2006a). Hancock and Shamblin (1998) reported that crystalline sugars and sugar derivatives may experience deliquescence at RHs higher than 65%. Deliquescence may accelerate chemical reactions and physical changes in powdered systems (Hancock and Shamblin 1998).

Hiatt and others (2008) conducted a study examining the chemical stability of vitamins B_1, B_6, and C in powdered blends. Exploring powdered blends is important because deliquescence lowering occurs, meaning the critical RH of the mixture (RH_{0mix}) is lower than RH_0 of the individual components, so deliquescence occurs more readily. Consequently, this may also increase chemical reactivity. The study explored single, binary, ternary and quaternary combinations of thiamin HCl, fructose, sodium ascorbate, and pyridoxine. Environmental chambers and salt solutions were used to create RHs of 43%, 54%, 59%, 64%, 85% and 98%. Powders at the various RHs were stored for 1, 2, 4, 8, and 12 weeks. Critical relative humidities were determined using moisture sorption isotherms and saturated salt solutions, and powders were stored above and below these RHs. Physical characteristics and moisture contents were monitored. Chemical

degradation was determined using high-performance liquid chromatography (HPLC) (Hiatt and others 2008).

Following 12 weeks at 98% RH, thiamin HCl was fully deliquesced and sodium ascorbate deliquesced to a dark brown solution with some precipitate. Both powders experienced some caking at 54% RH. Pyridoxine did not experience deliquescence even when stored at 94% RH whereas fructose deliquesced at 64% RH. The mixtures deliquesced at RHs below the RH_0 values of the separate powders. Thiamin and ascorbate acid experienced the greatest degradation above the $RH_{0,}$ while pyridoxine experienced minimum degradation (Hiatt and others 2008).

Deliquescence lowering in binary mixtures has also been observed in pharmaceutical ingredients (Salameh and Taylor 2005). Salameh and Taylor (2006a) looked at caked-induced deliquescence in various single and binary mixtures of different particle sizes (ground and unground). Caked-induced deliquescence can be attributed to the formation of liquid bridges that occur due to partial deliquescence. Following partial deliquescence, recrystallization can occur that causes solid bridge formation and caking. Citric acid, fructose and glucose were stored at RHs below and above their critical relative humidities as single components and as binary mixtures. Relative humidities of 33%, 54%, 65%, 71%, 81%, 85%, and 94% were created using saturated salt solutions and designated powders were exposed to each condition. Various cycles were used to expose samples to selected RHs, with one cycle storing the sample above the RH_{0mix} for 3 days and below the RH_{0mix} for 3 days. A total of three different blends of ground and unground samples were used and they included the following: 50:50 ratio of unground

compounds, 50:50 ratio of ground compounds, and a 50:50 ratio of unground and ground compounds. Cake mechanical strength was tested using a three point beam-bending method. Scanning electron microscopy was used to observe deliquescence-induced caking. Critical relative humidities were established through vapor sorption profiles and using a water activity meter (Salameh and Taylor 2006a).

Like the previous study discussed, mixed powders (fructose and citric acid) began deliquescing at a lower RH (RH_{0mix}) than the critical relative humidities of either powder. A considerable amount of caking was observed in both single and binary powdered systems. The control mixtures that were stored below the RH_{0mix} remained free flowing and free of caking. Single powder controls that were stored above and below $RH_{0mix,}$ but not above $RH_{0,}$, formed fragile cakes. The unground and ground particles had minimum differences in vapor sorption below the RH_0 where deliquescence occurs. The physical mixtures that experienced caking were cycled at an RH above and below RH_{0mix}, but not above RH_0 of individual components. Cycling was identified as a key factor in cake formation. The ground citric acid/glucose mixture experienced increased caking. This phenomenon could be possibly explained by smaller particle sizes (sucrose) having higher water vapor sorption, which results in an increased tendency to cake (Roge and Mathlouthi 2003; Mathlouthi and Roge 2003). Due to the reduction in particle size, there is a larger surface area which leads to more surface water adsorption. Another possible mechanism is an increased number of liquid bridges formed, following by recrystallization and formation of solid bridges which strengthen caking. Like the previous study, the RH_{0mix} was an indicator of stability, most importantly physical stability (Salameh and Taylor 2006a).

The previous study focused on deliquescence in relation to physical stability. Salameh and Taylor (2006b) also studied how deliquescence affects chemical stability. Similar methods were used as stated in the previous section to determine critical relative humidities of crystalline sucrose, citric acid, β-D-fructose and α-glucose monohydrate. Saturated salt solutions were used to create RHs of 43, 54, 65, 71, 75 and 85%. A 50:50 ratio of sucrose and citric acid was placed in 20-mL vials and stored at the previously mentioned RHs. Sucrose/citric acid solutions at concentrations of 10% w/v and 33% w/v were utilized to study sucrose inversion kinetics. Sucrose served as a control and was stored at 71 and 85% RH. Polarimetry was used to measure sucrose inversion kinetics. Deliquescence was also visually examined using a microscope. Moisture was monitored by preparing sucrose samples and mixtures of sucrose and citric acid, and placing them at all RHs, and at various time points weighing them (Salameh and Taylor 2006b).

The results from the study revealed that all RH_0 values obtained using single powders by the moisture sorption data extrapolating method were close to the equilibrium water activities of saturated solutions obtained from the water activity meter. The sucrose inversion reaction occurred more rapidly in solution than in powdered form. For example, the citric acid/sucrose powder at 85% RH took 24 d for complete hydrolysis compared to 9 d in the 33% w/v solution. The visual microscopy study provided additional evidence that deliquescence took place in the 72% RH mixture of citric acid and sucrose. Samples that were stored at 65% RH or above were completely deliquesced, and sucrose was completely inverted. The moisture study revealed that as time increased, so did moisture content in sucrose/citric acid mixtures stored at 65% RH and above.

Conversely, control samples and mixtures stored below 65% RH had a moisture content of less than 0.04% w/w (Salameh and Taylor 2006b).

Salameh and others (2006) conducted a study to investigate deliquescence lowering in food ingredients while comparing two different methods of measuring RH_0 (water activity of equilibrated saturated solutions vs. moisture sorption isotherm). The food ingredients included the following: sodium citrate tribasic dehydrate, sodium ascorbate, citric acid anhydrous and crystalline sugars with the exception of lactose (Salameh and others 2006).

The dynamic moisture sorption method required samples that ranged from 20-25 mg in weight. The samples were prepared by geometrically mixing the individual ingredients. Geometrically mixing involves mixing the minor component with the major component to achieve a 50/50% w/w premix. Next, an equal amount of the remaining major component is added and mixed to the premix. This method is replicated until the entire quantity of the major component is utilized and the final composition is obtained. Samples containing the hydrate form were not dried in the sorption analyzer. However, samples that contained anhydrous components were dried at 50°C in the sorption analyzer prior to conducting the experiment. The equilibrium criterion set for the experiments was 0.01% w/w in 2 minutes, with a drying time of no greater than 60 minutes. The samples were exposed to increasing RH, with 2-3 data points collected above and below RH_0. At each step in the isotherm, samples must equilibrate to ≤0.01% w/w in 15 minutes with a maximum step time of 90 minutes (Salameh and others 2006). RH_0 was determined using the isotherm generated. Based on extrapolating the linear

portions of the curve before and after deliquescence, the intersection value was determined and identified as RH_0 (Salameh and others 2005).

The second method used to determine the RH_0 of single and multi-component samples was water activity measurements. Water activity was measured in duplicate using the AquaLab 3TE (Decagon, Pullman, WA., U.S.A). Approximately 4 g of the physical mixtures were mixed with 250 to 500 μL of double distilled water and samples were allowed to equilibrate in the water activity meter for 24 hours before data were collected. Optical microscopy was also used to visually examine deliquescence in solid mixtures at 25°C (Salameh and others 2006).

Results for the water vapor sorption isotherm for sucrose showed that the RH_0 was approximately 85%. Agreement between the two methods (moisture sorption isotherm and water activity meter) used to determine RH_0 values of individual ingredients were fairly consistent, with the exception of lactose anhydrous and citric acid. These compounds have the ability to form hydrates, which may cause an increase in $a_{w.}$ Spectroscopy showed that during the measurement period, a phase conversion to a monohydrate occurred. Because the hydrate form was less soluble than the anhydrate form, a spike in a_w was observed. The sorption isotherm for the anhydrous citric acid, fructose and 3 mixtures of 10:90, 50:50 and 90:10 w/w blends showed that the mixtures deliquesced at a significantly lower RH than the individual components, thus displaying deliquescence lowering. Deliquescence lowering occurred in binary, tertiary and quaternary blends of the various food ingredients ($P<0.01$) (Salameh and others 2006).

Optical microscopy was carried out using one crystal of citric acid anhydrous and fructose. The two crystals were placed in contact with each other to mimic a mixture at

56% RH and 25°C. Obvious signs of deliquescence were observed after 60 minutes. Individual crystals were also stored at the same conditions with no contact. These crystals showed no signs of deliquescence. The RH_{0mix} for the citric acid/fructose mixture was 48% RH. A similar procedure was used for sucrose and citric acid anhydrous. The RH_{0mix} was 64% RH. The individual RH_0 values of sucrose, fructose and anhydrous citric acid are 85%, 62% and 75% RH, respectively. This result clearly shows that deliquescence lowering occurred when the ingredients were combined. There was some disagreement between the two methods used to determine critical relative humidity as the number of components increased in the mixture (Salameh and others 2006).

The last study focused on the combined effect of temperature and RH on stability. Hiatt and others (2010) investigated the effect of temperature and RH on chemical stability of two types of vitamin C. Vitamin C samples were obtained and stored at the following RHs: 54%, 64%, 75%, 85%, and 98% using desiccators. Drierite (0% RH) was used for the control. The desiccators were placed at temperatures including the following: 4, 25, 35, and 40°C. A microplate reader was used to analyze amount of ascorbate remaining following the treatment period. A moisture sorption isotherm was used to determine the RH_0 of the vitamin C at 25 and 40°C. A kinetic study was also conducted on vitamin C in solution (Hiatt and others 2010).

The RH_0 at 25°C for ascorbic acid and sodium ascorbate was 98% RH and 85% RH, respectively. Increasing the temperature to 40°C resulted in decreased RH_0 values of 86% RH (ascorbic acid) and 82% RH (sodium ascorbate). At 40°C below $RH_{0,}$ ascorbic acid was generally stable, but when RH was increased above RH_0 there was significant degradation. Following 8 weeks of storage at 98% RH/25°C, ascorbic acid had some

yellow colored crystals with moisture present, but was not fully deliquesced. At 98% RH/40°C, the ascorbic acid was a dark brown liquid. Likewise, sodium ascorbate was also stable at RH values below RH_0 at 25°C. However, after eight weeks, complete degradation was observed at RH values near 85% RH and above. Room temperature did not have a large impact on degradation until values near RH_0 were approached or exceeded (Hiatt and others 2010).

Sodium ascorbate and ascorbic acid were stable at 0% RH at all temperatures. At 75% RH/4°C and 75% RH/25°C, sodium ascorbate was stable, but at 35 and 40°C it had almost undergone complete degradation after 8 weeks. Overall, increased moisture adsorption led to greater vitamin instability. As temperature and RH increased, the rate constants generally increased, with 98%RH/40°C having the highest rate constant for degradation. The study showed that both, temperature and RH may have a pronounced effect on chemical stability. At times, they may behave synergistically. For example, a higher temperature may lower the RH_0, therefore causing the material to become more susceptible to instability (Hiatt and others 2010).

The previous articles demonstrate how deliquescence may alter chemical and physical stability in powdered systems. Information regarding the structures and moisture contents were obtained. Physical caking observed before the onset of deliquescence may be an indication of deteriorating chemical stability. Deliquescence lowering was explored by combining various powdered ingredients, which caused the RH_{0mix} to become lower than the critical RHs of the individual ingredients. Lastly, the synergistic effect of temperature and RH on physical and chemical stability was investigated.

The studies demonstrate the importance of dry ingredient physical and chemical stability to the food industry as well as other industries. The bakery, vitamin, pharmaceutical, and spice industries are drastically affected if functionality of dry ingredients fails. Dry ingredients may be susceptible to caking, stickiness and agglomeration and eventually deliquescence. Consequently, problems in product formulation, storage and mixing may occur. Therefore, care must be taken to limit the exposure of dry ingredients to harsh environmental conditions, such as heat and moisture. Taking time to investigate proper storage environments for food powders will save money, time and resources.

Another powdered ingredient that may be affected adversely by harsh environmental conditions and undergo deliquescence is tagatose. Although numerous studies have been conducted studying the health benefit, tolerance, and food application of tagatose, there have been limited studies examining the stability of tagatose. Tagatose may be incorporated into various food, hygienic and beauty products. In addition, tagatose may potentially have a large impact on health. It appears to have prebiotic properties, which promote satisfactory colon health. Due to these attributes, it is necessary to investigate how environmental factors may impact the bulk storage of powdered tagatose.

Tagatose Properties and Applications

D-Tagatose is a naturally occurring six carbon monosaccharide that is an epimer of D-fructose (i.e. the hydroxyl group inverted at carbon 4). Trivial amounts of tagatose may be found in dairy products, including infant formula, certain cheeses, yogurt and various processed milk. The amounts range from 4 mg/kg in infant formula to 6500

mg/kg in medications (Levin 2002). It was determined to be 92% as sweet as sucrose when both tagatose and sucrose were tested in 10% aqueous solutions. It is a white anhydrous crystalline powder that has a melting point of 134°C. Research has shown that tagatose is a full bulk, low calorie sweetener with a caloric value of 1.5 kcal/g (Levin 2002).

Tagatose has a wide range of applications in a number of industries because of its highly desirable attributes. Tagatose may be used as a flavor enhancer in dairy products (yogurt), bakery products and confectionery products. Preliminary studies indicate that tagatose may be used to replace sucrose (1:1 ratio) in chocolates and gum; the sensory and physical properties were similar to the target product (Levin 2002). Moreover, since it is a low calorie sweetener, it may be used in low carbohydrate diets, soft drinks, cereals and health bars (Oh 2007). Some of the nonfood uses for tagatose include using it as a sweetener in nonchronic prescription medication for children and adults. Moreover, tagatose may be used in the cosmetic industry as a sweetener in lipstick, mouthwash and toothpaste (Levin 2002).

Tagatose is mass produced using lactose in a two step method. First, lactose is enzymatically hydrolyzed into galactose and glucose. The second step is performed under alkaline conditions using calcium hydroxide and involves isomerization of galactose to D-tagatose. Calcium hydroxide causes a shift in the isomerization equilibrium between D-tagatose and galactose, favoring D-tagatose and consequently forming an insoluble complex with calcium hydroxide due to the high pH. Sugars usually experience side reactions due to the elevated pH, however because D-tagtose is in the complex it is protected and does not react. Lastly, the suspension is treated with carbon dioxide, which

precipitates the calcium in the form of calcium carbonate, thus releasing tagatose (Bar 2004). The tagatose may be further treated by purifying, crystallizing and drying. Other potential manufacturing techniques include using L-arabinose isomerase to catalyze the conversion of galactose to tagatose. However, this technique is challenging due to enzyme activity, yield and shelf life (Lu and others 2008).

Tagatose as a Prebiotic

The term “functional food” has created quite a stir among consumers and food scientists alike. Due to illnesses, such as diabetes, hypertension and cancer, consumers have become more educated on foods that may promote better health. In addition, food scientists have begun to incorporate functional foods in products and label packaging with identifying words like “antioxidants” or “prebiotics.” Functional foods describe foods that offer an added health benefit in addition to the nutritional value (Siro 2008).

Functional foods may include both probiotics and prebiotics. Probiotics are defined as live microbial food substances that have health benefits. Two of the most common genera that are referred to as probiotics are lactobacilli and bifidobacteria. These organisms are commonly found in fermented dairy products, including yogurt. After the probiotics are passed through the stomach and small intestines, some cultures survive and briefly interact with the large intestine. Consequently, the colon’s fermentation ability is affected, and there are an increased number of bacteria in human feces. Some benefits of ingesting foods that contain probiotics include: improved lactose tolerance, immune enhancement, cholesterol reduction and decline in mutagenicity and enzymes (Roberfroid 2000). Moreover, research has provided evidence that probiotics may reduce the risk of

colon cancer by reducing the number of aberrant crypt foci (colon cancer marker) (Roberfroid 2000).

Prebiotics are described as "nondigestible food ingredients that benefit the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the colon" (Roberfroid 2000). Research has shown that native or enzymatically hydrolyzed inulin, synthetic fructooligosaccharides and hydrolyzed oligofructose have prebiotic properties. Prebiotics are usually not digested and have been referred to as colonic food, which means they function as a substrate for inherent bacteria and provide energy and metabolic substrates (Roberfroid 2000). Tagatose has been labeled as a prebiotic due to its ability to undergo fermentation by intestinal bacteria and produce short chain fatty acids as well as serve as a substrate for some beneficial bacteria, such as lactic acid bacteria.

One study that looked at the prebiotic attributes of tagatose was conducted by Venema and others (2005). There have been a number of studies conducted *in vitro* on the prebiotic effect of tagatose (Laerke and Jenson 1999; Laerke and others 2000; Bertelsen and others 2001), however limited human studies have been conducted. Venema and others (2005) utilized an *in vitro* and human study to investigate the effect of tagatose on the production of microbial metabolites and composition of microbiota using a large intestinal model. Moreover, tagatose was compared with subjects receiving fructooligosaccharides and sucrose. A mixture of tagatose and fructooligosaccharides (FOS) was also investigated for prebiotic properties. A total of 18 healthy women and 13 healthy men participated in the study for approximately four months. The study was carried out as a double-blind, cross-over, randomized, reference substance-controlled

design. The study contained five treatment periods lasting 14 days, which were separated by 14 day wash-out periods. Participants consumed 30 g of raspberry jam daily for breakfast containing two different doses, one being a high dose (12.5 g) and one a low dose (7.5 g). Participants also consumed 7.6 g of tagatose and 7.5 g of FOS in a mixture, sucrose alone or fructooligosaccharides alone. Following each treatment period, stools were collected in a box that maintained anaerobic conditions and processed for microbial analyses. Fasted urine and blood samples were collected and gastrointestinal (GI) symptoms were monitored using questionnaires. The composition of the fecal matter was determined using a variety of agar plates. Other data was also gathered from the fecal matter such as pH, dry matter content and short chain fatty acid composition (Venema and other 2005).

