THERMAL STABILITY OF TAGATOSE IN SOLUTION

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THERMAL STABILITY OF TAGATOSE IN SOLUTION

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THESIS ABSTRACT

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Tagatose is a minimally absorbed monosaccharide that has prebiotic properties. To achieve this prebiotic benefit, tagatose in foods and beverages must not be lost during processing. However, data on the thermal stability of tagatose are lacking. The objective of this study was to evaluate the thermal stability of tagatose in solutions.

Tagatose solutions (0.05 M or about 1%) were prepared in 0.02 and 0.1 M phosphate and citrate buffers at pH 3 and 7. A second set of similar solutions was also made, only differing from the first set in that they contained 0.05 M glycine. All of the solutions were held at 60, 70, and 80ºC for a minimum of 5 hours. At least 11 samples were removed at regular time intervals during the study for analysis. Tagatose analysis occurred via reverse-phase HPLC while browning was measured using a spectrophotometer at 420 nm.

In the solutions without glycine, minimal tagatose was lost at 60-80ºC in citrate and phosphate buffers at pH 3. For these solutions, slight browning was observed at all temperatures. At pH 7, tagatose loss was enhanced. The pseudo-first-order rate constants (k_{obs}) for tagatose degradation at pH 7 were greater in phosphate buffer than citrate buffer. Higher buffer concentrations and higher temperatures also increased k_{obs}. Enhanced browning accompanied the tagatose degradation in all buffer solutions at pH 7.

In the solutions containing tagatose as well as glycine, tagatose degraded faster at pH 7 than pH 3. Tagatose degradation was again greater in phosphate buffer than citrate buffer, and at the higher buffer concentration. Temperature also affected tagatose degradation, with faster tagatose loss occurring as the temperature increased. With glycine present in the solutions, enhanced browning occurred, but tagatose degradation rates were similar to those of the solutions without glycine.

For both the solutions containing tagatose as well as those containing tagatose and glycine, the most reactive solution was 0.1 M phosphate at pH 7. Using the activation energies for tagatose degradation, it was predicted that less than 0.5 and 0.02% tagatose would be lost during basic vat and HTST pasteurization, respectively, regardless of whether or not glycine was present.

Based on the results from this study, it was determined that although tagatose does breakdown at elevated temperatures, the amount of tagatose lost during the times and temperatures associated with typical thermal processing conditions would be virtually negligible. Due to minimal tagatose degradation during typical thermal processing techniques, the majority of tagatose would remain present in a beverage after pasteurization, allowing its presence to provide the consumer with prebiotic benefits.

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CHAPTER 1: INTRODUCTION

Tagatose is an emerging sweetener with many desirable attributes that may lend it to possible applications, such as a sugar substitute or use as a dietary supplement. Tagatose is a low-calorie sweetener with a level of sweetness similar to that of sucrose. It is found naturally in some dairy products, but is commercially manufactured from lactose (Oh 2007). Tagatose is classified as a monosaccharide, with a structure similar to that of fructose, only differing in the position of the hydroxyl group on the fourth carbon. Because tagatose is only minimally absorbed in the upper gastrointestinal tract, the majority of it travels to the colon where it is fermented by indigenous microflora. Carbohydrate fermentation in the intestinal tract leads to the production of short chain fatty acids (SCFAs), carbon dioxide, hydrogen, and methane. SCFAs are believed to be important because of their role in the welfare of the colonic mucosa. Due to the fact that tagatose is fermented to provide SCFAs and provides minimal to no calories, it is classified as a prebiotic. Along with the prebiotic benefit of tagatose, it does not promote dental caries (Arla Foods 2002), and it does not significantly increase blood glucose or insulin levels (Donner and others 1999).

Tagatose can be used in various products as a bulk sweetener, humectant, texturizer, stabilizer, and it may act as a flavor enhancer in products like mint and lemon flavored chewing gum and mints (Calorie Control Council 2007; Rulis 2001; Skytte 2006). In October 2001, the FDA agreed with Arla's findings that tagatose is Generally

Recognized as Safe (GRAS) when used in specific beverages and food products up to certain maximum allowable levels (Rulis 2001; Skytte 2006). Outside of the United States, many other countries have also approved tagatose as a food ingredient (Skytte 2006), leading to the addition of tagatose into various food products (Damhert 2008). While the properties and behavior related to the use of tagatose as a food ingredient have been studied quite thoroughly, other aspects remain relatively unexplored.