The results showed that tagatose and the tagatose/FOS combination caused the most GI symptoms. There was no significant difference in the number of stools in the first week, however; in the second week the number of stools was significantly higher in the tagatose and tagatose + FOS treatments compared to the negative reference (sucrose). Blood work and fasting serum lipids were unaffected by the treatment. The participants that received the high dose had a larger number of lactobacilli (7.66 ± 1.14 log cfu/g), compared to the low dose group (6.95 ± 1.16 log cfu/g), but not significantly. Following test tube incubation of fresh fecal matter in sucrose, tagatose, FOS or no substrate, the SCFAs were significantly higher after two weeks in all samples except for the positive reference (fructo-oligosaccharides). Butyric acid production was higher after high tagatose, tagatose + FOS and low tagatose treatments compared to the negative reference (sucrose). Higher propionic acid contents were also observed in the high tagatose

treatment group compared to FOS. There were no differences among the treatments in lactic acid, valeric acid, iso-valeric acid and iso-butyric acid (Venema and other 2005).

The result of the *in vitro* (large intestine model) experiment showed that the concentration of the majority of microbial groups (Bacteroides, Bifidobacterium, Enterobacteriaceae, Enterococcus, sulphite-reducing Clostridium) did not increase with the exception of lactobacilli, which increased. The human study revealed that tagatose in the allotted dosages did not alter the amounts of butyrate and total SCFAs in the feces of women or men. An increase in butyrate and total SCFAs was only observed after incubation of the fecal matter with tagatose. Similarly, the *in vitro* study showed an in increased butyrate production. Due to the outcome of this study, tagatose may be regarded as a prebiotic substrate (Venema and other 2005).

Laerke and others (1999; 2000) also observed an increase in butyrate, propionate and valerate in a digestability study which suggests that tagatose may potentially have health benefits. Laerke and others (2000) conducted an *in vitro* study in pigs. The study had three objectives: to investigate the effect of tagatose on SCFA production and lactic acid production, to approximate degradation of tagatose and fermentation products, and to determine the amount of tagatose-degrading bacteria in the feces of the pigs. The study also aimed to understand the unadapted vs. adapted gut microbiota as it related to tagatose degradation (Laerke and others 2000).

The study included 16 pigs, 8 each for the control and experimental groups. At 7 day intervals, each group was given low-basal diet with 150 g/kg sucrose (control) or 50 g/kg + 100 g/kg of tagatose (experimental). The pigs had a 2 day (day-4 and day-3) adaptation period that consisted of consuming a traditional Danish diet followed by 2

days (day-2 and day-1) of a 1:1 mixture of the experimental diet and standard feed. Next the pigs were fed the experimental diet twice a day for 18 days, (day 0 to day 17). Fecal matter was obtained on days -3, 1, 8 and 15 of the study to determine total anaerobic bacteria, pH, dry matter and D-tagatose-degrading bacteria. On day 17, the pigs were killed using a lethal injection of pentobarbital sodium. The gastrointestinal tract was removed and divided into segments according to Laerke and Jensen (1999). A 20 g/100 g slurry of gastrointestinal contents in 100 mmol/L Na-phosphate buffer was used to determine the production rate of lactic acid and SCFA in the gastrointestinal tract. The rate and composition of fermentation products were determined by exposing a slurry of bacteria from the pig's stomach to tagatose. Remaining tagatose was quantified using HPLC. The total number of anaerobic and tagatose-degrading bacteria was determined using the most probable number technique (MPN). SCFA and lactic acid were also determined (Laerke and others 2000).

The data was analyzed using SAS. Results showed that the quantity of tagatose-degrading bacteria in the feces of the pigs fed the tagatose diet was ten times higher compared to the control diet. In both the control and experimental groups, the fermentation products produced in the stomach and distal small intestines (SI) were primarily lactic acid, acetic acid, and formic acid. The SCFA that were produced from the cecal material of the pigs fed the tagatose diet included the following (least to greatest): formic, valeric, butyric, acetic and propionic acids. Propionic production in the cecum of the pigs fed tagatose was more than twice as high compared to the control. The overall amount of SCFA produced in the cecum was 10.7±2.1 mmol/h and 3.0±2.7 mmol/h for the tagatose and control groups, respectively (P=0.06). Exposing the tagatose

to bacterial slurries from the stomach and SI caused no increase in SCFA levels, showing that these portions of the GI tract were unable to degrade tagatose *in vitro*. Due to the small amount of degradation, energy values for the unadapted microbiota were not determined. Compared to the control, hydrogen production was increased due to the *in vitro* incubation of tagatose with bacteria taken from the cecum and colon of the tagatose-fed pigs. Likewise, methane production (mid-colon) was also higher in the tagatose-fed pigs. This suggests that some of the energy was lost as gases (Laerke and others 2000). This study showed a higher level of tagatose-degrading bacteria as well as SCFA, which provides additional evidence that tagatose displays prebiotic properties.

Tolerance of Tagatose

Many studies have examined the consumption of tagatose at various levels to determine the acceptable intake as well as tolerance. Buemann and others (1999) conducted three mini studies to look more closely at how the body responds to tagatose. The studies included a screening study, an adaptation study and a metabolic study. Human subjects consumed 30 g of tagatose in each study and reported symptoms, such as increased flatulence, nausea and headache. In the screening study, 73 males were given cake that contained the tagatose in the afternoon followed by a three hour fasting period. Subjects recorded symptoms and severity of symptoms for the remainder of the day as well as the next day. The screening study was conducted to eliminate subjects from the adaptation and metabolic studies who displayed strong gastrointestinal symptoms. Subjects were responsible for recording and rating the severity of symptoms during the day of consumption and the subsequent day. The main goal of the adaptation study was to identify possible metabolic effects of tagatose consumption by 24 h indirect

calorimetry before and after a 2 week adaption period. The report did not present results of the metabolic study but focused only on tagatose tolerance. During the adaptation test, sucrose (control) and tagatose were administered in a randomized, blind crossover study. More than 2 months separated the treatments. Subjects recorded symptoms at specified times during days of indirect calorimetry. Most symptoms were moderate to light and no vomiting occurred. The authors explained that such symptoms may occur due to unabsorbed sugars that ferment in the large intestines. Although no analysis was performed on the data, the adaptation study revealed there was not an improvement in tolerance to tagatose, just adaptive changes in the microbiology of the colon. In the screening study, more symptoms were recorded where as in the adaptation (2 week) and metabolic studies less were recorded. In the screening test a 15.1% incidence of nausea was reported and a 31.5% incidence of diarrhea was reported. The author concluded that less than 30 g of tagatose should be consumed per eating occasion and tolerance varies (Buemann and others 1999).

Another study was conducted to compare gastrointestinal disturbances of sucrose, tagatose and lactitol when incorporated into chocolate (Lee and Storey 1999). Two 40 g chocolate candy bars containing 20 grams of each ingredient were randomly given to 50 unadapted subjects (25 male and 25 female). The experiment was designed as a double-blind, controlled, crossover study. Twenty-five of the subjects consumed the chocolate bars in the following order: day 1-lactitol bar, day 9-sucrose bar and day 17-tagatose bar. The additional subjects had the tagatose bar on day 1, sucrose bar on day 9 and lactitol bar on day 17. Subjects were given sheets to record gastrointestinal symptoms (incidence and extent), such as colic, bloating, flatulence, thirst, nausea, etc on a scale of 0 (normal)

to 3 (debilitating) during the 24 hour period after consumption. Toilet visits and consistency of bowel movements were also monitored (Lee and Storey 1999).

When compared to lactitol, tagatose chocolate had no significant differences in frequency of symptoms, except for an increased amount of thirst. When tagatose was compared to sucrose, significantly more subjects felt symptoms, such as thirst, appetite loss, nausea, bloating and borborygmi. Also compared to sucrose, individuals who consumed tagatose chocolate passed more watery bowel, but not more than those who consumed lactitol. Only one subject recorded a debilitating symptom after consumption of lactitol or tagatose. This study indicated that a 20 gram dose of tagatose may be better tolerated than the same dosage of lactitol (Lee and Storey 1999).

Buemann and others (2000) completed another study examining the acute effect of tagatose on *ad libitum* food intake. Twenty male subjects were required to undergo a pre-screening test in which they had to tolerate 30 g of tagatose without significant symptoms. The tests were separated by 4 days or more. Following a 12 h fast, the breakfast was consumed in 20 min. The subjects received a 1.6 MJ breakfast, which had either a 29 g dose of tagatose or sucrose. After breakfast, a duration of four hours passed in which subjects were not allowed to eat or drink. Subsequently, lunch was served *ad libitum* and plates were weighed to monitor intake. After lunch subjects were allowed to leave, but were supplied with lunchboxes. They were instructed to eat at will but to save leftovers for weighing. Dinner was served 5 h later and subjects were allowed to eat *ad libitum.* Similarities were observed in energy intake of the tests at lunch and snack, but a lower energy intake (15%) was observed during dinner in the tagatose group (Buemann and others 2000).

The results of the study revealed tagatose did not affect total macronutrient composition of total post lunch food. However, tagatose caused a lower fat intake (% of energy intake) at dinner. The liquid intake of lunch and snack combined was 11% higher after tagatose partially due to a lower intake at dinner. Two cases of nausea and one case of strong flatulence were reported with tagatose intake (Buemann and others 2000). Buemann and others (1999) reported that symptoms such as vomiting, nausea and perceived distention could be caused by an osmotic effect in the small intestine of unabsorbed tagatose. Moreover, the heightened flatulence may be attributed to the fermentation of tagatose in the large intestine. Diarrhea may be caused by osmotic effects in the colon from tagatose that was not digested. In addition, diarrhea may also be caused by the increased fermentation producing poorly absorbed short chain fatty acids (Buemann and others 1999).

Metabolism of Tagatose

Livesey and Brown (1996) examined the thermic effect of tagatose and determined it supplied zero net metabolizable energy (NME). This study utilized rats that consumed sucrose (control) and tagtatose (1.8 grams) incorporated in a basal diet to investigate the thermic effect of tagatose. The NME value of tagatose was calculated using equations based on the influence of body makeup. All substrate-induced energy losses, such as feces and urine, were accounted for. No symptoms, such as diarrhea, were observed in the rats as reported later in human studies conducted by Buemann and others (1999). The tagatose did not supply significant amounts of beneficial energy—the calculated metabolizable energy value was -0.4 ±2.2 kJ/g (Livesey and Brown 1996). Moreover, Moyer and Roden (1993) also found that sugars, such as tagatose, have

diminished net energy contents because of poor digestibility. Although this study (Livesey and Brown 1996) confirmed the thermogenic effect of tagatose, Buemann and others (1998) conducted a study that was not able to confirm that tagatose minimal net energy is a result of a thermogenic effect. Although Livesey and Brown (1996) determined tagatose had a substantially lower caloric content, Levin (2002) reported that the caloric content may range from 1.1 to 1.4 Kcal/g. Differences in caloric content maybe attributed to experimental procedures, variables and result interpretation. Therefore, the Food and Drug Administration approved the caloric content of tagatose as 1.5 Kcal/g (Levin 2002).

Another study examined the digestibility of tagatose in pigs (Laerke and Jensen 1999). This study was designed to investigate the ileal and fecal digestibility of tagatose in pigs. In addition, this study looked at the effect of ileal and fecal digestion on macronutrients and changes in microbial activity, pH, and concentration of short chain fatty acids (SCFAs) in the gut contents. Seven days separated two experiments that were performed using two groups of eight pigs. There was an adaptation period of two days, two days of consuming a 1:1 ratio of a mixture of control and experimental diet and eighteen days of the experimental diet. The control group consumed a low fiber diet with sucrose (150 g/kg) and the experimental group consumed a low fiber diet with tagatose (100 g/kg) and sucrose (50 g/kg). Both diets also contained chromic oxide, which served as a digestibility marker. Analysis was conducted on the upper gut (stomach and three equal segments of small intestine [SI]) and the lower gut (cecum and three equal segments of the colon) (Laerke and Jensen 1999).

The digestibility of tagatose in the distal portion of the small intestine measured by chromic oxide was 25.8 ± 5.6%. No significant differences between groups in the SI digestibilites of macronutrients were found, but there was a lower digestibility of sucrose in the pigs fed the tagatose diet. Significant differences were observed in the total microbial activity in the cecum and colon of the control and test pigs. An increase in butyrate, propionate and valerate were observed in the test pigs, which suggests that tagatose may potentially have health benefits. Tagatose in the large intestine of adapted pigs was completely fermented—this played a role in the overall energy balance with a high production of SCFAs (Laerke and Jensen 1999). Buemann and others (1998) also suggested that some tagatose is not absorbed, but fermented in the large intestine.

In the small intestine, absorption and digestion of tagatose is minimal (approximately 25%), with the large majority of tagatose being fermented in the large intestines (Laerke and Jensen 1999). Although metabolism is similar to other monosaccharides, it may occur at a slower rate (Levin 2002). Moreover, the study was also comparable to a study that used rats with radiolabeled tagatose where only 15-20% of tagatose was absorbed across the small intestine (Saunders 1999).

Tagatose in Foods

For decades, consumers as well as the food industry have searched for a solution to enjoy palatable foods, but without the added calories and sugar. Tagatose is a prime candidate and is currently being incorporated into various food products. Currently, marketed products that have tagatose include the following: Shurgr by Swiss Diet, Pasco Light and Tasty Juice, 7-Eleven's Diet Pepsi Slurpee, SweetFiber by Dr. Murray Natural

Living, Miada Chocolite, and Therasweet by Living Fuel (Wise 2008). Some of the geographical locations where these products are found include the United States, European Union, South Korea, Australia and New Zealand (Peckenpaugh 2006). The Food and Drug Administration maximum specification levels for tagatose include the following: diet and/or sugar free carbonated soda and teas-1%, sugar free gum -60%, icings and glazes- 30%, diet soft candies and low fat and reduced fat energy/nutrient bars-10%, powdered products made with milk-5 g/per serving, diabetic and regular hard candies-15%, frozen novelties- 3% and ready-to-eat cereals- 3 g/serving (FDA 2001).

Taylor and others (2008) conducted a study examining the physical properties and consumer preference of cookies formulated with tagatose. In addition to control cookies prepared with 100% sucrose, eight experimental formulations were prepared using fructose and tagatose at 25%, 50%, 75% and 100%. The cookies were prepared according to McWatters and others (2003). Textural properties of the cookie dough were quantified using the Texture Analyzer. Physical properties of the baked cookies were also monitored. They included height and diameter, color and hardness. A total of 53 untrained panelists evaluated 3 different formulations (100% tagatose, 100% sucrose and 50/50 blend of tagatose and sucrose) on texture, color, sweetness and overall liking (Taylor and others 2008).

The Texture Profile Analysis revealed results concerning the resilience, hardness, adhesiveness and cohesiveness of the cookie dough. There was no significant difference in resilience between the 100% tagatose cookie dough and control (100% sucrose). Moreover, there were no significant differences in hardness, adhesiveness and cohesiveness of doughs made with 100% sucrose and 100% tagatose. The rheological

properties of the dough that was made with tagatose were similar to that made with sucrose. In contrast, the dough made with fructose was softer and stickier and did not closely resemble the rheological properties of the dough made with sucrose. The diameter of cookies made with fructose and tagatose were significantly smaller than the cookies made with sucrose. Compared to the control, cookies made with 100% tagatose were significantly harder. Cookies made with tagatose and fructose were darker in color compared to the control. The sensory evaluation of the cookies revealed that overall acceptance of the 100% sucrose cookie was significantly higher than that of the 100% tagatose cookie, but not significantly different from the 50/50 tagatose/sucrose formulation. Based on the study, it appears possible to formulate an acceptable cookie made with tagatose, but not by completely replacing sucrose with tagatose (Taylor and others 2008).

Armstrong and others (2009) investigated whether 1% and 2% tagatose affected the flavor of bakery products when determined by an untrained sensory panel. Cinnamon muffins, chocolate cakes and lemon cookies with 1-2 % tagatose were formulated and prepared. Standard recipes were used for the cinnamon muffins and lemon cookies, whereas a premixed formula was used for the chocolate cake. Control bakery products were prepared with sucrose using the same concentrations. Untrained panelists performed a triangle test and hedonic test. The triangle test revealed panelists could not differentiate between the formulations at the 1% and 2% levels. Therefore, incorporating tagatose into the bakery products does not significantly impact the flavor profile. The hedonic scores indicated the panelists liked the tagatose and control products similarly (Armstrong and others 2009).

Stability of Tagatose in Solution

A few studies have investigated the stability of tagatose in solution (Ryu and others 2003; Dobbs and Bell 2010; Luecke and Bell 2010). One study that investigated the effect of temperature and pH on the non-enzymatic browning reaction involving tagatose was conducted by Ryu and others (2003). The effect of temperature on non-enzymatic browning was investigated by heating a solution of 0.2 M tagatose and 0.2 M glycine in a water bath for 5 hours at 70, 80, 90 and 100°C with no pH control. To determine the effect of pH on non-enzymatic browning, tagatose/glycine solutions ranging from pH 3 to 7 were heated at 100°C. Two other studies looked specifically at temperature and pH effects on tagatose only. A 5% solution of tagatose was heated to 100°C for 5 hours at pH 3, 4 and 5. The effect of temperature on tagatose was investigated using a 10% tagatose solution and heating it to 100°C for 5 hours. In all studies, samples were removed hourly for analyses, including HPLC, spectroscopy, and color measurements. The study found that the rate of browning increased as temperature increased. Generally, as pH increased, browning also increased, except at pH 6 where a slight decrease in browning was observed. Tagatose alone did not experience any browning or degradation. However, when combined with glycine both were observed; this result was attributed to the Maillard reaction (Ryu and others 2003).

Dobbs and Bell (2010) investigated the storage stability of tagatose in buffer solutions at 20, 30 and 40°C. Tagatose was placed into two buffer (citrate and phosphate) solutions at a concentration of 0.05 M or 1% and adjusted to a pH of 3 and 7, respectively. The concentrations of the buffer were 0.02 and 0.1 M. Each sample was

stored at 20, 30 and 40°C in triplicate. Over the nine month period, 11-13 samples were obtained (Dobbs and Bell 2010).

Concentrations of tagatose were analyzed using reverse phase high performance liquid chromatography (HPLC), whereas brown pigmentation was determined using spectrophotometry (wavelength = 420 nm). Results indicate that pH, temperature, buffer type and concentration all impacted tagatose degradation. Tagatose degradation rate was fastest at the greater pH (i.e., pH 7) whereas at pH 3 the tagatose degradation rate was not significantly different from zero. The tagatose solution consisting of 0.1 M phosphate buffer at pH 7 and 40°C experienced a 29% loss of tagatose in 30 days. Tagatose also degraded faster in phosphate buffer than in citrate buffer. In addition, the higher the buffer concentration, the more rapid was tagatose degradation. At pH 7, the tagatose degradation rate constants for 0.1 M phosphate buffer were 2.5-4.8 times higher than the lower buffer concentration (0.02 M). As expected, the rate constants increased as temperature increased. Browning was also most pronounced at the highest temperature (40°C). In summary, this study found that tagatose degradation was highest at higher temperatures, pHs, and buffer concentrations. The study suggests that tagatose may be permitted in mildly acidic products such as fruit juices and yogurts. Moreover, beverages that take advantage of tagatose should be exposed to low temperatures, low buffer concentrations and low pHs to minimize tagatose degradation and browning (Dobbs and Bell 2010).

Luecke and Bell (2010) conducted a similar study that investigated the thermal stability of tagatose when exposed to temperatures of 60, 70 and 80°C. Similar methods were used for this study as the previous study. Citrate and phosphate buffers were

prepared at 0.02 M and 0.1 M concentrations and adjusted to pHs 3 and 7. A total of eight buffer solutions contained 0.05 M tagatose. The kinetic studies were carried out in a water bath that maintained temperatures of 60, 70, and 80°C. The tagatose solutions were transferred to NMR tubes, which were placed in the water bath and reached the specified temperature within a minute. Tubes were moved at regular time intervals and transferred to an ice bath to halt further reaction (Luecke and Bell 2010).

Tagatose analysis was similar to previous study and was carried out via HPLC and spectroscopy (Dobbs and Bell 2010). Pseudo-first-order kinetics were used to model tagatose degradation. Rate constants as well as 95% confidence intervals were determined. Moreover, activation energies were determined for each experiment using the rate constants and average temperature. The effect of pH, buffer type and concentration, and temperature on tagatose was determined. Similar results were noted when compared to the previous study. Tagatose degradation occurred slower at pH 3 than pH 7. The concentration of tagatose decreased by 26% when stored at the following conditions for 7.5 hours: 0.1 M citrate buffer, pH 7 and 80°C (Luecke and Bell 2010). Tagatose degradation was also more pronounced in the phosphate buffer when stored at the higher pH. The phosphate buffer accelerated tagatose degradation as compared to the citrate buffer. Buffer concentration also impacted tagatose loss, with greater loss being observed at higher buffer concentrations. With a pH of 7, the rate constant for tagatose loss in a 0.02 M phosphate buffer solution was five times less ($0.00566 \pm 0.00071\ h^{-1}$) than in 0.1 M phosphate buffer ($0.0301 \pm 0.0022\ h^{-1}$). Likewise, as temperature increased from 60° to 80°C, degradation also increased. Pseudo-zero-order rate constants were calculated for browning of the tagatose solutions. Browning was faster at higher

temperatures and higher pHs. Browning was also faster at increased buffer concentrations and in phosphate buffer. Based on this study, tagatose may be used in applications, such as beverages, which are subjected to pasteurization without the loss of its prebiotic property or browning due to the very short time of thermal exposure (Luecke and Bell 2010).

Often times, food manufacturing facilities are not air conditioned and relative humidity is not controlled. Consequently, extreme environmental conditions may occur, causing powdered ingredients to be adversely affected. As discussed previously, water can impact powdered ingredients, like tagatose, to varying extents causing caking, clumping, loss of flow ability and even deliquescence. Although some studies have focused on the chemical stability of tagatose in solution, none have addressed the physical and chemical stability of powdered tagatose. Therefore, the purpose of this study was to investigate the physical stability (caking, deliquescence) and chemical stability (degradation, browning) of tagatose powder subjected to various temperatures and relative humidities.

Chapter 3: Materials and Methods

Sample Preparation

Food grade tagatose was obtained from Arla Food Ingredients (Basking Ridge, N.J., USA). According to the product specification sheet, it had a purity of greater than 99%. Ash (<0.1%), water (<0.5%) and, non-tagatose monosaccharides (<0.4%) made up the remainder of the percentage. Approximately 150 g tagatose were placed in beakers over anhydrous calcium sulfate indicating Drierite (W.A. Hammond Drierite Company, LTD.) for one month under vacuum to remove any residual moisture present. Three 400-mL beakers contained about 50 g tagatose each to ensure efficient distribution. Following the one month drying time, 0.3-0.5 g of tagatose were analytically weighed into 338 20-mL glass vials making certain to distribute tagatose evenly over the entire surface area of the vial. A total of 26 vials were placed in each of 13 desiccators (environmental chambers) prepared for the experiment. The chambers were vacuum sealed to maintain a constant environment. One desiccator served as the control, and contained Drierite. The remainder of the desiccators contained one of four saturated salt solutions in triplicate, with one of each being stored at 20, 30 and 40°C.

Particle Size Characterization

Some studies show that particle size influences moisture adsorption and deliquescence (Mathlouthi and Roge 2003; Salameh and Taylor 2006a; Kwok and others 2010); therefore the particle size of tagatose was investigated. Tagatose samples were

placed in a Sieve Shaker (CSC Scientific Company, Inc, Fairfax, VI) for 5 min. Three trials were done to determine the average particle size of the tagatose available to the food industry. A total of 29-34 g of tagatose were used for each trial. To characterize the powdered tagatose sample, its particle size distribution was analyzed using the following sieve sizes: 1) 250 µm, 2) 355 µm 3) 500 µm and 4) 710 µm. The amount retained by each sieve was weighed. Particles that remained at the top of the 710 µm sieve were a particle size greater than 710 µm. Likewise, those particles that were sieved through the 250 µm sieve were less than 250 µm. The distribution of the particle sizes is given in Table 3.1. Particle size may influence powder physical stability; therefore, a small side study was conducted to investigate this possibility. Further detailed information about this side study appears in Appendix D.

Table 3.1. Average particle size distribution of food grade tagatose as a percentage with standard deviation.

Particle size	**%**
< 250 µm	7.1±0.8
250-355 µm	39.8±1.4
355-500 µm	46.1±1.8
500-710 µm	6.6±0.5
>710 µm	0.3±0.1

Preparation of Saturated Salt Solutions

Laboratory grade certified A.C.S. salts (Fisher Scientific, Fair Lawn, N.J.) magnesium chloride ($MgCl_2$) crystals, magnesium nitrate [$Mg(NO_3)_2$], sodium chloride (NaCl) and potassium chloride (KCl) were obtained to make saturated salt solutions, which created relative humidities of 33%, 54%, 75% and 85% at 20°C. A 1000-mL

beaker with a stir bar was filled with approximately 500 mL of distilled water. The beaker was placed on a hot plate/stir plate and monitored with a thermometer until it reached a temperature ranging from 55-60°C. Upon reaching the desired temperature, the specified salt (i.e., $MgCl_2$, $Mg(NO_3)_2$, NaCl or KCl) was stirred into the water in increments. A critical amount of crystals was stirred into the beaker to create a saturated slurry of salt crystals in water. The salt solutions were allowed to cool and equilibrate in a sealed jar at the desired temperature (20, 30, 40°C) for approximately one month. All saturated salt solutions were transferred into desiccators, which were returned to the appropriate incubator and allowed to re-equilibrate for approximately one week. The relative humidities of the salt solutions were verified using an AquaLab (Decagon, Pullman, WA, USA) water activity meter and compared to literature values. Table 3.2 shows the salt solutions that were used and the relative humidity as a function of temperature.

Experimental Overview

Following the placement of all tagatose samples in the desiccators, they were allowed to equilibrate to the specified %RH for one month (time 0). After the equilibration period, 2 vials from each desiccator at each temperature were removed monthly for 12 months. The end of the equilibration time was termed time 0 and each month thereafter was termed time 1 through time 12. Desiccators were checked weekly to ensure they remained vacuum sealed. Following sample removal, physical descriptors and photographs were used to characterize the powdered tagatose. The physical descriptors included the following: free flowing, partially caked, fully caked, partially deliquesced and fully deliquesced. In addition, the tagatose was weighed to determine

water loss/gain. After assessing the physical appearance and morphology of the tagatose, 20 mL of distilled water was volumetrically added. The vials were vortexed and stored at 4°C for 4-5 days to allow complete dissolution of the tagatose before chemical analyses. The concentration of tagatose and brown pigment formation were determined using these solutions. The pHs of the solutions were also determined using a pH meter (model 920A, Orion Research Inc, Boston, MA) at times 6, 9 and 12 months. Throughout the study care was taken to vacuum seal each desiccator after sample removal and place it back in the proper environmental storage conditions.

Table 3.2. Saturated salt solution relative humidity as a function of temperature (Bell and Labuza 2000).

Salt Solution	**20°C**	**30°C**	**40°C**
$MgCl_2$	33.1	32.4	31.6
$Mg(NO_3)_2$	54.4	51.4	48.4
NaCl	75.5	75.1	74.7
KCl	85.1	83.6	82.3

Physical Stability

Based on the movement and physical state of the samples, they were characterized as either free flowing, partially caked, fully caked, partially deliquesced or fully deliquesced. Upon removing the samples from the desiccators at each time period, they were visually inspected and lightly moved around to characterize the powder. If the powder moved freely throughout the vial, it was considered free flowing. If the powder was caked, but had still had some portions of free flowing powder, it was considered partially caked. If there was no movement in the vial, the powder was labeled fully

caked; this powder was fixed to the bottom of the vial. If the sample had some crystals that were dissolved and others that were still in crystal formed, it was labeled partially deliquesced. The sample was very similar to a "slurry." It contained some solids (tagatose crystals) and some liquid (deliquesced tagatose crystals). Lastly, if the sample was fully liquefied (deliquesced), it was termed fully deliquesced. There were no crystals present.

Moisture Content

Moisture content was monitored to give an indication of when the vials containing tagatose equilibrated in the desiccators. An initial weight of the vials was obtained before placement in the desiccators. Each month (time 0 – time 12), 2 vials were weighed upon removal from each desiccators to obtain moisture gain or moisture loss. The following equation (3.1) was used to calculate moisture content (M) as a percentage on a dry weight basis:

$$M = 100 \times \frac{w_f - w_i}{w_i} \qquad 3.1$$

In the equation, w_f refers to the final weight of the tagatose and w_i refers to the initial weight of the dried tagatose.

Photographs

Photographs were taken using a Hewlett Packard Photosmart R725v digital camera (Hewlett-Packard Company, Palo Alto, CA, USA). Photographs of tagatose vials were taken at time 6, 9 and 12 to assess browning as well as morphology of the tagatose. Initially pictures were not taken because browning was not noticeable. Upon removing the vials from the desiccators, triplicate pictures of each vial were taken with no flash. A

ring stand was used to make certain the camera was approximately at the same distance to ensure pictures were as comparable as possible; white paper served as the background. The primary lighting consisted of overhead fluorescent light. Due to differences in window lighting, slight differences in lighting occurred.

Critical Relative Humidity Determination

When determining the deliquescence point of a sample, an assumption is made that the solid particles are surrounded by a saturated solution at the critical relative humidity (RH_0). Water activity can be defined as RH/100. Consequently, water activity measurements of saturated solutions can be used to determine RH_0, which in turn predicts deliquescence points (Mauer and Taylor 2010). The critical relative humidity (RH_0) of tagatose was obtained using a saturated solution of the sugar and distilled water. The tagatose solution and water activity devices were allowed to equilibrate at each temperature for at least 2-3 days before duplicate readings were obtained. To obtain the RH_0 at 40°C, the sample was allowed to equilibrate for approximately 24 hours. This action was taken because tagatose degradation was observed at 40°C. Saturated solutions have been used in other studies to determine critical relative humidities (Salameh and Taylor 2005; 2006a; 2006b). Both the AquaLab (Decagon, Pullman, WA, USA) and Rotronic (Rotronic Instruments Corp, Huntington, NY, USA) water activity devices were used to determine RH_0 at 20, 30 and 40°C. Saturated salt solutions and water were used to verify accuracy of equipment.

Chemical Degradation Analysis

Chemical degradation of tagatose was determined by analyzing the tagatose concentration in each sample (i.e., tagatose dissolved in 20 mL H_2O) using reverse phase

high performance liquid chromatography (HPLC). The mobile phase used was a 91%/9% (v/v) acetonitrile/water solution that had a flow rate of 3 mL/min. Separation was carried out using a 250 x 4.6 mm Luna 5 µ amino column (Phenomenex, Torrance, CA) in a column oven set at 40°C. Tagatose detection occurred through the use of a refractive index detector RID-10A (Shimadzu, Kyoto, Japan). A Hewlett-Packard Integrator 3395 was used to integrate the data, which allowed the generation of the chromatogram. The tagatose eluted at approximately 7 minutes.

A total of seven standard solutions with a known concentration of tagatose were analyzed with the experimental samples at each time period. The peak area from the chromatogram and concentration of the standards were used to generate a linear standard curve. The area was plotted on the x-axis and the concentration was plotted on the y-axis. A linear trend line was obtained from the curve, which allowed the concentration of the experimental samples from each time period to be determined. The area of the tagatose peak was recorded. The concentration of tagatose was determined from the peak area and standard curve. This value was converted into percent recovery, accounting for the initial sample mass and moisture content. The average percent recovery for the control held over drierite was 98.3%, and the coefficient of variation was less than 3% (n=26). This result is similar to that reported by Luecke and Bell (2010).

Graphs were generated that plotted percent tagatose remaining versus time (days). No rate constants were determined for tagatose degradation because only one sample (85%RH/40°C) showed substantial degradation, but its loss plateaued at 6 months. Tagatose did not follow the customary degradation kinetic models during the 12 month study.

Browning of Tagatose

Brown pigmentation was measured monthly for 12 months for all tagatose samples using a spectrophotometer (DU 640, Beckman Instrument Inc, Fullterton, CA). After tagatose was removed from each desiccator and physical appearance was evaluated, 20 mL of distilled water was added to each vial using an electronic pipette. Approximately 1 mL aliquots of each sample were filtered using 5 mL sterile syringes and 0.45 µm nylon filters, and transferred into disposable methacrylate cuvettes (Fisher Scientific Co LLC, Suwanee, GA). Distilled water was used as a blank to zero the instrument. Preliminary browning reactions were measured at 280 nm, whereas the later stage brown pigment formation was measured at 420 nm, which has been noted in the literature (Karmas and others 1992).

The rate of browning was statistically analyzed using Microsoft® Excel® (Microsoft Corporation, Redmond, WA). Due to the natural off-white color that tagatose displayed, the optical densities (OD) of all samples were corrected using the control samples, which were stored over drierite. The OD/g of these duplicate samples (i.e., stored over drierite) were averaged at each time. The average OD/g values were subtracted from the OD/g of the samples to account for the slight background pigmentation. The resultant OD/g represented the actual discoloration of the tagatose sample associated with a particular relative humidity and temperature. Graphs were generated with time (days) on the x-axis and the corrected absorbance (OD/g) on the y-axis. As outlined by Labuza and Kamman (1983), least squares analysis was used to calculate pseudo zero-order rate constants for tagatose browning with 95% confidence intervals. When select treatments were graphed, some had lag phases. To obtain better

R^2 values and rate constants, only the linearly increasing portion of the plot was used. Eliminating the lag phase was determined by evaluating the R^2 value as well as the appearance of the plot.

pH

The pH was measured at times 6, 9 and 12 months because when monosaccharide degradation occurs in solution, acidic products are formed (de Bruijn and others 1986). Following HPLC analysis and spectroscopy, a small aliquot of the tagatose solution was transferred to a 20 mL vial and pH measurements were taken using a pH meter with a combination electrode (model 920A, Orion Research Inc, Boston, MA).

Chapter 4: Results and Discussion

Because relative humidities within the environmental chambers decreased with increasing temperature (Table 4.1), all samples will be identified by the RH at 20°C. This notation will allow for an easier read of this section. For example, 33%RH/30°C refers to an actual RH of 32.4% at 30°C.

Table 4.1. Actual RH values associated with temperatures and the notation that will be used throughout this section.

Salt Solution	**20°C**		**30°C**		**40°C**	
	Sample Notation	**Actual RH (%)**	**Sample Notation**	**Actual RH (%)**	**Sample Notation**	**Actual RH (%)**
$MgCl_2$	33%RH/20°C	33.1	33%RH/30°C	32.4	33%RH/40°C	31.6
$Mg(NO_3)_2$	54%RH/20°C	54.4	54%RH/30°C	51.4	54%RH/40°C	48.4
NaCl	75%RH/20°C	75.5	75%RH/30°C	75.1	75%RH/40°C	74.7
KCl	85%RH/20°C	85.1	85%RH/30°C	83.6	85%RH/40°C	82.3

Moisture Sorption

Moisture contents were monitored throughout the study to verify that samples equilibrated at each environmental storage condition. The samples stored over $MgCl_2$ solutions (33% RH) adsorbed less than 1% moisture (db) over the 12 month period. The samples stored at 54 and 75% RH adsorbed less than 2% moisture (db) throughout the study. Tagatose stored above KCl solutions (85%) gained substantially more moisture with the amount of moisture decreasing as temperature increased (Figure 4.1). At 20, 30

and 40°C tagatose equilibrated at an average of 80%, 66%, and 53% moisture (db), respectively (Figure 4.1). As shown in the figure, the samples stored at 20°C equilibrated at a slower rate than the samples stored at 30 and 40°C. The equilibrium moisture content was plotted as a function of %RH in Figure 4.2. This graph is the moisture sorption isotherm for tagatose. The sudden gain in moisture at 85% RH was due to deliquescence.

Salameh and Taylor (2006b) formed moisture sorption profiles for individual solids (fructose, citric acid) and mixtures. The profiles clearly show that minimal moisture is adsorbed prior to the deliquescence point. When the deliquescence point is reached, there is a significant increase in moisture and complete dissolution of the solid (Salameh and Taylor 2006b). Moisture uptake profiles were also generated for a mixture (RH_{0mix}= 64%) of sucrose and citric. For the samples that were stored above or at 65% RH, moisture content increased as a function of time. However, samples stored below 65% RH equilibrated with a moisture content of less than 0.4% w/w (Salameh and Taylor 2006b). Although the tagatose study involved a single component, it followed a similar pattern. Minimal adsorption (< 2%) occurred in all samples stored below 85% RH, with increased moisture adsorption at 85% RH (Figure 4.2).

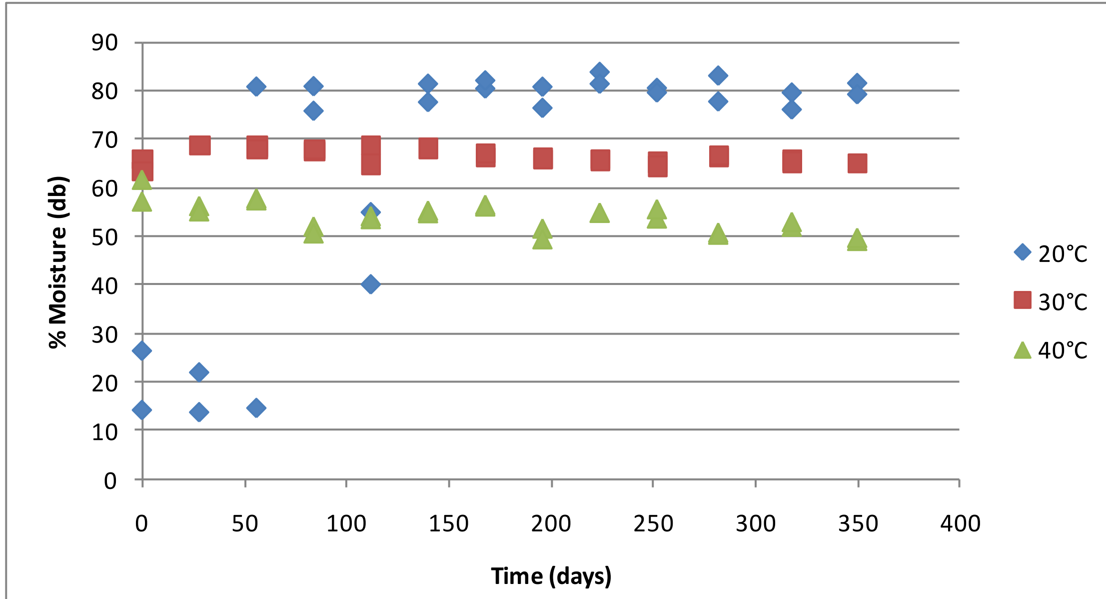


Figure 4.1. Moisture adsorption profile of samples stored at 85% RH at 20, 30 and 40°C.