Tagatose appears to have many of the attributes that would allow it to be a successful substitute for commonly used sugars, specifically sucrose. However, there are still properties of tagatose that have not been studied thoroughly. The stability of tagatose under thermal processing conditions and the ability of tagatose to cause browning have only undergone minimal evaluation (Ryu and others 2003). Because tagatose must be present in food to offer any prebiotic benefit and foods are generally processed at high temperatures, studying high temperature stability is important. Browning is desirable in some applications, while undesirable in others, so determining the relationship between tagatose and browning is vital in the production of high quality products containing tagatose. The objective of this study is to determine the effect of pH, temperature, buffer concentration, and buffer type on the thermal stability of tagatose. The extent of browning will also be evaluated.

CHAPTER 2: LITERATURE REVIEW

In a world where trends and ideals are constantly changing, the food industry is expected to develop and discover new ingredients and food products to keep up with the current demands of today's society. It is apparent through television commercials, magazine articles, and food labels that consumers are becoming more health-conscious. As the link between diet and disease becomes more prominent, consumers have become increasingly concerned about what they are consuming and the effects it may have on their health. One area that has been receiving a lot of attention lately is nutraceuticals. Nutraceuticals are, "foods, or parts of foods, that provide medical or health benefits, including the prevention and treatment of disease" (National Nutraceutical Center 2005). Categories of nutraceuticals include dietary supplements (vitamins, minerals, ginseng, gingko biloba) and functional foods. Functional foods are foods that exert a specific beneficial effect on health beyond their basic nutritional properties. They include foods such as tomatoes (contains lycopene which contributes to prostate health), wheat bran (contains insoluble fiber which helps maintain a healthy digestive tract), and foods classified as prebiotics (IFIC 2006). Prebiotics are beneficial to the body since they selectively stimulate the favorable growth or activity of certain indigenous bacteria **(**Reid and others 2003**)**. Tagatose, a relatively new sweetener, has been shown to display prebiotic characteristics (Bertelsen and others 1999).

Chemistry and Properties of Tagatose

Tagatose is a monosaccharide that has a structure similar to that of fructose, only differing in the position of the hydroxyl group on the fourth carbon. Acyclic D-fructose is oriented with a hydroxyl group on the right side of the fourth carbon, whereas this hydroxyl group is on the left side of the fourth carbon on acyclic D-tagatose. Figure 2.1 shows the β-pyranose form of tagatose. This slight difference in structure only allows tagatose to be absorbed minimally in the upper gastrointestinal tract, resulting in a caloric value of less than 1.5 kcal/g (Levin 2002; Levin and others 1995; Livesey and Brown 1996). The tagatose that is not absorbed in the upper gastrointestinal tract travels to the colon where it is fermented to produce short chain fatty acids (SCFAs) including propionate and butyrate (Bertelsen and others 1999; Bertelsen and others 2001; Laerke and Jensen 1999; Laerke and others 2000; Venema and others 2005). Tagatose is therefore classified as a prebiotic, which makes it a very attractive food ingredient for companies looking to boost the overall health content of their product.

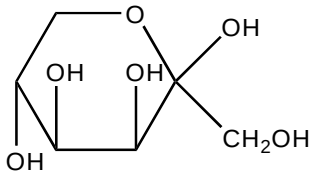

Figure 2.1. Structure of β-D-Tagatose

Although tagatose has a structure similar to fructose, its taste is comparable to that of sucrose, with no cooling effect, aftertaste, or off-flavors (Levin and others 1995). A standard taste panel test on 10% aqueous solutions of tagatose showed it to be 92% as

sweet as sucrose (Levin and others 1995). With a sweetness level and bulking ability similar to that of sucrose, tagatose may also have potential as a low calorie substitute for sucrose. Research has also found that tagatose blunts the rise in blood glucose and insulin typically observed after glucose or sucrose loading, and it produces exceptionally low glycemic and insulinemic responses, which links tagatose to possibly being beneficial in products geared towards individuals with diabetes (Gaio® 2003; Lu and others 2008).

Even though tagatose is a relatively new sweetener, a moderate amount of research has been done to evaluate the properties and applications of this monosaccharide. A look at numerous past studies on tagatose will provide a better understanding of this sugar.

Tagatose Production

While tagatose is found naturally in a variety of dairy products including sterilized cow's milk, hot cocoa, powdered cow's milk, and certain yogurts, the amount of tagatose in these products is so minimal that for economic purposes it is must be manufactured by chemical or biological methods (Levin and others 1995; Oh 2007). Using the chemical method, tagatose is produced from lactose through a two-step process, where initially lactose is split into glucose and galactose; the galactose is then isomerized to create tagatose by adding calcium hydroxide (Beadle and others 1992). The biological method of tagatose production involves converting galactose to tagatose using an immobilized L-arabinose isomerase enzyme (Oh 2007). Upon completion of either the chemical or biological method, tagatose appears as a white, odorless, crystalline product.

Digestion and Absorption

Numerous studies, mainly done on animals, led researchers to determine that only about 15-20% of tagatose is absorbed in the upper gastrointestinal tract (Laerke and Jensen 1999; Saunders and others 1999b). One study that helped researchers come to this conclusion involved 11 rats (Saunders and others 1999b). For this study, each of the rats was treated with two of the following treatment variations: conventional (normal levels of gut microflora), germ-free (no gut microflora), adapted (fed a diet containing tagatose for 28 days prior to the study), or unadapted to tagatose. Out of the 11 rats involved in the study, four were adapted conventional, three were unadapted conventional, two were unadapted germ-free, and all three groups were all dosed orally. The final two rats were unadapted conventional, and were dosed intravenously. All of the rats received a single dose (220-380 kBq) of tagatose before being placed in a metabolism chamber where samples of carbon dioxide, urine, and feces were taken at regular intervals. Analysis of the results concluded that the intestinal absorption of tagatose in the rat was approximately 20%. Since carbon dioxide is one of the products of carbohydrate fermentation, and the percentage of carbon dioxide produced by the conventional rats was greater than the carbon dioxide produced by the germ-free rats, the researchers were able to conclude that the tagatose was being fermented by bacteria in the large intestine. Also, there was 93% less tagatose in the feces of the adapted conventional rats, compared to the rats that were unadapted conventional, demonstrating the importance of adaptation in the microflora's ability to ferment tagatose (Saunders and others 1999b).

The low digestibility of tagatose in the small intestine was also observed in two similar studies done on pigs (Laerke and Jensen 1999; Laerke and others 2000). Both

studies followed the same method, while focusing on different variables including tagatose absorption, adaptation, and SCFA production. In both studies, eight pigs were fed a low fiber diet with 15% of the caloric value of the diet coming from sucrose. A second group of eight pigs was fed a similar diet to the first group of pigs except that a majority of the sucrose was replaced with tagatose. Also, a portion of the pigs in the second group underwent a two-day adaptation period to tagatose prior to the study. The pigs were fed their respective diets for 18 days, and on the 18th day they were killed, and their gastrointestinal contents were analyzed. In the studies, it was found that tagatose was digested and absorbed very minimally in the small intestine of pigs (Laerke and Jensen 1999), and no microbial fermentation of tagatose took place in the stomach or small intestine (Laerke and others 2000), but instead took place in the cecum and the colon (Laerke and Jensen 1999; Laerke and others 2000). The fermentation of tagatose led to the production of many beneficial SCFAs, in particular, formate, acetate, propionate, butyrate, valerate, and caproate (Laerke and Jensen 1999; Laerke and others 2000). The amount of microbial fermentation of tagatose in the cecum and colon was dependent on the amount of tagatose in the diet (Laerke and others 2000). The rate of microbial fermentation of tagatose was much higher in the pigs that were adapted to tagatose before starting the study (Laerke and Jensen 1999; Laerke and others 2000). The colon of the adapted pigs was shown to produce proportions of butyric and valeric acid that were two to three times higher than those seen in the colon of unadapted pigs (Laerke and others 2000).

A study done on the small intestine absorption of tagatose in ileostomy subjects resulted in conflicting findings to the other results on tagatose absorption in the small

intestine (Normen and others 2001). In this study, six individuals, who had well-functioning ileostomies with less than 10 cm of their terminal ileum removed, were studied to determine the absorption of tagatose in the small intestine. The subjects in the study were fed a specific diet for two periods of two days during three consecutive weeks. In one of the periods, 15 g of tagatose were added to the daily diet of the subjects. Ileostomy effluents were freeze-dried and analyzed to determine the tagatose absorption. Contrary to other studies (Laerke and Jensen 1999; Laerke and others 2000), it was found that 81% of tagatose was absorbed in the small intestines of the subjects in this study. While the results in this study differ dramatically from the results of the previous two studies, fermentation of tagatose by the indigenous microflora, which was not analyzed in this study, cannot be ruled out as a factor for such high absorption values (Normen and others 2001).