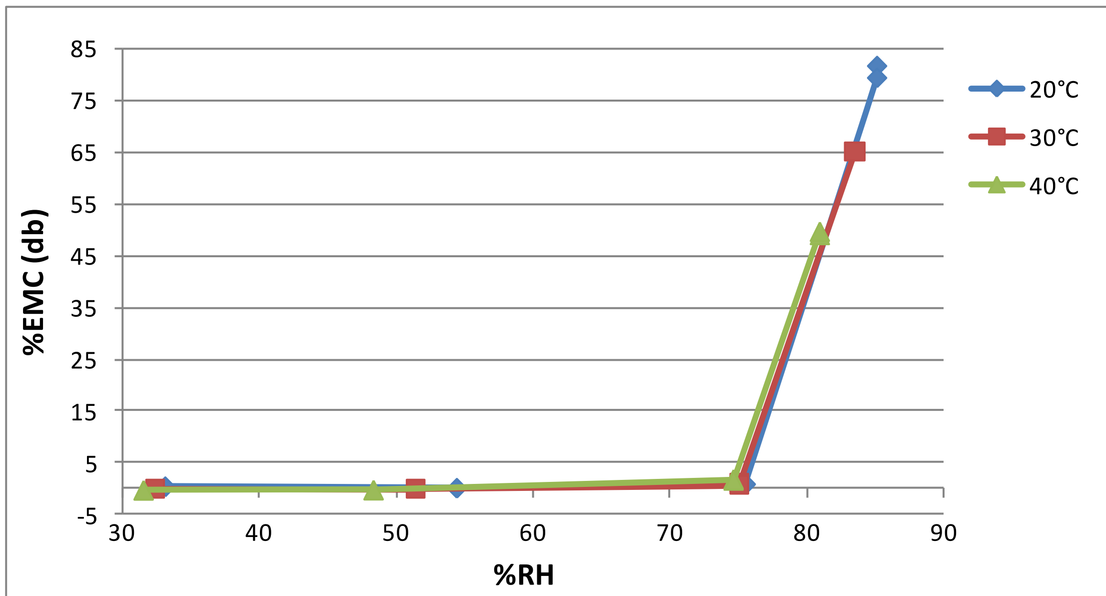


Figure 4.2. Moisture sorption isotherm for tagatose at 20, 30 and 40°C after exposure for 12 months.

Physical Stability

For a control, tagatose was stored over drierite. The control remained free flowing throughout the study with no noticeable browning or degradation. Depending upon storage relative humidity and temperature, various physical changes in tagatose occurred. Tagatose stored at 33%RH/20°C remained free flowing throughout the study. All other samples experienced some degree of caking and deliquescence. For example, samples stored at 33%RH/30°C were partially caked after 1 month and remained in that state for the duration of the study. At 33% RH, as the temperature of the samples increased, the more the physical stability was affected (Table 4.2). The samples went from being free flowing at 33%RH/20°C to fully caked at 33%RH/40°C after 12 months. All other samples were fully caked, except for samples held at 85% RH. Samples stored at 85% RH at 20, 30 and 40°C were all fully deliquesced after 12 months. However, full deliquescence occurred at various time points including the following: 85%RH/20°C-5 months, 85%RH/30°C-2 months and 85%RH/40°C-1 month. This result was due to the 20°C sample being held slightly below RH_0, the 30°C sample being held slightly above RH_0, and the 40°C sample being held further above RH_0, as will be discussed later. The higher temperature also improves dissolution. Due to the samples being stored at the higher RH, the physical stability was more adversely affected. Table 4.2 shows the physical characteristics of all samples at 12 months.

Pictures were also taken and show the physical state of the samples, more specifically deliquescence although some caking is also visible. Figure 4.3 shows caking in the sample stored at 75%RH/20°C for 12 months. Deliquescence is visible in all samples stored at 85% RH (Figure 4.3, 4.4 and 4.5).

As mentioned earlier, one of the most common mechanisms through which caking of water soluble solids occurs is particle surface wetting. Particle surface wetting may occur through accidental wetting or condensation of atmospheric moisture. This in turn leads to plasticization of amorphous regions, which facilitate the formation of liquid bridges. The liquid bridges increase the risk of powder stickiness and cohesiveness. Crystallization of the amorphous regions releases moisture and solid bridges are formed, which promote additional caking (Peleg 1983). Furthermore, raising the temperature increases dissolution of particles, which may alter the crystalline form and result in caking and flow problems (Teunou and Vasseur 1996). This mechanism may explain the caking in the tagatose stored below RH_0 where there was minimal water adsorption.

Table 4.2. Physical characteristics of tagatose as affected by RH and temperature after 12 months and time at which final physical state was observed.

RH (%)	**Temperature (°C)**		
	20°C	**30°C**	**40°C**
33%	free flowing (12 months)	partially caked (1 month)	fully caked (1 month)
54%	fully caked (10 months)	fully caked (3 months)	fully caked (9 months)
75%	fully caked (1 month)	fully caked (1 month)	fully caked (1 month)
85%	fully deliquesced (5 months)	fully deliquesced (1 months)	fully deliquesced (1 month)

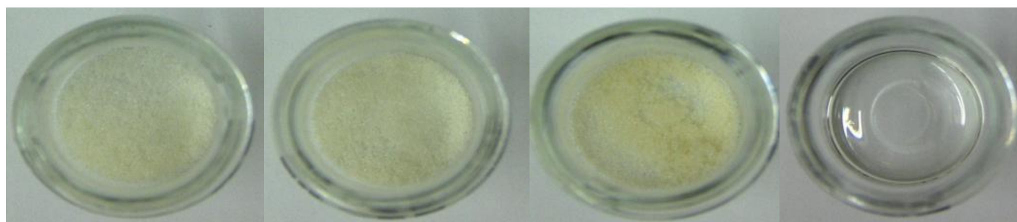


Figure 4.3. Tagatose at 20°C after 12 months, left to right, 33, 54, 75 and 85% RH.

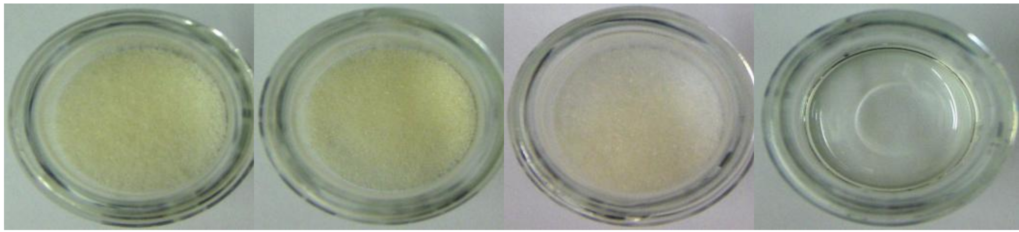


Figure 4.4. Tagatose at 30°C after 12 months, left to right, 33, 54, 75 and 85% RH.

Figure 4.5. Tagatose at 40°C after 12 months, left to right, 33, 54, 75 and 85% RH.

Salameh and Taylor (2006b) investigated caking in single and binary physical mixtures by cycling the mixtures above and below RH_0. The study found a large amount of caking occurred for single components and mixtures that were exposed to RH above RH_0 and RH_{0mix} and subsequently stored at a lower RH. Control mixtures that were stored below RH_{0mix} remained free flowing. Single components that served as controls were cycled above and below RH_{0mix}, but below the RH_0 of the individual components formed fragile cakes (Salameh and Taylor 2006b). Conversely, components that were cycled above RH_0 formed strong cakes. This study demonstrated that cycling (exposing ingredients above and below RH_0) contributes to caking and the importance of storing components below their RH_0 or RH_{0mix} for optimum physical stability.

Critical Relative Humidity

Because deliquescence was observed, it is desirable to identify the critical relative humidity (RH_0) at which tagatose deliquesces. The average RH_0 values obtained by measuring the water activity of saturated tagatose solutions at 20, 30, and 40°C were 85.5%, 83.3% and 81.0%, respectively. The actual RHs of the desiccators stored at 20, 30 and 40°C were 85.1, 83.5 and 82.3%, respectively. Samples stored at 85%RH/30°C and 85%RH/40°C were slightly above their RH_0s (Table 4.3), causing complete deliquescence to occur faster than at 20°C. Numerous studies have shown that if ingredients are stored below their RH_0, they are less susceptible to physical and chemical instability (Salameh and others 2006; Salameh and Taylor 2006a, Mauer and Taylor 2010). Mauer and Taylor (2010) reported that RH_0 can vary depending upon temperature. This largely depends on the solubility of the ingredient. For example, the RH_0 of sucrose decreases from approximately 86% RH at 20°C to 83% RH at 40°C. Another compound whose RH_0 is drastically affected by changes in temperature is ranitidine HCl (active compound in Zantac™). Increasing the temperature from 15 to 40°C caused RH_0 to decrease from 79% to 70% (Mauer and Taylor 2010). Both temperature and RH work together to influence the physical stability of powdered ingredients.

Table 4.3. Environmental RH in which tagatose deliquescence was observed and RH_0 of tagatose at 20, 30 and 40°C.

Temperature (°C)	RH[a]	RH_0[b]
20	85.1	85.5
30	83.5	83.3
40	82.3	81.0

[a] from Bell and Labuza (2000)
[b] averages obtained from water activity meters

Tagatose Browning

During storage, powdered tagatose was observed to brown to varying extents depending upon RH and temperature. Tagatose browning was more extensive at 40°C. Browning was quantified by measuring the optical density of tagatose dissolved in 20 mL water and subtracting the OD/g of the control (i.e., held over drierite). The tagatose stored at 85%RH had the largest absorbance for preliminary browning (280 nm) as shown in Figure 4.6. Tagatose at 75%RH had the largest absorbance for brown pigment formation (420 nm) as shown in Figure. 4.7. The rate constants (OD/g/d) for preliminary activity and brown pigment formation were obtained using the slopes of each line in Figures 4.6 and 4.7. The line for tagatose stored at 85%RH/40°C had the largest slope for preliminary activity, and therefore the greatest rate constant (Figure 4.6 and Table 4.4). Likewise, the slope of the line at 75%RH/40°C was the greatest for brown pigment formation, so the rate constant was the largest (Figure 4.7 and Table 4.4). The rate constants for early stages of browning and brown pigment formation generally increased as temperatures increased, with the highest rate constants at 40°C (Table 4.4). At 40°C, all samples showed visible browning at each RH with pronounced browning at 75 and 85% RH, whereas no visible browning was observed at 20°C. Figure 4.5, presented

earlier, shows pictures of the enhanced browning of samples stored at 40°C as well as deliquescence of the samples at 85% RH.

When observing the qualitative data, it appears the samples became browner as time increased (Figure 4.8), which is reflected in the quantitative data (Figures 4.6 and 4.7). Results indicated that the rate of brown pigment formation was highest at 75%RH/40°C. Due to the phase change (deliquescence), which was associated with high moisture gain, the reactants leading to brown pigment formation became diluted at 85%RH/40°C. The pH of this sample also decreased (data shown later), which reduces the rate of browning reactions (Hodge 1953). Therefore, the rate constant for brown pigment formation was reduced. Samples stored at 33 and 54% RH at 40°C browned similarly as reflected by their brown pigment formation rate constants (Table 4.4) and pictures (Figure 4.8). However, as the RH continued to increase, samples became noticeably darker with a caramel-like color. When examining the plot for early stages of browning, the lines for 33 and 54% RH are virtually flat with no slope, indicating there was minimal reactivity occurring. Similar results were seen in the plot for brown pigment formation.

In nonenzymatic browning at water activity values of 0.5-0.8, the browning rate usually increases with water activity (Labuza and Baiser 1992). Increased a_w results in greater mobility as well as dissolution of solutes, which causes the material to become rubbery. However, at low a_w the water is bound via hydrogen bonds and reactions do not take place readily (Labuza and Baiser 1992). A maximum is usually obtained followed by a decline due to dilution of reactants (Vaikousi and others 2008). This trend was observed in the current study as shown in Figure 4.9, where a maximum in browning was

reached around 75% RH. This maximum was followed by a decline in reactivity around 85% RH. Although Labuza and Baiser (1992) were investigating Maillard browning, a similar trend in browning was observed in the powdered tagatose, which was presumed to be caused by caramelization.

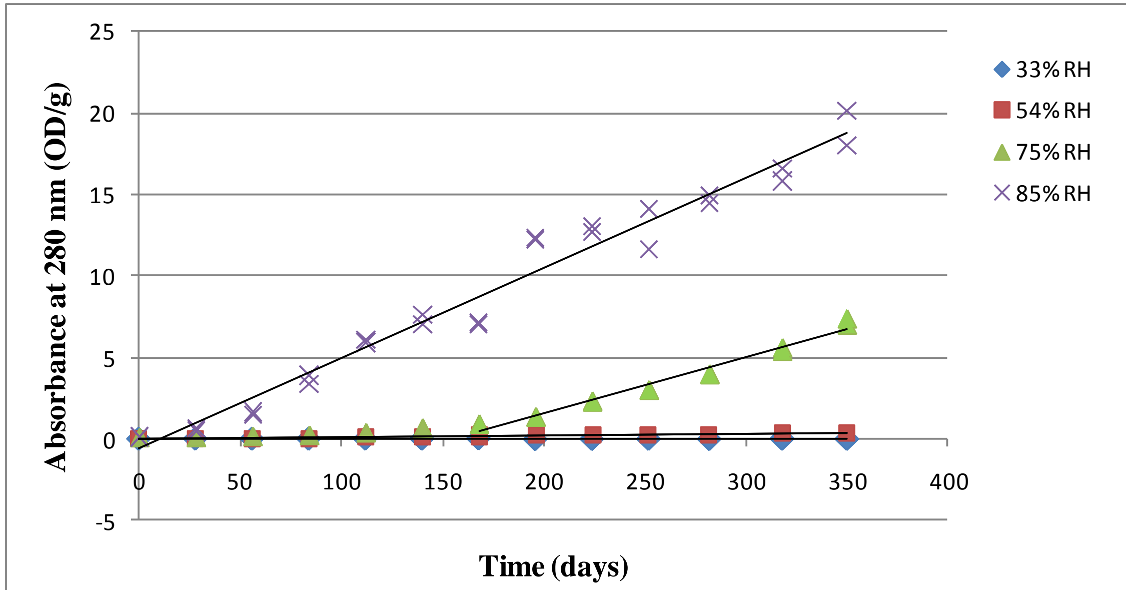


Figure 4.6. Early stages of browning in tagatose at 40°C as affected by RH.

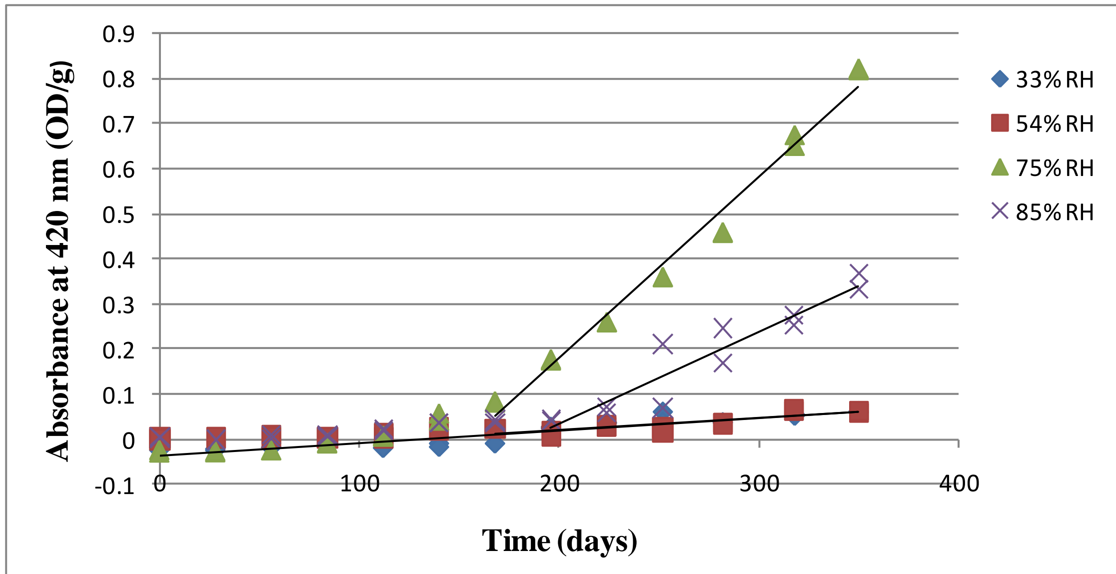


Figure 4.7. Brown pigment formation in tagatose at 40°C as affected by RH.

Table 4.4. Pseudo-zero order rate constants (OD/g/d) with 95% CL times 10^4 for browning of tagatose at 20, 30 and 40°C.

Relative Humidity	20°C	30°C	40°C
280 nm[a]			
33%	0[c]	1.3±0.47	11.5±1.4
54%	0.59±0.39	2.5±0.56	11.1±0.76
75%	2.4±1.1	0[c]	345±35
85%	2.8±1.3	38.5±10.5	554±40.2
420 nm[b]			
33%	0[c]	0.63±0.44	2.7±0.45
54%	0[c]	0[c]	2.7±0.1
75%	0.94±0.32	0.41±0.38	40.2±3.2
85%	0[c]	0.48±0.25	20.5±4.8

[a]= early stages of browning, [b]= brown pigmentation formation, [c]= rate constants that were statistically equivalent to zero based on the 95% CL.

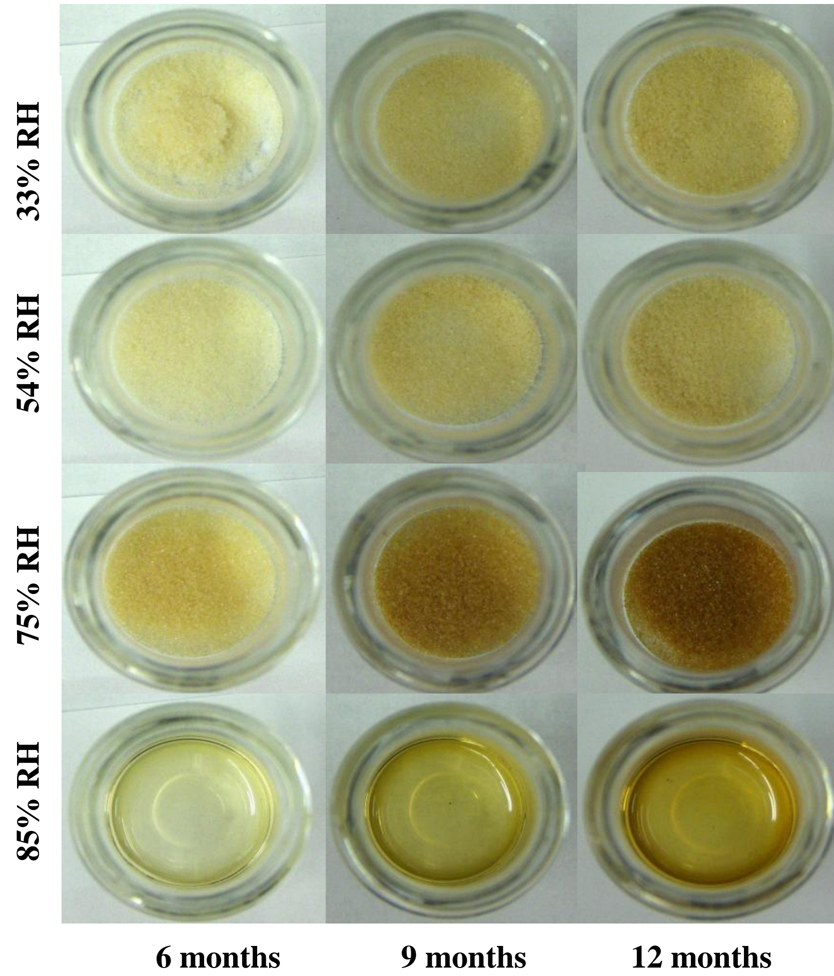


Figure 4.8. The effect of time on browning of tagatose at 40°C, left to right, after 6 months, 9 months and 12 month. Tagatose at 33, 54, 75 and 85% RH top to bottom.

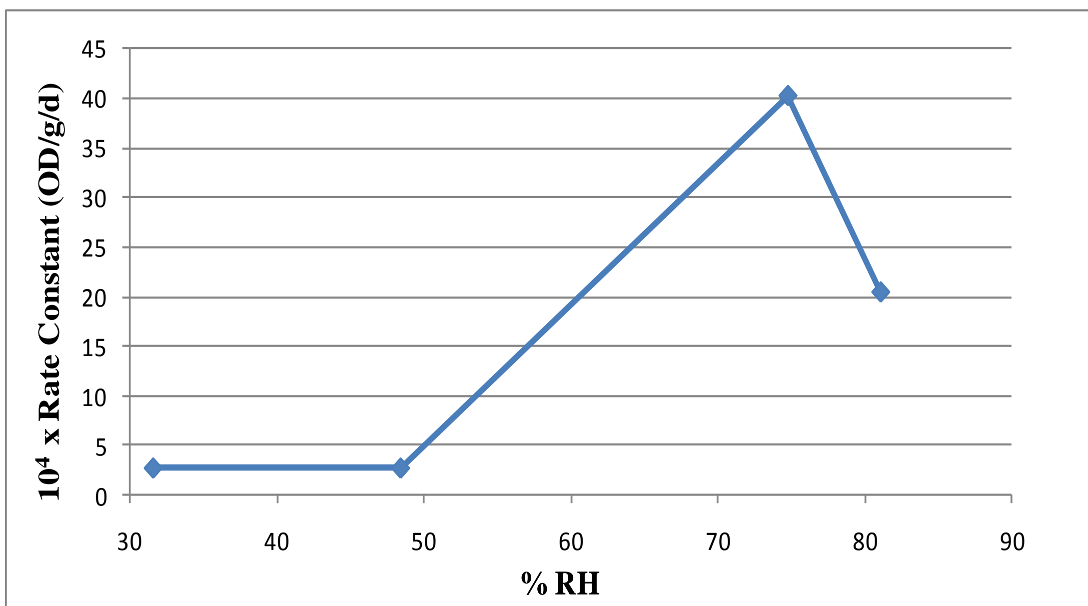


Figure 4.9. Rate constants for tagatose browning (420 nm) as a function of %RH at 40ºC.

Hiatt and others (2008; 2010) also captured deliquescence and browning in sodium ascorbate at 85 and 98% RH. In one study (Hiatt and others 2008), a combination of pyridoxine HCl and sodium ascorbate also showed browning at 98% RH after 12 weeks. Binary (thiamin HCl/ sodium ascorbate) and tertiary (thiamin HCl/sodium ascorbate/ pyridoxine HCl) also showed browning at the same conditions.

Similarly, browning was observed in tagatose solutions held at various conditions (Dobbs and Bell 2010; Luecke and Bell 2010). Dobbs and Bell (2010) observed enhanced browning at 40°C. Browning of tagatose was most enhanced in the sample with 0.1 M phosphate buffer at pH 7 at 40°C. Luecke and Bell (2010) noticed that as temperature increased, the rate of browning also increased. The rate constants obtained for tagatose browning in 0.1 M phosphate buffer at pH 7 at 60 and 80°C were 0.00661±0.00044 h^{-1} and 0.0756±0.0070 h^{-1}, respectively. Likewise, faster browning

was also observed in the samples exposed to higher concentrations of phosphate buffer at the higher pH (Dobbs and Bell 2010; Luecke and Bell 2010). Since there were no amino acids present in the solutions, the reaction taking place was believed to be a caramelization reaction in both studies (Dobbs and Bell 2010; Luecke and Bell 2010). Conversely, Ryu and others (2004) did not observe any browning or degradation in 10% tagatose solutions heated up to 100°C for 5 hours. However, when combined with glycine, browning and degradation were observed; this was attributed to the Maillard reaction (Ryu and others 2003). Although the powdered tagatose stored at 40°C was generally in a solid form (exception is 85%RH/40°C), it also probably underwent a caramelization reaction.

Caramelization can be defined as a "non-enzymatic sugar browning reaction…which generates a brown color and a caramel-like flavor" (Lee and Lee 1997). Caramelization may be characterized by heat-induced decomposition of monosaccharides. Caramelization includes reactions such as enolization, dehydration, dicarboxylic cleaving and aldol condensation. A release of H^+ usually accompanies the reaction, thus decreasing the pH (Kroh 1994).

Chemical Stability

A combination of high RH and high temperature was required to achieve chemical instability in the tagatose samples. Tagatose degradation only occurred at one environmental condition (85%RH/40°C), which was slightly above RH_0. This sample had the largest rate constant for preliminary browning and was fully deliquesced. During the 12 month period, about a 20% loss of tagatose occurred in the sample, but the loss leveled off around 6 months without further degradation. Although all samples

deliquesced at 85% RH, only the sample stored at 40°C displayed measurable degradation. Samples stored at 33, 54 and 75% RH at 40°C showed approximately 100% tagatose remaining upon HPLC analysis (Figure 4.10). No loss was observed at 20 and 30°C either.

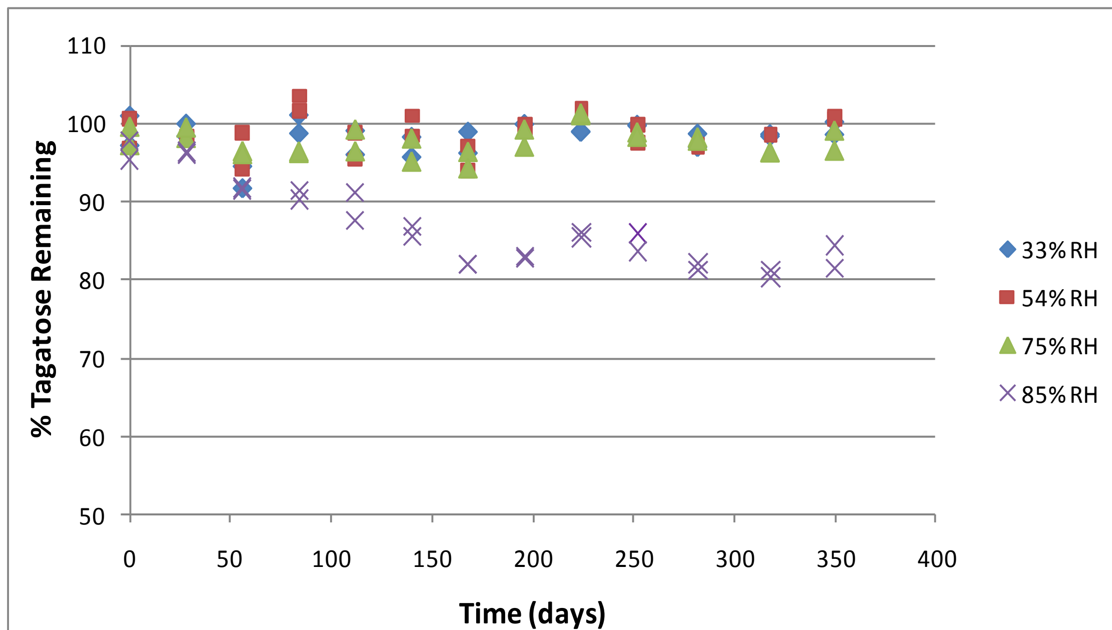


Figure 4.10. Chemical degradation of powdered tagatose at 40°C as affected by RH.

A combination of factors caused the dissolution and degradation of tagatose at 85%RH/40°C. The higher temperature contributed to a greater amount of energy, which may have accelerated the reaction. Likewise, the higher RH may have also accelerated the reaction by causing reactants to become more mobile, therefore increasing interaction. Mauer and Taylor (2010) reported that crystalline solids generally have greater chemical stability than amorphous materials or solutions. Mobility is generally limited in crystalline solids, whereas it is higher in solutions (Mauer and Taylor 2010).

Furthermore, if a crystalline ingredient, such as tagatose, undergoes deliquescence, chemical stability will most likely be affected adversely.

Although the mechanism for degradation of powdered tagatose is unclear, assumptions can be made based on the pH and appearance (caramelization) of the tagatose. In addition, some similarities to tagatose degradation in solution can be identified. The pH values of tagatose dissolved in water were obtained at 6, 9 and 12 months. The pH values of the control samples were 5.8-6.0 at 6 months. The solution pH of tagatose held at 85%RH/40°C was approximately 4 at all time periods. On the other hand, all other samples had pH values ranging from approximately pH 5-6.3. When tagatose stability was investigated in solution, pH decreased from neutral to mildly acidic as tagatose degraded (Dobbs and Bell 2010; Luecke and Bell 2010). The lower pH was attributed to the acidic degradation products and was believed to be responsible for stopping degradation. A plateau was also associated with tagatose loss (Dobbs and Bell 2010), which was also observed in the deliquesced tagatose around 6 months (Figure 4.10). The initial tagatose loss may be the result of a similar alkaline degradation reaction as described by Dobbs and Bell (2010). In solution, monosaccharides undergo a series of rearrangements including, ionization, mutarotation, enolization and isomerization to create an enediol anion species. The species may then participate in several other reactions including β-elimination, retro-aldol reaction, and aldolization reactions. The series of reactions result in carboxylic acid degradation products, thus lowering the pH and slowing the reaction (de Bruijn and others 1986). It is important to note that caramelization may also decrease pH.

Based on the similarities to the studies mentioned above (degradation plateau, pH decrease, brown pigment formation), it can be inferred that the tagatose at 85%/40°C may be participating in a similar alkaline degradation reaction. Tagatose at 85%/40°C was fully deliquesced and brown, so it may very well display behavior similar to alkaline degradation in solution. The behavior is not exactly like the tagatose solutions due to buffers that were present in solution, which allowed the systems to resist changes in pH and also catalyze the reaction (Dobbs and Bell 2010; Luecke and Bell 2010).

As mentioned earlier, chemical instability was observed in tagatose solutions (Dobbs and Bell 2010; Luecke and Bell 2010). Tagatose in solution was affected by temperature, buffer type and buffer concentration in both studies. Dobbs and Bell (2010) investigated tagatose loss in solution at 20, 30 and 40°C. Following one month at 40°C, Dobbs and Bell (2010) observed a 29% loss of tagatose in 0.1M phosphate buffer at pH 7 compared to less than 1% loss at pH 3 under the same conditions. The citrate buffer also resulted in less loss compared to the phosphate buffer. Luecke and Bell (2010) investigated tagatose loss at 60, 70 and 80°C and similar results were observed in the study. The interesting observation is that tagatose degradation was favored at the higher pH in higher concentrations of phosphate buffer. Degradation was greatly reduced at lower pH levels and in the absence of buffer. The powdered tagatose, once deliquesced, displayed degradation without buffer present and at relatively low pH levels. Dobbs and Bell (2010) reported the greatest tagatose loss at an acidic condition to be 5% in 6 months (0.1 M citrate buffer at pH 3 and 40°C). The deliquesced tagatose at 85% RH and 40°C experienced approximately 20% loss in 6 months.

Hiatt and others (2008) also looked at the effect of deliquescence on the chemical stability of vitamins B_1, B_6, and C in powder blends. In the study, thiamin HCl (RH_0= 89%) degradation ranged from 0-29.8%. Thiamin HCl (vitamin B_1) that was stored below the RH_0 (54%) had no chemical degradation and showed slight caking. However, samples that were stored above (98% RH) or near the RH_0 (85%) displayed significant degradation compared to the samples below RH_0. In addition, the thiamin HCl stored at 98% RH deliquesced after 12 weeks (Hiatt and others 2008). Pyridoxine HCl (vitamin B_6) degradation ranged from 7-25.3%. The degradation of pyridoxine was not greatly affected by RH because it is less soluble, has a higher RH_0 and normally undergoes degradation via light.

Salameh and Taylor (2006) also conducted a study looking at the role of deliquescence lowering in increasing chemical reactivity in physical mixtures. The ingredients that were investigated included crystalline sucrose, β-D-fructose, and α-glucose monohydrate. The RH_0 of mixtures (RH_{0mix}) has been shown to create more chemical instability because when two ingredients are mixed, the RH_{0mix} is even lower than the individual component RH_0 values. The RH_{0mix} for a sucrose/citric acid mixture was 64% RH. When the mixture was exposed to a RH at or above 65% RH, complete sucrose inversion occurred. Sucrose inversion is an indication of degradation because the sucrose is breaking down into fructose and glucose. Control samples of sucrose (RH_0=85%) alone that were stored at 85 and 71% showed no degradation. Likewise, sucrose/citric acid mixtures stored below the RH_{0mix} at 54 and 43% RH showed no discernible degradation. The previous two studies give a clear indication that if ingredients are stored above the RH_0 or RH_{0mix}, chemical degradation can occur.

Another explanation for the tagatose degradation is the synergistic effect of RH and temperature. Hiatt and others (2010) conducted a study that looked at the effect of temperature (4, 25, 35 and 40°C) and RH (54, 64, 75, and 85% RH) on vitamin C stability. The study found that both RH and temperature impacted vitamin C stability, but the impact of RH was greater. Temperature and RH behaved synergistically. Higher temperatures caused better vitamin C solubility, which resulted in a reduction in RH_0. Consequently, the vitamin C experienced deliquescence, degradation, and browning (Hiatt and others 2010). Temperature and RH also worked synergistically to cause deliquescence, degradation, and browning in tagatose.

Chapter 5: Summary and Conclusions

Powdered tagatose physical and chemical stabilities were affected to varying extents by RH and temperature. All samples experienced varying amounts of physical instability except for the sample stored at 33% RH/20°C. This sample remained free flowing and was very similar to the control tagatose sample stored over drierite. All samples stored at 85% RH deliquesced and adsorbed large quantities of water. Although samples experienced varying amounts of caking and browning, chemical degradation only occurred in the tagatose sample stored at 85%RH/40°C. This sample had a 20% loss following 6 months of storage. Browning was most pronounced in all samples stored at 40°C. Although no correlation was made between chemical degradation and the prebiotic property of tagatose, it is predicted that the beneficial property will be lessened.

It is unlikely that tagatose would be stored for 1 year without use by a food company since materials are ordered as needed and the shelf life is listed as 6 months by the manufacturer. The specifications included with the powdered tagatose stated that it could be stored at 65% RH at 20°C for 6 months. Based on the study conducted, tagatose would cake at this storage condition. The study provides evidence that tagatose should be stored at 33% RH/20°C to remain a free flowing powder with minimal to no chemical degradation. It is also evident that although chemical degradation is not likely to occur, adverse physical changes are probable. Therefore, steps should be taken to keep tagatose stored in a cool, dry location. The shelf life could possibly be extended if stored at these conditions.

References

Citation Style: Journal of Food Science

Adhikari B, Howes T, Bhandari BR, Truong V. 2001. Stickiness in foods: a review of mechanisms and test methods. Int J Food Prop 4(1):1-33.

Aguilera, JM, Del Valle JM, Karel M. 1995. Caking phenomena in amorphous food powders. Trends Food Sci Tech 6:149-155.

Armstrong LM, Luecke KJ, Bell LN. 2009. Consumer evaluation of bakery product flavor as affected by incorporating the prebiotic tagatose. Int J Food Sci Technol 44(4):815-819.

Bar A. 2004. D-Tagatose, Dossier prepared and submitted on behalf of Arla Food Ingredients amba, Viby, Denmark for evaluation pursuant to EU Novel Foods Regulation (EC) 258/97 by the UK Advisory Committee on Novel Foods and Processes. Available from http://www.food.gov.uk/multimedia/pdfs/tagatoseapplicationdossier.pdf (Accessed January 18, 2010).

Bell LN. 2007. Moisture effect on food's chemical stability. In Barbosa-Canovas GV, Fontanta AJ, Schmidt SJ, Labuza TP. Water Activity in Foods. Ames, Iowa: Blackwell Publishing and Institute of Food Technologists. p 173-198.

Bell LN, Labuza TP. 2000. Moisture: Sorption Practical Aspects of Isotherm Measurement and Use. 2nd ed. St. Paul, MN: American Association of Cereal Chemists, Inc. p 1-6.

Bertelsen H, Andersen H, Tvede M. 2001. Fermentation of d-tagatose by human intestinal bacteria and dairy lactic acid bacteria. Microb Ecol Health Dis 13(2):87-95.

Buemann B, Toubro S, Astrup A. 1998. D-Tagatose, A stereoisomer of d-fructose, increase hydrogen production in humans without affecting 24 –hour energy expenditure or respiratory exchange ratio. J Nutr 128(9):1481-1486.