Since tagatose is only absorbed minimally in the small intestine, the majority of it enters the colon where it is fermented by saccharolytic bacteria, producing SCFAs, carbon dioxide, methane, and hydrogen. SCFAs have been associated with the reduced risk of some diseases, including irritable bowel syndrome, inflammatory bowel disease, cardiovascular disease, and cancer (Hijova and Chmelarova 2007). Upon absorption of the SCFAs in the cecum and colon, they are mainly metabolized in three sites in the body. One site in the body where SCFAs can be metabolized is the cells of the ceco-colonic epithelium. This site uses butyrate as a major substrate for the maintenance of energy producing pathways. A second site where metabolism of SCFAs occurs is in the liver cells, where butyrate and propionate are used for gluconeogenesis. Finally, the muscle cells are able to metabolize acetate and generate energy from its oxidation

(Hijova and Chmelarova 2007). Roles of SCFAs include being modulators of colonic and intracellular pH, cell volume, and other functions associated with ion transport. They are also nutrients for the colonic epithelium, and they act as regulators of proliferation, differentiation, and gene expression (Hijova and Chmelarova 2007). SCFAs have many important functions throughout the body, and tagatose is beneficial to the body since it promotes the production of these SCFAs.

Various studies have been conducted in order to determine the specific SCFAs that are produced through the fermentation of tagatose (Bertelsen and others 1999; Bertelsen and others 2001; Laerke and Jensen 1999; Laerke and others 2000; Venema and others 2005). In one study (Bertelsen and others 1999), a group of eight pigs was fed a standard diet plus 15% sucrose (unadapted pigs), while a second group of eight pigs was fed a standard diet plus 5% sucrose and 10% tagatose (adapted pigs). All 16 pigs were fed their specified diets for a total of 17 days, and on the 17th day the pigs were killed 3 hours after their morning feed, and their entire gastrointestinal tract was sectioned. Only contents from the mid-colon were used for *in vitro* fermentation assays. The contents of the intestinal tract were diluted with water to create 20% slurries. After the slurries were incubated at 37°C under anaerobic conditions for four hours, with or without 1% tagatose added, it was found that the colon contents from the adapted pigs fermented tagatose and produced a low acetate, high butyrate, high valerate environment. This differed from the unadapted pigs whose colon contents produced a SCFA profile that was more normal, containing 20% less butyrate and a higher level of acetate (Bertelsen and others 1999).

Bertelsen and others (1999) had another part to their study. In this study, there were three groups containing two pigs each; the first group of pigs received a standard diet plus 20% sucrose, the second group of pigs received a standard diet plus 10% sucrose and 10% tagatose, while the third group of pigs was fed a standard diet plus 20% tagatose for a total of 33 days. Six hours after their morning feed on the 33rd day, the pigs were killed and their entire gastrointestinal tract was divided into eight segments and each segment was analyzed for the concentrations of various SCFAs. Contents from each of the eight segments were also used for 12 hour *in vitro* incubations at 37°C under anaerobic conditions. The researchers found that at the time of slaughtering, butyrate was only present in the hind gut, and that the more tagatose the pigs were fed, the more butyrate was found in the hind gut. After 12 hours, it was also found that there was a direct relationship between tagatose consumption and butyrate production. The more tagatose the pigs were fed, the more butyrate was produced in the colon. Acetate production was found to decrease in the gastrointestinal tract of the pigs that consumed tagatose, with more of a reduction occurring in the pigs that consumed larger amounts of tagatose (Bertelsen and others 1999).

After the *in vitro* studies done on pigs resulted in findings that were very similar, the researchers looked at the *in vivo* absorption of SCFAs in the blood of pigs (Bertelsen and others 1999). Blood samples were taken from the same five pigs, 12 hours after morning feeding, on three different occasions. The first blood sample was taken after seven days of adaptation to a standard pig diet plus 20% sucrose. The second blood sample was taken on the first day the pigs were switched to a standard pig diet plus 20% tagatose (unadapted pigs). The third and final blood sample was taken after the pigs had

adapted to a pig diet plus 20% tagatose for seven days (adapted pigs). In order to determine the intestinal absorption of SCFAs, the researchers measured the difference between the SCFA content in the samples taken from blood found in the portal vein and those taken from the arterial blood. The results from this study showed that when the pigs were on the 20% sucrose diet, the butyrate concentrations were very low. The butyrate concentrations increased slightly when the pigs first began the 20% tagatose diet, but increased dramatically after the pigs had been on the 20% tagatose diet for seven days. Intestinal absorption of acetate and propionate did not increase when tagatose was added to the diet of the pigs (Bertelsen and others 1999).

Pigs were used in the previous studies because their digestive tracts are similar to those of humans, and they both have qualitatively the same types of indigenous intestinal bacteria. In order to get a better idea of how tagatose reacts in humans, *in vitro* studies were carried out where tagatose was added to human faecal slurries (Bertelsen and others 1999). In this study, there were 16 human volunteers who provided the researchers with faecal samples before consuming tagatose, as well as 14 days after consuming 10 g of tagatose three times a day. The study participants maintained a controlled diet for four days before each faecal sample was taken. The faecal slurries were incubated at 37°C under anaerobic conditions for either 4 or 48 hours. The faecal slurries analyzed after 4 hours showed that the samples taken after the humans had consumed tagatose in their diet for 14 days contained much higher amounts of total SCFAs and butyrate than the samples taken before tagatose consumption. After 48 hours, there was no longer any difference in the amount of SCFAs produced, and the butyrate production was high in both the samples taken before tagatose consumption as well as those taken after tagatose

consumption. Also, the number of lactic acid bacteria were significantly higher in the faecal samples collected from humans who had consumed tagatose for 14 days, compared to the faecal samples taken from humans before tagatose consumption (Bertelsen and others 1999).

A second study on humans also looked at how tagatose consumption affected the production of SCFAs in the colon (Venema and others 2005). In the *in vivo* part of this study, 12 men and 18 women consumed 30 g of raspberry jam containing either 7.5 g or 12.5 g tagatose, 7.8 g fructooligosaccharides, 7.6 g tagatose plus 7.5 g fructooligosaccharides, or 15.1 g sucrose at breakfast for two weeks each, in varying orders. After each two-week period, test-tube incubations of faecal slurries were processed for microbiological analyses within two hours after voiding. There was also an *in vitro* part to the study that involved a model simulating the large intestine to observe the mechanistic effect of tagatose on SCFA production. Results from both the *in vitro* study and the *in vivo* study indicated that there was an increase in butyrate production after all treatments with tagatose (Venema and others 2005).

In another study, 45 isolates of normal or pathogenic human enteric bacteria were chosen for fermentation of tagatose *in vitro* (Bertelsen and others 2001). Also, 107 lactic acid bacteria strains were used for fermentation of tagatose *in vitro*. All samples were incubated for 48 hours in order to determine which intestinal bacteria were able to ferment tagatose. It was found that the fermentation of tagatose is not very widespread among the different genera of human enteric bacteria. Only five of the 45 human enteric bacteria studied were able to ferment tagatose. The five human enteric bacteria that were able to ferment tagatose include two strains of *Lactobacillus*, two strains of *Clostridium*,

and *Enterococcus faecalis*. A majority of the lactic acid bacteria showed tagatose fermentation, with strong fermentation in 61 strains, and in 38 of these strains the fermentation of tagatose was very extensive (Bertelsen and others 2001).

From the research, it was determined that only about 15-20% of tagatose is absorbed in the upper gastrointestinal tract (Laerke and Jensen 1999; Saunders and others 1999b). The remaining tagatose makes its way into the colon where it is able to undergo fermentation by indigenous microflora, in particular strains of *Lactobacillus*, *Clostridium*, and *Enterococcus faecalis* (Bertelsen and others 2001; Laerke and Jensen 1999; Laerke and others 2000). The fermentation of tagatose leads to the production of SCFAs, specifically butyrate and valerate, which are beneficial to our colonic health (Bertelsen and others 1999; Bertelsen and others 2001; Laerke and Jensen 1999; Laerke and others 2000; Venema and others 2005).