Buemann B, Toubro S, Astrup A. 1999. Human gastrointestinal tolerance to d-tagatose. Regul Toxicol Pharm 29(2):S71-S77.

Buemann B, Toubro S, Raben A, Blundell J, Astrup A. 2000. The acute effect of d-tagatose on food intake in human subjects. Brit J Nutr 84(2):227-231.

Chirife J, Jr. Fontana AJ. 2007. Introduction: Historical highlights of water activity research. In Barbosa-Canovas GV, Fontanta AJ, Schmidt SJ, Labuza TP. Water Activity in Foods. Ames, Iowa: Blackwell Publishing and Institute of Food Technologists. p 3-9.

De Bruijn JM, Kieboom APG, van Bekkum H, van der Poel PW. 1986. Reactions of monosaccharides in aqueous alkaline solutions. Sugar Technol Rev 13:21-52.

Dobbs CM, Bell LN. 2010. Storage stability of tagatose in buffer solutions of various compositions. Food Res Int 43:382-386.

Farkas, BE and Singh RP. 1991. A study of the structure of air and freeze-dried white chicken meat using scanning electron microscopy. J Food Sci 56(3):611-615.

FDA. 2001. Agency Response Letter, GRAS Notice No. GRN 000078. Available from http://www.fda.gov/ohrms/DOCKETS/dailys/02/Apr02/042302/02p-0177-cp00001-03-Tab-02-vol1.pdf (Accessed on July 10, 2009).

Hancock BC, Shamblin SL. 1998. Water vapour sorption by pharmaceutical sugars. Pharm Sci Tech Today 1(8):345-351.

Hiatt AN, Ferruzzi MG, Taylor LS, Mauer LJ. 2008. Impact of deliquescence on the chemical stability of vitamins B1, B6, and C in powder blends. J Agric Food Chem 56(15):6471-6479.

Hiatt AN, Taylor LS, Mauer LJ. 2010. Influence of simultaneous variations in temperature and relative humidity on chemical stability of two vitamin C forms andi for shelf life models. J Agric Food Chem 58(6):3552-3540.

Hicsasmaz Z, Clayton JT. 1992. Characterization of the pore structure of starch based food materials. Food Struct 11:115-132.

Hodge J. 1953. Dehydrated foods chemistry of browning reactions in model systems. J Agric Food Chem 15(1):928-943.

Karmas R, Buera MP, Karel M. 1992. Effect of glass transition on rates of nonenzymatic browning in food systems. J Agric Food Chem 40:873-879.

Kelley FHC, Mak FK, Shah D. 1974. Some hygroscopic properties of refined sugar. Int Sugar J 7 (912):361–363.

Kroh LW. 1994. Caramelization in food and beverages. Food Chem 51(4):373-379.

Kwok K, Mauer LJ, Taylor LS. 2010. Phase behavior and moisture absorption of deliquescent powders. Chem Eng Sci 65(21):5639-5650.

Labuza TP, Altunakar B. 2007. Water activity prediction and moisture sorption isotherms. In Barbosa-Canovas GV, Fontanta AJ, Schmidt SJ, Labuza TP. Water Activity in Foods. Ames, Iowa: Blackwell Publishing and Institute of Food Technologists. p 109-151.

Labuza TP, Baisier WM. 1992. The kinetics of nonenzymatic browning. In Schwartzberg HG, Hartel RW. Physical Chemistry of Foods. New York, NY: Marcel Dekker, Inc. p 595-646.

Labuza TP, Kamman JF. 1983. Reaction kinetics and accelerated tests simulation as a function of temperature. In Saguy I. Computer-aided techniques in food technology. New York: Marcel Dekker. p 71-115.

Laerke HN, Jensen BB. 1999. D-Tagatose has low small intestinal digestibility but high large intestinal fermentability in pigs. J Nutr 129(5):1002-1009.

Laerke HN, Jensen BB, Hojsgaard S. 2000. In vitro fermentation pattern of d-tagatose is affected by adaptation of the mcrobiota from the gastrointestinal tract of pigs. J Nutr 130:1772-1779.

Lee GC, Lee CY. 1997. Inhibitory effect of caramelization products on enzymatic browning. Food Chem 60(2):231-235.

Lee A, Storey DM. 1999. Comparative gastrointestinal tolerance of sucrose, lactitol, or d-tagatose in Chocolate. Regul Toxicol Pharm 29(2):S78-S82.

Levin GV. 2002. Tagatose, the new GRAS sweetener and health product. J Med Food 5(1):23-36.

Livesey GV, Brown JC. 1996. D-Tagatose is a bulk sweetener with zero energy determined in rats. J Nutr 126(6):1601-1609.

Lu Y, Levin GV, Donner TW. 2007. Tagatose, a new antidiabetic and obesity control drug. Diabetes Obes Metab 10(2):109-134.

Luecke KJ, Bell LN. 2010. Thermal stability of tagatose in solution. J Food Sci 75(4): C346-C351.

Mathlouthi M, Roge B. 2003. Water vapour sorption isotherms and the caking of food powders. Food Chem 82(1):61-71.

Mauer LJ, Taylor LS. 2010. Water-solids interactions: deliquescence. Annu Rev Food Sci Technol 1:41-63.

McWatters KH, Ouedraogo JB, Resurreccion A, Hung YC, Phillips RD. 2003. Physical and sensory characteristics of sugar cookies containing mixtures of wheat, fonio (*digitaria exilis*) and cow pea (*vigna unguiculata*) flours. Int J Food Sci Technol 38(4):403-410.

Moyer AE, Rodin J. 1993. Fructose and behavior: does fructose influence food intake and macronutrient selection? Am J Clin Nutr 58(5):810S-814S.

Oh D. 2007. Tagatose: properties, applications, and biotechnological processes. Appl Microbiol Biotechnol 76(1):1-8.

Peckenpaugh DJ. 2006. Tagatose Wins EU Approval. Available from http://www.foodproductdesign.com/news/2006/02/tagatose-wins-eu-approval.aspx (Accessed January 23, 2010).

Peleg M. 1983. Physical characteristics of food powders. In Peleg M, Bagley EB. Physical Properties of Foods. Westport, CT: AVI Publishing Company. p 293-323.

Peleg M, Mannheim CH. 1969. The mechanism of caking powdered onion. J Food Process Preserv 1(1):3-11.

Reid DS. 2007. Water activity: fundamentals and relationships. In Barbosa-Canovas GV, Fontanta AJ, Schmidt SJ, Labuza TP. Water Activity in Foods. Ames, Iowa: Blackwell Publishing and Institute of Food Technologists. p 15-28.

Roberfroid MB. 2000. Prebiotics and probiotics: are they functional foods? Am J Clin Nutr 71(6):1682S-1687S.

Roos YH. 2007. Water activity and glass transition. In Barbosa-Canovas GV, Fontanta AJ, Schmidt SJ, Labuza TP. Water Activity in Foods. Ames, Iowa: Blackwell Publishing and Institute of Food Technologists. p 29-45.

Roudaut G. 2007. Water activity and physical stability. In Barbosa-Canovas GV, Fontanta AJ, Schmidt SJ, Labuza TP. Water Activity in Foods. Ames, Iowa: Blackwell Publishing and Institute of Food Technologists. p 199-210.

Ryu SY, Roh HJ, Noh BS, Kim SY, Oh DK, Lee WJ, Yoon JR, Kim SS. 2003. Effects of temperature and pH on the non-enzymatic browning reaction of tagatose-glycine model system. Food Sci Biotechnol 12(6):675-9.

Salameh AK, Taylor LS. 2005. Deliquescence in binary mixtures. Pharm Res 22(2):318-323.

Salameh, AK, Mauer LJ, Taylor LS. 2006. Deliquescence lowering in food ingredient mixtures. J Food Sci 71(1):E10-E16.

Salameh AK, Taylor LS. 2006a. Deliquescence –induced caking in binary powder blends. Pharm Dev Technol 11(4):453-464.

Salameh AK, Taylor LS. 2006b. Role of deliquescence lowering in enhancing chemical reactivity in physical mixtures. J Phys Chem B 110(20):10190-10196.

Saunders JP, Zehner LR, Levin GV. 1999. Disposition of D-[U-14C] tagatose in the rat. Regul Toxicol Pharm 29(2):S46-S56.

Siro I, Kapolna E, Kapolna B, Lugasi A. 2008. Functional food. Product development, marketing and consumer acceptance—A review. Appetite 51(3):456-467.

Taylor TP, Fasina O, Bell LN. 2008. Physical properties and consumer liking of cookies prepared by replacing sucrose with tagatose. J Food Sci 73(3):S145-S151.

Teunou E, Vasseur J. 1996. Time flow function: means to estimate water effect on dissoluble bulk materials flow. Powder Hand Proc 8(2):111-116.

Troller JA, Christian JHB. 1978. Water Activity and Food. New York, New York: Academic Press Inc. 61-65.

Vaikousi H, Koutsoumanis K, Biliaderis CG. 2008. Kinetic modeling of non-enzymatic browning of apple juice concentrates differing in water activity under isothermal and dynamic heating conditions. Food Chem 107(2):785-796.

Van Campen L, Amidon GL, Zografi G. 1983. Moisture sorption kinetics for water-soluble substances I: Theoretical considerations of heat transport control. J Pharm Sci 72(12):1381-1388.

Venema K, Vermunt SHF, Brink EJ. 2005. D-Tagatose increases butyrate production by the colonic microbiota in healthy men and women. Microbial Ecol 17(1):47-57.

Wise BC. 2008. Try Tagatose, An Artificial Sweetener. Available from http://www.associatedcontent.com/article/778721/try_tagatose_an_artificial_sweetener.html?cat=5 (Accessed January 23, 2010).

Xiong X, Narsimhan G, Okos, MR. 1991. Effect of composition and pore structure on binding energy and effective diffusivity of moisture in porous food. J Food Eng 15:187-208.

Appendix A

Moisture content, physical appearance, optical density and % tagatose remaining of tagatose powder stored at all environmental conditions

Table A1. Moisture content, physical appearance, optical density and tagatose remaining of samples stored over drierite at room temperature.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	Drierite 1A	0.03043	free flowing	0.171	0.00487	97.54
0	Drierite 1B	0.09944	free flowing	0.169	0.00365	99.22
28	Drierite 2A	0.2510	free flowing	0.172	0.00628	99.57
28	Drierite 2B	0.2405	free flowing	0.171	0.00601	97.87
56	Drierite 3A	0.2617	free flowing	0.167	0.00552	100.38
56	Drierite 3B	-0.02443	free flowing	0.172	0.00562	94.83
84	Drierite 4A	0.3689	free flowing	0.178	0.00851	100.18
84	Drierite 4B	0.2800	free flowing	0.178	0.00653	97.70
112	Drierite 5A	0.2485	free flowing	0.182	0.00963	93.85
112	Drierite 5B	-0.2968	free flowing	0.179	0.00759	92.06
140	Drierite 6A	0.09271	free flowing	0.183	0.00618	99.98
140	Drierite 6B	0.3424	free flowing	0.192	0.00871	99.96
168	Drierite 7A	0.3827	free flowing	0.176	0.00902	90.44
168	Drierite 7B	0.4760	free flowing	0.174	0.00254	94.66
196	Drierite 8A	0.3645	free flowing	0.203	0.0129	99.40
196	Drierite 8B	0.5068	free flowing	0.172	0.0101	99.65
224	Drierite 9A	0.6930	free flowing	0.176	0.00181	102.54
224	Drierite 9B	0.7203	free flowing	0.180	0.00345	99.50
252	Drierite10A	0.4956	free flowing	0.157	-0.00204	99.31
252	Drierite 10B	0.7982	free flowing	0.165	0.0128	99.70
282	Drierite11A	0.8173	free flowing	0.164	0.0389	100.12
282	Drierite 11B	0.6422	free flowing	0.171	0.0428	99.69
318	Drierite12A	0.6834	free flowing	0.194	-0.00746	98.51
318	Drierite 12B	0.5270	free flowing	0.188	-0.00620	98.06
350	Drierite13A	0.6878	free flowing	0.193	0.00807	101.37
350	Drierite 13B	0.5159	free flowing	0.180	0.00593	100.25

Table A2. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 33% RH at 20°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	33-20-1A	0.4268	free flowing	0.187	-0.00711	98.78
0	33-20-1B	-0.3216	free flowing	0.181	-0.00397	97.35
28	33-20-2A	0.2613	free flowing	0.188	-0.00402	99.13
28	33-20-2B	0.0377	free flowing	0.198	-0.00264	98.86
56	33-20-3A	0.3212	free flowing	0.187	-0.00505	98.38
56	33-20-3B	-2.685	free flowing	0.192	-0.00290	95.53
84	33-20-4A	-0.1361	free flowing	0.185	-0.0127	92.85
84	33-20-4B	0.03965	free flowing	0.208	-0.00833	95.63
112	33-20-5A	-0.2936	free flowing	0.203	-0.00822	94.40
112	33-20-5B	0.3106	free flowing	0.198	-0.00580	97.26
140	33-20-6A	-0.2446	free flowing	0.204	-0.00828	100.52
140	33-20-6B	0.2629	free flowing	0.212	-0.00627	99.17
168	33-20-7A	0.4711	free flowing	0.211	-0.00621	100.84
168	33-20-7B	0.3718	free flowing	0.210	-0.0117	99.92
196	33-20-8A	0.2969	free flowing	0.179	0.00959	96.78
196	33-20-8B	0.4288	free flowing	0.175	0.00453	99.11
224	33-20-9A	0.6711	free flowing	0.176	0.00183	100.98
224	33-20-9B	0.5110	free flowing	0.182	0.00266	101.61
252	33-20-10A	0.3055	free flowing	0.166	-0.00131	98.70
252	33-20-10B	0.5215	free flowing	0.166	-0.000869	98.89
282	33-20-11A	0.5495	free flowing	0.169	0.0132	98.81
282	33-20-11B	0.6721	free flowing	0.161	0.0103	96.76
318	33-20-12A	0.4355	free flowing	0.238	-0.000207	97.63
318	33-20-12B	0.3607	free flowing	0.200	-0.00180	97.68
350	33-20-13A	0.4109	free flowing	0.193	0.00799	100.34
350	33-20-13B	0.3375	free flowing	0.192	0.00675	100.32

Table A3. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 33% RH at 30°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	33-30-1A	-0.1355	partially caked	0.196	-0.00697	98.66
0	33-30-1B	0.04187	partially caked	0.195	-0.0105	98.72
28	33-30-2A	-0.02240	fully caked	0.205	-0.0125	98.84
28	33-30-2B	-0.09508	fully caked	0.211	0.00837	101.35
56	33-30-3A	-0.2052	slightly caked	0.212	-0.0117	96.69
56	33-30-3B	0.0000	slightly caked	0.204	-0.0113	105.28
84	33-30-4A	0.08339	slightly caked	0.209	-0.0136	100.16
84	33-30-4B	-0.3167	slightly caked	0.210	-0.0174	100.34
112	33-30-5A	0.1436	free flowing	0.215	-0.00923	97.23
112	33-30-5B	0.0000	free flowing	0.218	-0.0113	95.71
140	33-30-6A	-0.6682	slightly caked	0.229	-0.0124	100.60
140	33-30-6B	0.01962	slightly caked	0.231	-0.0129	100.68
168	33-30-7A	0.4324	partially caked	0.243	-0.00827	95.50
168	33-30-7B	0.2383	partially caked	0.239	-0.00814	96.82
196	33-30-8A	0.1434	partially caked	0.212	0.00771	99.61
196	33-30-8B	0.1776	partially caked	0.212	0.00888	99.18
224	33-30-9A	0.3393	partially caked	0.230	0.0132	101.37
224	33-30-9B	0.3537	partially caked	0.219	0.00791	101.61
252	33-30-10A	0.3484	partially caked	0.202	0.00261	99.95
252	33-30-10B	0.2839	partially caked	0.209	0.00454	99.24
282	33-30-11A	0.3387	partially caked	0.240	0.0134	98.60
282	33-30-11B	0.05767	partially caked	0.217	0.00961	98.90
318	33-30-12A	0.2733	partially caked	0.272	0.00605	97.41
318	33-30-12B	0.06333	partially caked	0.260	0.00274	98.28
350	33-30-13A	0.0000	slightly caked	0.260	0.0197	98.63
350	33-30-13B	0.05673	partially caked	0.274	0.0151	101.21

Table A4. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 33% RH at 40°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	33-40-1A	-0.4622	fully caked	0.0624	-0.0153	101.07
0	33-40-1B	-0.3610	fully caked	0.211	-0.0214	97.23
28	33-40-2A	-0.4404	fully caked	0.257	-0.0167	98.45
28	33-40-2B	-0.1940	fully caked	0.245	-0.0199	100.04
56	33-40-3A	-0.07278	fully caked	0.267	-0.0138	91.68
56	33-40-3B	-0.2684	fully caked	0.266	-0.0137	94.53
84	33-40-4A	-0.2178	fully caked	0.320	-0.00139	101.17
84	33-40-4B	-0.06306	fully caked	0.342	-0.000210	98.80
112	33-40-5A	-0.2400	fully caked	0.358	-0.0122	99.15
112	33-40-5B	-0.1930	fully caked	0.359	-0.0113	96.04
140	33-40-6A	-0.2877	fully caked	0.414	-0.00310	98.33
140	33-40-6B	-0.02470	fully caked	0.406	-0.0109	95.73
168	33-40-7A	0.09706	fully caked	0.451	-0.00461	99.00
168	33-40-7B	0.1419	fully caked	0.453	-0.00497	96.25
196	33-40-8A	0.0000	fully caked	0.446	0.0376	99.99
196	33-40-8B	0.04792	fully caked	0.431	0.0345	99.98
224	33-40-9A	0.1444	fully caked	0.473	0.0390	98.98
224	33-40-9B	0.4352	fully caked	0.452	0.0369	99.05
252	33-40-10A	0.2407	fully caked	0.498	0.0652	99.79
252	33-40-10B	0.3840	fully caked	0.468	0.0420	99.89
282	33-40-11A	0.2687	fully caked	0.512	0.0764	97.02
282	33-40-11B	0.3068	fully caked	0.518	0.0771	98.75
318	33-40-12A	-0.07065	fully caked	0.545	0.0440	98.67
318	33-40-12B	0.02430	fully caked	0.596	0.0476	98.43
350	33-40-13A	-0.1942	fully caked	0.613	0.0650	98.64
350	33-40-13B	-0.1671	fully caked	0.620	0.0683	100.28

Table A5. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 54% RH at 20°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	54-20-1A	-0.6346	fully caked	0.174	0.00562	98.30
0	54-20-1B	0.09198	fully caked	0.156	0.00515	98.50
28	54-20-2A	0.01977	slightly caked	0.152	0.00119	102.31
28	54-20-2B	0.2851	slightly caked	0.187	0.0101	100.06
56	54-20-3A	-0.4618	slightly caked	0.184	0.00482	100.46
56	54-20-3B	0.1961	slightly caked	0.177	0.00349	99.81
84	54-20-4A	-0.3034	slightly caked	0.170	0.00433	93.33
84	54-20-4B	-0.4213	free flowing	0.162	0.00441	96.14
112	54-20-5A	-0.4829	slightly caked	0.189	0.00984	95.75
112	54-20-5B	-0.2141	slightly caked	0.175	0.00707	101.80
140	54-20-6A	0.3120	slightly caked	0.185	0.00716	98.85
140	54-20-6B	-0.5856	slightly caked	0.195	0.00390	99.21
168	54-20-7A	-0.2871	partially caked	0.180	0.00390	100.94
168	54-20-7B	0.3696	partially caked	0.181	0.00564	97.97
196	54-20-8A	0.06025	partially caked	0.181	0.00562	99.84
196	54-20-8B	-0.3788	partially caked	0.176	0.000421	99.78
224	54-20-9A	0.1482	partially caked	0.187	0.00296	103.60
224	54-20-9B	-2.038	partially caked	0.186	0.00530	98.96
252	54-20-10A	0.6647	partially caked	0.174	0.00236	97.15
252	54-20-10B	0.4300	fully caked	0.171	0.000748	98.99
282	54-20-11A	0.2944	fully caked	0.169	0.0269	98.72
282	54-20-11B	0.3030	fully caked	0.180	0.0277	99.13
318	54-20-12A	-0.2224	fully caked	0.223	0.00425	98.32
318	54-20-12B	0.2023	fully caked	0.202	-0.00465	98.44
350	54-20-13A	0.08963	fully caked	0.211	0.00605	100.34
350	54-20-13B	0.1116	fully caked	0.196	0.00357	100.93

Table A6. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 54% RH at 30°C.