Caloric Value

Due to the minimal absorption of tagatose in the small intestine, researchers have found tagatose offers the body fewer calories than typical carbohydrates, including sucrose (Livesey and Brown 1996). Tagatose is estimated to provide less than 1.5 kcal/g, whereas sucrose provides 4 kcal/g. In one study (Livesey and Brown 1996), two groups of 30 rats were fed a diet containing either sucrose or tagatose for 21 days prior to the study in order to allow for an adaptation period to the sugar, either sucrose or tagatose. After the 21 day adaptation period, the 30 rats from each group were broken into three groups containing ten rats each. One group immediately underwent body composition analysis, the second group had the test (or reference) carbohydrate removed from their diets for about 6 weeks, and the third group continued to receive a daily diet

supplemented with 1.8 g of the test carbohydrate, either sucrose or tagatose, for about 6 weeks. The rats that consumed the diet supplemented with sucrose showed an increase in growth, protein, and lipid deposition, while the rats consuming a diet supplemented with tagatose did not show any measurable changes in those three parameters. Based on the results, the researchers were able to calculate that tagatose contributed -3±14% of its heat of combustion to net metabolizable energy, therefore tagatose effectively had a zero energy value (Livesey and Brown 1996).

Arla Foods, the company that distributes tagatose, used a different technique to determine the caloric value of tagatose (Levin 2002). Using a factoral method, they estimated that the metabolizable energy provided by tagatose ranged from 1.1 to 1.4 kcal/g. Depending on the experimental procedure, the interpretation of results, and the factors applied, it could be even lower (Levin 2002).

Because tagatose provides the body with few to no calories, one would expect weight loss to occur in subjects consuming a diet where a portion of the sucrose is substituted with tagatose. Levin and others (1995) conducted a study where rats in groups of six each were fed a standard diet plus 15% tagatose for an adaptation period of 14 days. After those 14 days one group of rats continued with the previous diet, while the other group of rats was fed a standard diet plus sucrose at an equal weight basis as the tagatose fed to the first group of rats. After undergoing a 60 day comparison between the tagatose-fed rats and the sucrose-fed rats, it was found that the sucrose-fed rats showed a greater than 7% weight gain than the tagatose-fed rats (Levin and others 1995).

A second study looked at the effect of tagatose on food intake of human subjects (Buemann and others 2000c). In this study, the food intake patterns of 19 normal-weight

men were studied after 29 g of tagatose or 29 g of sucrose were substituted into their breakfast meal. Following the consumption of either tagatose or sucrose at breakfast, the food intake of the study subjects was evaluated at lunch, afternoon snack, and supper of the same day. Researchers found that the energy intake at lunch and afternoon snack was comparable after intake of the two sugars, but at supper the food intake was 15% lower after consuming tagatose at breakfast (Buemann and others 2000c). While there are a lot of factors that can influence an individual's food intake, this study does bring up the possibility of tagatose acting as some type of appetite suppressant.

Tolerance

Tagatose provides only a minimal amount of calories since it is not fully digested or absorbed in the small intestine, which leads to concerns about tolerance levels and possible negative side effects from the overconsumption of this sugar. Incomplete absorption of nutrients has been linked to gastrointestinal discomforts such as cramping, bloating, flatulence, and diarrhea. To determine how humans react to high levels of tagatose consumption, Lee and Story (1999) studied how the gastrointestinal tract of 50 subjects responded to 40 g of chocolate containing 20 g sucrose, lactitol, or tagatose. It was found that lactitol consumption was linked to a significant increase in the frequency of passing feces and the number of subjects passing watery feces, whereas tagatose consumption did not provoke either of these side effects. Tagatose consumption and lactitol consumption both resulted in significant increases in colic, flatulence, borborygmi (rumbling sounds caused by gas moving through the intestines), and bloating, but a majority of these symptoms were described as only "slightly more than usual" by the study participants. Also, a significant number of subjects reported nausea after

consuming the chocolate containing tagatose. While minor side effects were seen after tagatose consumption, in comparison to lactitol, it was found that 20 g of tagatose was well tolerated among the subjects (Lee and Storey 1999).

In a second study done on human gastrointestinal tolerance to tagatose, 73 healthy male subjects consumed cake containing 30 g of tagatose (Buemann and others 1999). Following tagatose consumption, the study participants reported any side effects using a questionnaire which explicitly referred to common symptoms such as heartburn, nausea, vomiting, stomach ache, flatulence, and diarrhea. Nausea was reported in 15.1% of the subjects, and 31.5% of the subjects suffered from diarrhea after consuming tagatose. An increase in flatulence was commonly reported and did not decline over a 15 day period where tagatose was consumed in one 30 g dose daily. While the symptoms were generally mild or moderate, this study showed that consuming 30 g of tagatose at one time may be more than the recommended limit (Buemann and others 1999).

Tagatose as a Prebiotic

Prebiotics are defined as "nondigestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria" (Reid and others 2003). Prebiotics differ from probiotics, because probiotics are "live microorganisms which, when administered in adequate amounts confer a health benefit on the host" (Reid and others 2003). As discussed earlier, the majority of tagatose consumed is not absorbed in the small intestine, thus allowing a substantial portion of tagatose to enter the large intestine where it increases the production of certain beneficial bacteria, ultimately leading to an increase in SCFA production. These qualities allow tagatose to be classified as a prebiotic.

Besides tagatose, other common carbohydrate sources of prebiotics include fructooligosaccharides, inulin, galactooligosaccharides, and lactulose. Prebiotics offer the body many benefits, including the ability to strengthen the immune system, inhibit the growth of harmful bacteria in the intestines, reduce the risk for colon cancer, and aid in digestion and laxation (Kraft® 2007).

While it is known that prebiotics are beneficial to the body, the amount required to gain the beneficial effects has not yet been determined. Based on studies mainly done on fructooligosaccharides, inulin, galactooligosaccharides, and lactulose (Macfarlane and others 2006), experts estimate an intake of 5-15 g of prebiotics daily is needed to see beneficial effects (Reid and others 2003; Tuohy and others 2005). More than 15 g/day may lead to undesirable side effects such as flatulence, bloating, and abdominal cramps (Kraft® 2007; Saavedra and Tschernia 2002). Because tagatose is also a carbohydrate-based prebiotic, it may behave physiologically in a similar fashion to fructooligosaccharides, inulin, lactulose, and galactooligosaccharides.

Health Effects of Tagatose

Diabetes

With carbohydrates being the main nutrient of concern when referring to individuals with diabetes and because diabetes is very prevalent in today's society, tagatose was tested in order to determine how its consumption affected blood glucose levels (Donner and others 1999; Gaio® 2003; Lu and others 2008). The glycemic and insulinemic responses of tagatose were tested by Sydney University's Glycaemic Index Research Service (SUGiRS) (Gaio® 2003). After 12 people consumed 50 g portions of tagatose and glucose dissolved in 200 mL of water, on different mornings, changes in

their blood glucose levels and insulin levels were measured over a two-hour period. While glucose had glycemic and insulinemic responses of 100%, tagatose only produced glycemic and insulinemic responses of 3% (Gaio® 2003).

In a second study, the glycemic effects of oral tagatose alone or in combination with oral glucose were studied in humans with and without type 2 diabetes mellitus (Donner and others 1999). This study was divided into two parts. In the first part of the study, there were eight participants with type 2 diabetes mellitus and eight participants without diabetes. On separate occasions, the participants consumed 75 g glucose, 75 g tagatose, or 75 g tagatose 30 minutes prior to a 75 g glucose load. Between test days, the participants received 10 g tagatose daily. In the second part of this study, ten participants with diabetes mellitus received separate 0, 10, 15, 20, and 30 g of tagatose prior to a 75 g oral glucose tolerance test. From this study, it was concluded that oral tagatose can significantly blunt the rise in blood glucose typically seen after oral glucose consumption in patients with diabetes mellitus. The ability of tagatose to reduce the rise in blood glucose occurs in a dose-dependant manner without significantly affecting insulin levels (Donner and others 1999).

Plasma Uric Acid Levels

Although only a small amount of tagatose is absorbed in the body, the part that is absorbed follows a metabolic pathway in the liver similar to that of fructose. The similarities in the way tagatose and fructose are metabolized concerned researchers because high oral doses of fructose are linked to increased uric acid levels in the blood, otherwise known as hyperuricemia. Individuals who suffer from fructose intolerance syndrome, which is a hereditary genetic defect in the aldolase B enzyme responsible for

cleaving fructose-1-phosphate into D-glyceraldehyde and dihydroxyacetone phosphate, experience hypoglycemia and hyperuricemia after the consumption of moderate amounts of fructose (Buemann and others 2000a). Even individuals without fructose intolerance syndrome may experience a slight hyperuricemic effect after large oral doses of fructose (Buemann and others 2000a).