Time (days)	**Sample**	**Moisture Content (%db)**	**Physical Appearance**	**OD/g (280 nm)**	**OD/g (420 nm)**	**% Tagatose remaining**
0	54-30-1A	-0.8316	fully caked	0.151	0.00151	100.84
0	54-30-1B	-0.3540	fully caked	0.155	0.00819	98.92
28	54-30-2A	0.0000	fully caked	0.162	0.00275	99.81
28	54-30-2B	-0.4003	fully caked	0.153	0.000445	98.62
56	54-30-3A	-0.09956	partially caked	0.185	0.00538	96.08
56	54-30-3B	-1.331	partially caked	0.156	0.00148	97.58
84	54-30-4A	-2.016	fully caked	0.174	0.00450	96.17
84	54-30-4B	1.154	fully caked	0.160	0.00266	93.30
112	54-30-5A	-0.7922	fully caked	0.190	0.0136	96.35
112	54-30-5B	-0.5736	fully caked	0.189	0.0111	95.67
140	54-30-6A	-0.04322	fully caked	0.198	0.00605	96.89
140	54-30-6B	-0.01981	fully caked	0.201	0.00436	98.32
168	54-30-7A	-0.3956	fully caked	0.231	0.0136	98.06
168	54-30-7B	-1.426	fully caked	0.199	0.00643	97.09
196	54-30-8A	-0.7758	fully caked	0.243	-0.00446	96.72
196	54-30-8B	0.08198	fully caked	0.209	0.0150	98.95
224	54-30-9A	-0.1951	fully caked	0.189	-0.0141	99.88
224	54-30-9B	0.08505	fully caked	0.183	-0.0117	101.34
252	54-30-10A	0.1071	fully caked	0.212	0.00514	97.31
252	54-30-10B	-1.041	fully caked	0.210	0.00167	98.17
282	54-30-11A	0.3175	fully caked	0.216	-0.00437	98.50
282	54-30-11B	-0.09398	fully caked	0.217	-0.00376	99.05
318	54-30-12A	-1.072	fully caked	0.240	-0.00445	96.90
318	54-30-12B	-0.07388	fully caked	0.237	-0.00351	97.38
350	54-30-13A	0.0000	fully caked	0.267	0.0128	101.12
350	54-30-13B	-0.1447	fully caked	0.273	0.0174	100.52

Table A7. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 54% RH at 40°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	54-40-1A	-0.07455	fully caked	0.183	0.00746	100.61
0	54-40-1B	-0.1608	fully caked	0.184	0.00299	96.91
28	54-40-2A	-0.1168	slightly caked	0.203	0.00974	98.21
28	54-40-2B	-0.1119	slightly caked	0.202	0.00918	98.43
56	54-40-3A	-0.3391	partially caked	0.230	0.0143	98.82
56	54-40-3B	-0.2393	partially caked	0.203	0.00892	94.13
84	54-40-4A	-0.1452	fully caked	0.235	0.0104	101.70
84	54-40-4B	-0.2432	fully caked	0.238	0.0107	103.54
112	54-40-5A	-0.2962	fully caked	0.270	0.0116	95.38
112	54-40-5B	-0.07378	fully caked	0.287	0.0219	98.77
140	54-40-6A	-0.07224	fully caked	0.357	0.0330	100.95
140	54-40-6B	-0.04912	fully caked	0.344	0.0270	98.43
168	54-40-7A	-0.04779	fully caked	0.362	0.0282	93.90
168	54-40-7B	0.04937	fully caked	0.361	0.0281	97.14
196	54-40-8A	-0.06745	fully caked	0.380	0.0297	99.22
196	54-40-8B	-0.04896	fully caked	0.385	0.0184	99.77
224	54-40-9A	0.2885	slightly caked	0.380	0.0300	101.60
224	54-40-9B	0.4177	slightly caked	0.395	0.0316	101.83
252	54-40-10A	0.3353	fully caked	0.440	0.0309	99.80
252	54-40-10B	0.4292	fully caked	0.443	0.0181	97.46
282	54-40-11A	0.2747	fully caked	0.441	0.0764	96.95
282	54-40-11B	0.4011	fully caked	0.456	0.0750	97.22
318	54-40-12A	-0.04898	fully caked	0.538	0.0544	98.62
318	54-40-12B	0.02459	fully caked	0.542	0.0578	98.61
350	54-40-13A	-0.1666	fully caked	0.582	0.0664	100.56
350	54-40-13B	-0.1160	fully caked	0.604	0.0705	100.92

Table A8. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 75% RH at 20°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	75-20-1A	0.5000	fully caked	0.204	-0.00860	98.87
0	75-20-1B	0.4958	fully caked	0.205	-0.00873	99.21
28	75-20-2A	0.6419	fully caked	0.225	-0.00553	97.68
28	75-20-2B	0.6198	fully caked	0.220	-0.00826	96.82
56	75-20-3A	0.6264	fully caked	0.234	-0.00304	99.27
56	75-20-3B	0.4172	fully caked	0.242	0.000199	97.51
84	75-20-4A	0.6639	fully caked	0.255	-0.00664	98.63
84	75-20-4B	0.6037	fully caked	0.247	-0.00765	97.93
112	75-20-5A	0.6375	fully caked	0.256	-0.00717	94.12
112	75-20-5B	0.4765	fully caked	0.250	-0.00834	98.15
140	75-20-6A	0.6575	fully caked	0.212	0.0125	97.82
140	75-20-6B	0.5989	fully caked	0.268	-0.00867	100.30
168	75-20-7A	0.7769	fully caked	0.262	-0.0133	97.48
168	75-20-7B	0.7914	fully caked	0.273	-0.0134	98.56
196	75-20-8A	0.8356	fully caked	0.233	0.0190	97.41
196	75-20-8B	-0.8554	fully caked	0.249	0.0210	98.59
224	75-20-9A	1.034	fully caked	0.217	0.00823	101.38
224	75-20-9B	0.9259	fully caked	0.224	0.00903	100.89
252	75-20-10A	1.108	fully caked	0.243	-0.000443	99.59
252	75-20-10B	1.000	fully caked	0.247	0.00652	99.14
282	75-20-11A	1.249	fully caked	0.246	0.0450	97.13
282	75-20-11B	0.9599	fully caked	0.250	0.0422	98.60
318	75-20-12A	0.8946	fully caked	0.305	0.0183	98.04
318	75-20-12B	0.7413	fully caked	0.292	0.0160	98.32
350	75-20-13A	0.8357	fully caked	0.387	0.0233	101.06
350	75-20-13B	0.9083	fully caked	0.364	0.0202	100.08

Table A9. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 75% RH at 30°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	75-30-1A	0.3403	fully caked	0.211	-0.0159	97.74
0	75-30-1B	-0.2569	fully caked	0.216	-0.0193	99.93
28	75-30-2A	0.2280	fully caked	0.214	-0.0174	97.84
28	75-30-2B	0.4321	fully caked	0.166	0.00518	97.94
56	75-30-3A	0.3850	fully caked	0.218	-0.0207	94.74
56	75-30-3B	-0.2365	fully caked	0.215	-0.0206	95.38
84	75-30-4A	0.3596	fully caked	0.159	-0.0161	97.96
84	75-30-4B	0.3620	fully caked	0.158	-0.0207	98.08
112	75-30-5A	0.3934	fully caked	0.219	-0.0221	94.13
112	75-30-5B	0.5348	fully caked	0.226	-0.0206	95.38
140	75-30-6A	0.3366	fully caked	0.238	-0.0206	97.49
140	75-30-6B	0.4791	fully caked	0.250	-0.0183	99.28
168	75-30-7A	0.6612	fully caked	0.185	0.000735	97.66
168	75-30-7B	0.7384	fully caked	0.238	-0.0174	96.74
196	75-30-8A	0.4010	fully caked	0.211	0.0110	97.24
196	75-30-8B	0.8158	fully caked	0.216	0.00258	97.33
224	75-30-9A	0.7635	fully caked	0.179	-0.0252	101.16
224	75-30-9B	0.9759	fully caked	0.397	0.000413	100.99
252	75-30-10A	0.9098	fully caked	0.199	-0.0215	101.37
252	75-30-10B	0.6388	fully caked	0.220	-0.00793	100.33
282	75-30-11A	0.8168	fully caked	0.203	0.0286	99.01
282	75-30-11B	0.8949	fully caked	0.199	0.0267	98.63
318	75-30-12A	0.7443	fully caked	0.217	-0.0271	95.92
318	75-30-12B	0.6527	fully caked	0.215	-0.0306	95.70
350	75-30-13A	1.135	fully caked	0.253	0.00817	100.63
350	75-30-13B	0.6405	fully caked	0.262	0.00941	100.59

Table A10. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 75% RH at 40°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	75-40-1A	0.2824	fully caked	0.228	-0.0212	97.25
0	75-40-1B	0.2633	fully caked	0.224	-0.0268	99.57
28	75-40-2A	-1.154	fully caked	0.235	-0.0244	98.16
28	75-40-2B	0.4170	fully caked	0.237	-0.0216	99.47
56	75-40-3A	0.4941	fully caked	0.306	-0.0205	96.48
56	75-40-3B	0.4867	fully caked	0.290	-0.0204	96.12
84	75-40-4A	0.7290	fully caked	0.364	-0.00142	96.25
84	75-40-4B	0.7907	fully caked	0.384	-0.00333	96.44
112	75-40-5A	0.7308	fully caked	0.539	0.0127	96.47
112	75-40-5B	0.8800	fully caked	0.539	0.0153	99.22
140	75-40-6A	1.118	fully caked	0.786	0.0474	98.08
140	75-40-6B	0.8525	fully caked	0.846	0.0632	95.16
168	75--40-7A	1.104	fully caked	1.08	0.0888	96.39
168	75-40-7B	1.150	fully caked	1.05	0.0858	94.31
196	75-40-8A	1.231	fully caked	1.54	0.188	97.05
196	75-40-8B	1.093	fully caked	1.51	0.185	99.22
224	75-40-9A	1.518	fully caked	2.44	0.262	101.03
224	75-40-9B	1.394	fully caked	2.50	0.261	101.26
252	75-40-10A	1.578	fully caked	3.18	0.365	98.87
252	75-40-10B	1.488	fully caked	3.15	0.364	98.30
282	75-40-11A	1.659	fully caked	4.12	0.498	98.22
282	75-40-11B	1.745	fully caked	4.17	0.500	97.81
318	75-40-12A	1.250	fully caked	5.60	0.668	96.37
318	75-40-12B	1.410	fully caked	5.81	0.644	96.32
350	75-40-13A	1.957	fully caked	7.60	0.826	96.54
350	75-40-13B	1.721	fully caked	7.22	0.830	99.08

Table A11. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 85% RH at 20°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	85-20-1A	26.48	partially deliq	0.177	0.00294	100.15
0	85-20-1B	14.31	partially deliq	0.239	0.00233	100.17
28	85-20-2A	22.04	partially deliq	0.148	0.00362	97.60
28	85-20-2B	13.85	partially deliq	0.232	0.0102	98.00
56	85-20-3A	14.72	partial deliq	0.179	0.00340	97.15
56	85-20-3B	80.79	fully deliq	0.147	0.00369	95.61
84	85-20-4A	80.87	fully deliq	0.224	0.00277	99.65
84	85-20-4B	75.81	fully deliq	0.174	-0.000407	100.19
112	85-20-5A	54.97	partial deliq	0.188	-0.00101	99.31
112	85-20-5B	40.14	partial deliq	0.213	-0.000863	96.97
140	85-20-6A	81.38	fully deliq	0.184	0.000820	96.93
140	85-20-6B	77.62	fully deliq	0.257	-0.00134	96.70
168	85-20-7A	80.40	fully deliq	0.242	-0.00366	98.97
168	85-20-7B	82.06	fully deliq	0.246	-0.00425	99.89
196	85-20-8A	76.41	fully deliq	0.225	0.00907	99.42
196	85-20-8B	80.76	fully deliq	0.255	0.00866	99.31
224	85-20-9A	83.80	fully deliq	0.188	-0.00139	100.33
224	85-20-9B	81.38	fully deliq	0.184	0.00158	97.72
252	85-20-10A	80.52	fully deliq	0.269	0.00197	96.55
252	85-20-10B	79.58	fully deliq	0.238	0.00977	96.16
282	85-20-11A	83.05	fully deliq	0.298	0.0280	95.80
282	85-20-11B	77.74	fully deliq	0.282	0.0300	94.51
318	85-20-12A	79.57	fully deliq	0.284	0.00264	96.57
318	85-20-12B	76.10	fully deliq	0.275	0.00392	97.59
350	85-20-13A	81.52	fully deliq	0.254	0.00185	97.34
350	85-20-13B	79.22	fully deliq	0.317	0.000942	100.03

Table A12. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 85% RH at 30°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	85-30-1A	65.70	fully deliq	0.177	-0.00488	98.73
0	85-30-1B	63.28	almost fully deliq	0.186	-0.00260	99.48
28	85-30-2A	68.70	fully deliq	0.230	-0.00552	102.64
28	85-30-2B	68.56	fully deliq	0.217	-0.00394	98.01
56	85-30-3A	68.51	fully deliq	0.196	-0.00812	96.31
56	85-30-3B	67.75	fully deliq	0.191	-0.00646	91.53
84	85-30-4A	67.55	fully deliq	0.320	-0.00514	98.46
84	85-30-4B	67.75	fully deliq	0.347	-0.00962	96.42
112	85-30-5A	64.79	fully deliq	0.524	-0.00477	96.28
112	85-30-5B	68.67	fully deliq	0.409	-0.00664	96.44
140	85-30-6A	68.09	fully deliq	0.690	-0.00797	97.82
140	85-30-6B	68.11	fully deliq	0.641	-0.00940	97.17
168	85-30-7A	66.10	fully deliq	0.764	-0.00569	97.25
168	85-30-7B	66.91	fully deliq	0.829	-0.00370	98.36
196	85-30-8A	65.68	fully deliq	0.848	0.00793	94.42
196	85-30-8B	65.97	fully deliq	0.829	0.00984	95.91
224	85-30-9A	65.73	fully deliq	1.36	-0.00129	96.70
224	85-30-9B	65.64	fully deliq	1.62	-0.00525	97.91
252	85-30-10A	65.20	fully deliq	1.54	0.00159	93.58
252	85-30-10B	64.35	fully deliq	1.07	0.00107	95.87
282	85-30-11A	66.32	fully deliq	1.59	0.0302	95.33
282	85-30-11B	66.65	fully deliq	1.65	0.0318	93.83
318	85-30-12A	65.60	fully deliq	1.00	0.00680	94.54
318	85-30-12B	65.18	fully deliq	1.02	0.0127	93.74
350	85-30-13A	65.08	fully deliq	0.820	0.00466	94.88
350	85-30-13B	65.02	fully deliq	1.47	0.00536	96.82

Table A13. Moisture content, physical appearance, optical density and tagatose remaining of samples stored at 85% RH at 40°C.

Time (days)	Sample	Moisture Content (%db)	Physical Appearance	OD/g (280 nm)	OD/g (420 nm)	% Tagatose remaining
0	85-40-1A	61.77	fully deliq	0.294	0.00706	97.86
0	85-40-1B	57.28	fully deliq	0.304	0.00838	95.34
28	85-40-2A	56.36	fully deliq	0.651	0.00468	96.10
28	85-40-2B	55.26	fully deliq	0.819	0.00432	96.43
56	85-40-3A	57.94	fully deliq	1.82	0.0158	91.58
56	85-40-3B	57.53	fully deliq	1.69	0.00745	91.79
84	85-40-4A	52.13	fully deliq	4.10	0.0169	91.43
84	85-40-4B	50.72	fully deliq	3.59	0.0137	90.26
112	85-40-5A	54.27	fully deliq	6.08	0.0312	87.68
112	85-40-5B	53.66	fully deliq	6.29	0.0274	91.25
140	85-40-6A	55.41	fully deliq	7.78	0.0440	86.78
140	85-40-6B	54.89	fully deliq	7.20	0.0435	85.60
168	85-40-7A	56.61	fully deliq	7.22	0.0431	81.96
168	85-40-7B	56.27	fully deliq	7.29	0.0492	81.97
196	85-40-8A	51.71	fully deliq	12.5	0.0518	83.01
196	85-40-8B	49.51	fully deliq	12.4	0.0540	82.68
224	85-40-9A	54.98	fully deliq	12.9	0.0620	86.15
224	85-40-9B	54.95	fully deliq	13.2	0.0750	85.41
252	85-40-10A	53.79	fully deliq	14.3	0.216	83.65
252	85-40-10B	55.70	fully deliq	11.8	0.075	85.96
282	85-40-11A	50.88	fully deliq	15.1	0.287	82.15
282	85-40-11B	50.46	fully deliq	14.6	0.210	81.19
318	85-40-12A	52.09	fully deliq	16.0	0.247	81.26
318	85-40-12B	53.08	fully deliq	16.8	0.268	80.33
350	85-40-13A	49.17	fully deliq	20.3	0.376	81.48
350	85-40-13B	49.81	fully deliq	18.2	0.340	84.45

Appendix B

pH values of tagatose obtained at time 6, 9 and 12.

B1. Duplicate pH values for tagatose at time 6, 9 and 12 months.

Sample	Time 6 pH	Time 9 pH	Time 12 pH
Drierite	5.755	6.201	5.567
Drierite	6.02	6.137	5.737
33%RH/20°C	4.98	5.626	5.326
33%RH/20°C	5.23	5.53	5.631
33%RH/30°C	5.43	5.029	5.445
33%RH/30°C	5.49	4.945	5.485
33%RH/40°C	5.09	4.894	5.352
33%RH/40°C	5.39	4.967	5.456
54%RH/20°C	5.22	5.121	5.435
54%RH/20°C	4.96	5.01	5.321
54%RH/30°C	4.94	5.055	5.298
54%RH/30°C	4.92	4.917	5.399
54%RH/40°C	5.08	4.945	5.551
54%RH/40°C	5.87	5	5.373
75%RH/20°C	5.59	5.657	5.867
75%RH/20°C	5.47	5.706	5.856
75%RH/30°C	5.29	5.36	5.806
75%RH/30°C	5.51	5.404	5.666
75%RH/40°C	5.65	5.898	5.636
75%RH/40°C	5.87	5.913	5.601
85%RH/20°C	5.52	6.045	6.158
85%RH/20°C	5.89	5.87	6.116
85%RH/30°C	5.28	4.792	5.132
85%RH/30°C	5.53	5.092	5.527
85%RH/40°C	4.06	3.955	4.052
85%RH/40°C	3.98	4.109	4.101

Appendix C

Pictures of tagatose at time 6, 9 and 12 months

Figure C1. Duplicate pictures of tagatose at time 6.

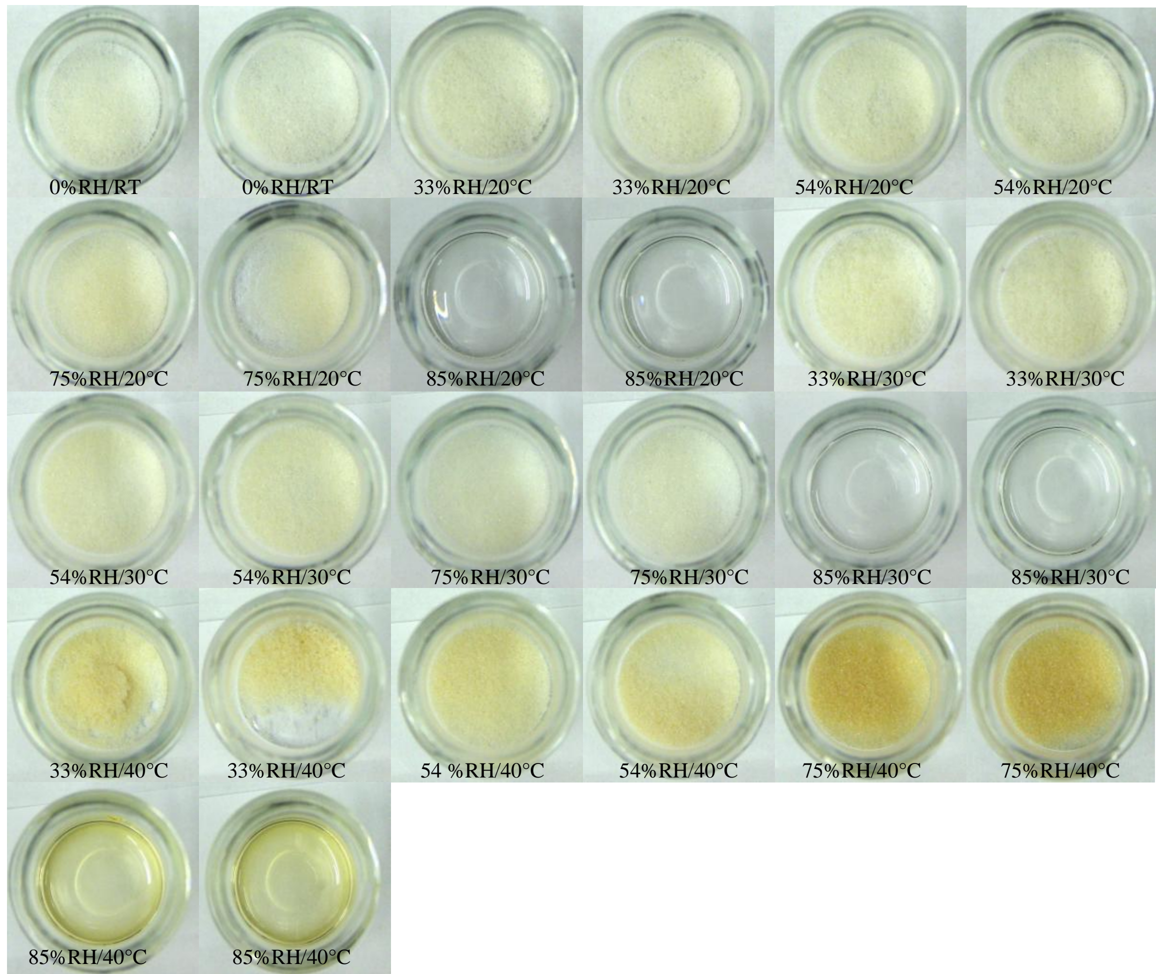


Figure C2. Duplicate pictures of tagatose at time 9.

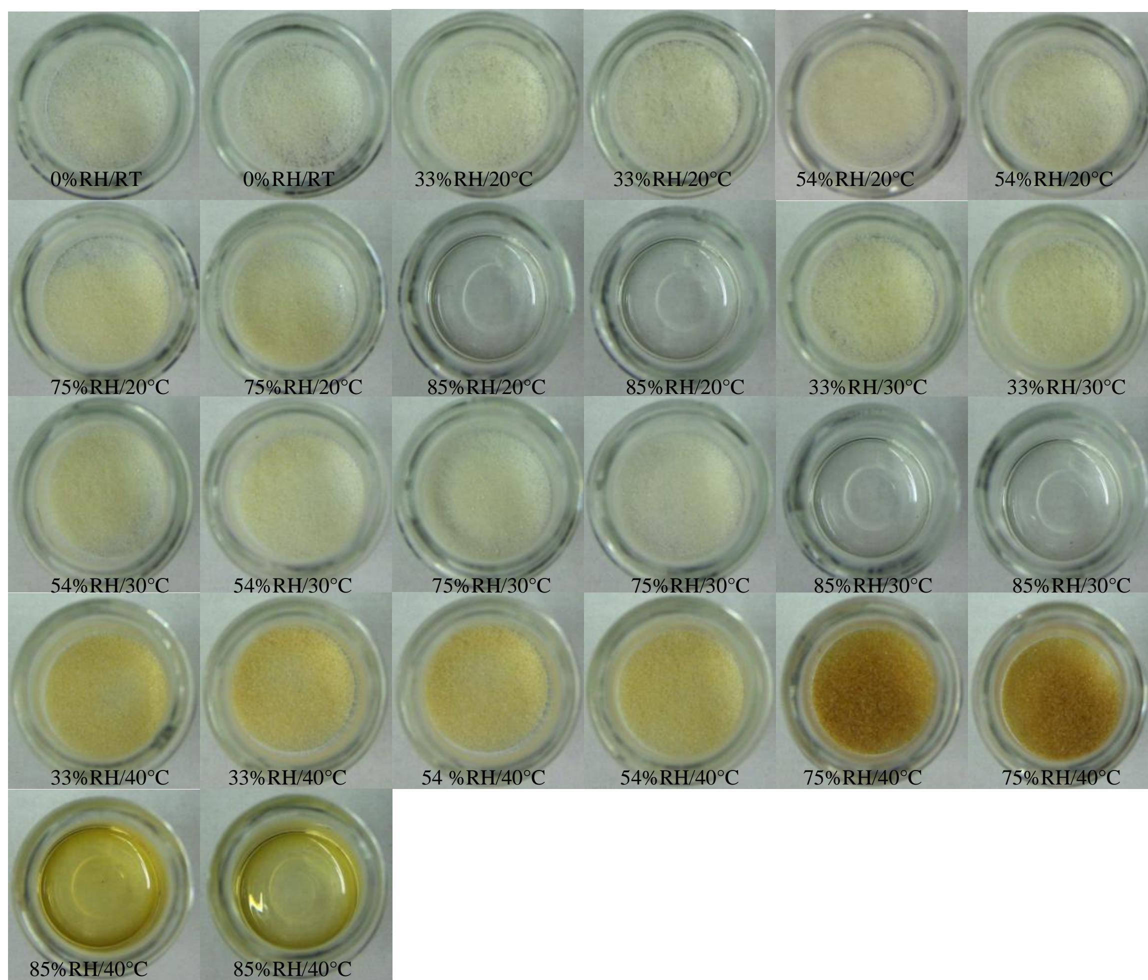
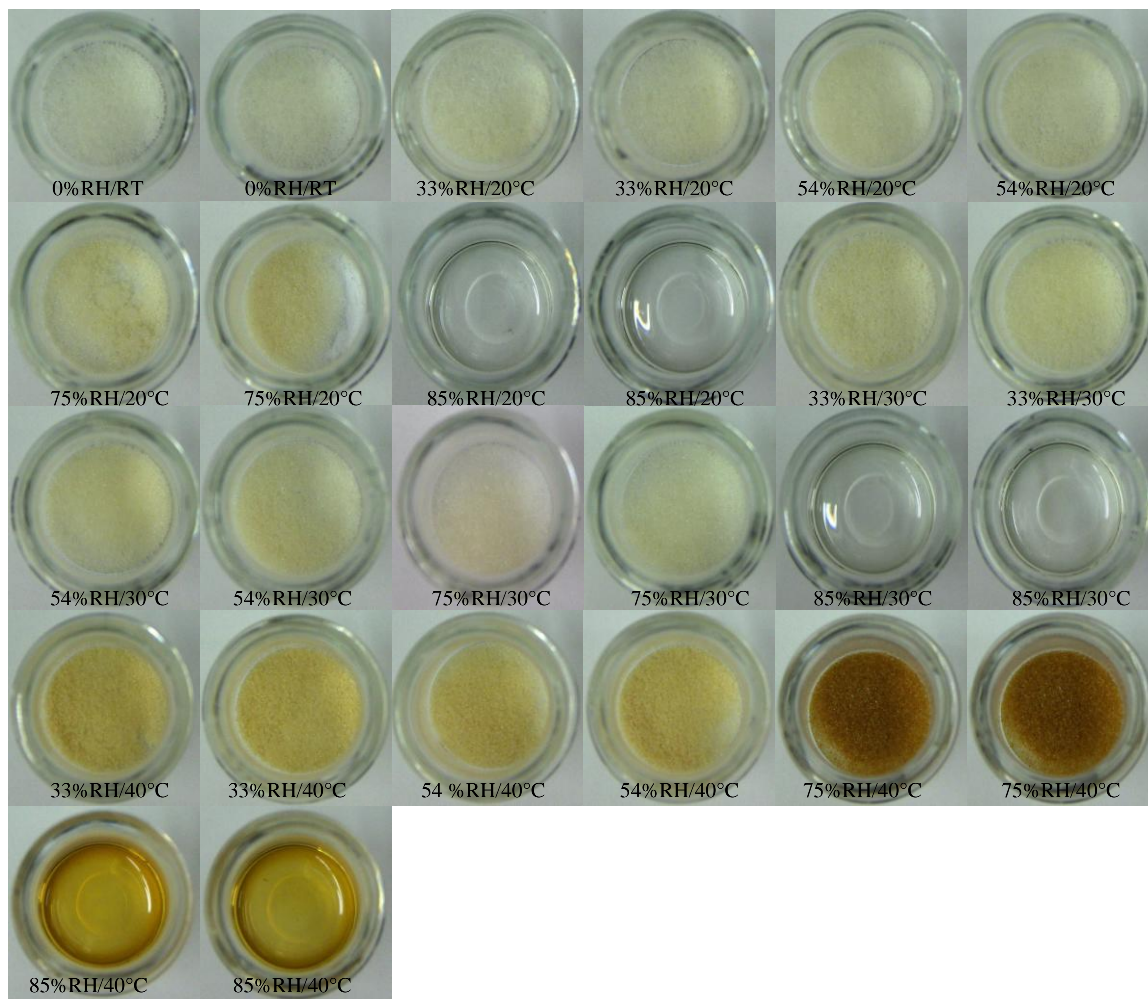


Figure C3. Duplicate pictures of tagatose at time 12.



Appendix D

Particle size study

Particle Size Study

As mentioned earlier in the Materials and Methods section, studies show that particle size influences moisture adsorption and deliquescence (Mathlouthi and Roge 2003; Salameh and Taylor 2006a; Kwok and others 2010); therefore the effect of particle size on deliquescence of tagatose was investigated. In the primary study, particle size was not taken into account. Therefore, to eliminate any particle size bias on deliquescence, a smaller study was conducted to see if smaller particles of tagatose would deliquesce at a faster rate than larger particles. It is important to note that the tagatose used for the primary study was food grade tagatose and would be the type normally used in an industrial setting.

Similar methods were used as mentioned in the particle size characterization section to obtain desired particle sizes. Tagatose that had a particle size greater than 500 µm was used to study the effect of larger particles on deliquescence. Likewise tagatose particles less than 250 µm were used to study the effect of smaller crystals on deliquescence. Triplicate vials were placed in four desiccators. The four desiccators were prepared at two different environmental conditions, 75% RH (NaCl) and 85% RH (KCl). Two of each desiccator were placed at 20 and 30°C. The desiccators were first checked daily and then weekly for a total of 17 weeks. Physical descriptions were recorded to monitor how fast samples deliquesced. Physical descriptors included: wet, slurry, partially deliquesced, fully deliquesced, caked and solid. Plots were generated that displayed moisture content (dry basis) vs. time (days). Standard deviations were represented with error bars on each chart to determine if samples of varying particle sizes equilibrated at a faster rate and/or adsorbed more water.

There was no conclusive evidence that suggested that smaller or larger particles of tagatose deliquesced at a faster rate. Samples that were stored at 75% RH at 20 and 30°C gained less that 2% moisture (db). Due to no significant water gain, no further analysis was done on those samples. Figures D1 and D2 show average moisture contents of the three samples on the y-axis and time on the x-axis. The plots look very similar. The larger particle six adsorbed water at a faster rate initially. For the most part, the profiles for small and larger particles of tagatose were similar. It was hypothesized that smaller particles would deliquesce at a faster rate.

Salameh and Taylor (2006a) investigated moisture sorption of unground and ground powders. Using a higher fraction of fine particles in the glucose/citric acid mixture led to increased cake mechanical strength. Similarly, another study observed increased water sorption in smaller particle sizes of sucrose. This led to increased caking, which was credited to the increased specific solid surface area which allowed more water adsorption (Roge and Mathlouthi 2003; Mathlouthi and Roge 2003). Kwok and others (2010) also explored moisture sorption of various particle sizes including the following: 425–600 mm (large particles), 150–212 mm (medium particles), <53 mm (small particles). Particle size did not have a large impact on the sodium chloride/sucrose system. However, in the fructose/citric acid system, the smallest particles absorbed the most moisture (Kwok and others 2010). Although tagatose particle size does not seem to drastically affect its moisture adsorption and subsequent deliquescence, particle size does have an effect on moisture adsorption for some powdered ingredients.

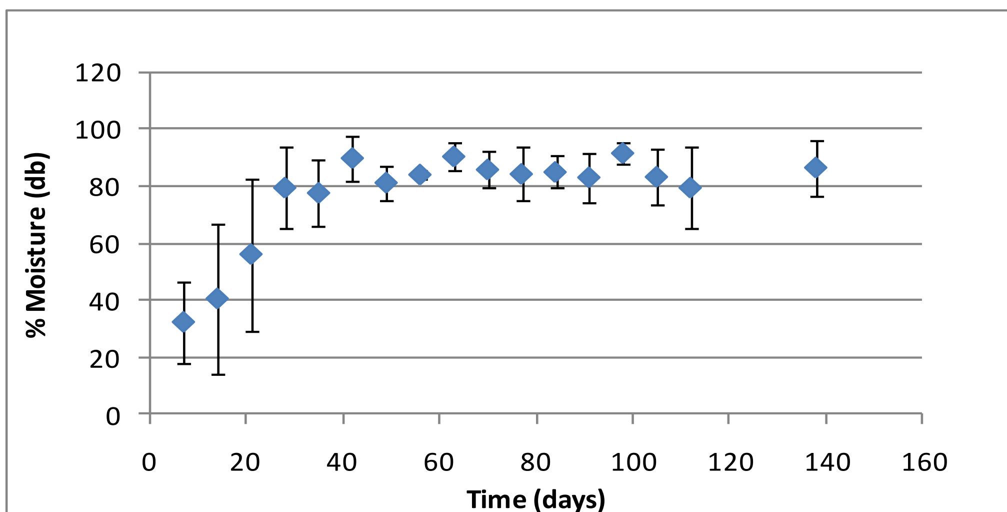


Figure D1. Moisture content (db) vs. time for particles < 250 nm at 85% RH/20°C.

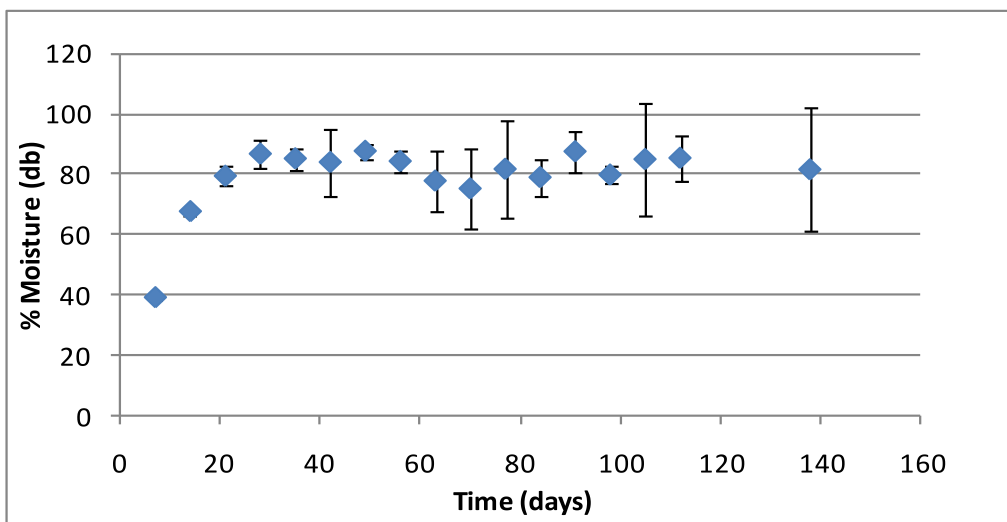


Figure D2. Moisture content (db) vs. time for particles > 500 nm at 85% RH/20°C.