The possibility of increased uric acid in blood due to the consumption of tagatose can be understood by following the metabolic pathway of tagatose in the liver. Tagatose is initially phosphorylated to D-tagatose-1-phosphate by the enzyme fructokinase. At this point, the enzyme aldolase cleaves D-tagatose-1-phosphate to D-glyceraldehyde and dihydroxyacetone phosphate. Since D-tagatose-1-phosphate is cleaved at a slower rate than fructose-1-phosphate, P_i (inorganic phosphate) is complexed by tagatose for a longer time than by fructose, which is likely to cause a reduction in hepatic P_i (Saunders and others 1999a). Since P_i is an inhibitor of adenosine deaminase, a rate-limiting enzyme in the degradation of adenosine monophosphate (AMP), a reduction of P_i in the liver may lead to increased degradation of purine nucleotides, which can result in a greater amount of uric acid being released from the liver (Saunders and others 1999a). Since the metabolism of tagatose could potentially lead to increased uric acid levels, studies were conducted to observe how varying levels of tagatose would affect the uric acid levels in the blood (Buemann and others 2000a; Buemann and others 2000b; Saunders and others 1999a).

In one study, eight normal human subjects and eight subjects with type 2 diabetes mellitus were adapted to tagatose for three days (given 5, 10, and 25 g single doses of tagatose for three consecutive days), and then on the fourth day they were given a 75 g,

3-hour oral tagatose tolerance test (Saunders and others 1999a). The results from this portion of the test revealed that for both the normal and diabetic subjects, plasma uric acid levels on average peaked 60 minutes into the tagatose tolerance test, although these increases were not statistically significant (Saunders and others 1999a). In the second part of this study, the eight normal subjects were randomly selected to receive either 75 g tagatose or 75 g sucrose daily for eight weeks. The eight diabetic subjects were given either 75 g tagatose or no sugar supplementation for eight weeks. The researchers found that no significant changes occurred in the fasting plasma uric acid levels within each treatment group over the period of the study (Saunders and others 1999a).

A second study compared the differences between how serum uric acid concentrations were affected by either 30 g of tagatose or 30 g fructose (Buemann and others 2000b). The uric acid concentrations of the eight male subjects that participated in the study were observed for seven hours after being administered either 30 g of tagatose or 30 g of fructose orally. Four hours and fifteen minutes after the respective sugar was consumed, the test subjects ingested a fixed lunch in attempt to observe any possible influence tagatose may have on the normal glucose and insulin responses seen during a typical meal. It was found that both the peak concentration and 4-hour area under the curve of serum uric acid were significantly higher after tagatose consumption than they were with either 30 g of fructose or plain water. Also, it was found that tagatose lessened the glycemic and insulinemic responses caused by the meal that was consumed (Buemann and others 2000b).

A third study on this topic attempted to clarify if the acutely increased blood concentration and renal excretion of uric acid can be associated with an accumulation of

D-tagatose-1-phosphate and alterations in ATP in the liver of normal human subjects (Buemann and others 2000a). For this study, five male subjects consumed 30 g tagatose or fructose orally, and were then studied by ^{31}P-magnetic resonance spectroscopy (^{31}PMRS). Blood and urine samples were also taken to observe any increase in uric acid production. Roughly 30 minutes after the tagatose had been administered to the subjects, a peak assigned as D-tagatose-1-phosphate equivalent to roughly 1 mmol/L was apparent on the spectrum for each one of the individuals, and it took about two hours for the baseline to return to normal. Also, around 30 minutes after tagatose consumption, ATP was reduced by roughly 12%. Fifty minutes after tagatose consumption, serum uric acid concentrations increased by 17%, and did not return to the baseline level for the entirety of the experiment (230 minutes after the load). There were no changes in the ^{31}PMRS spectra or serum uric acid concentrations after fructose consumption, leading to the conclusion that moderate tagatose consumption may affect liver metabolism (Buemann and others 2000a).

Tagatose in Food

In 2001, the United States Food and Drug Administration (FDA) agreed with Arla Food Ingredients, and found tagatose to be Generally Recognized as Safe (GRAS) (Rulis 2001). Maximum levels of tagatose allowed in specific products were outlined by the FDA: 1% in carbonated beverages, 1% in ready-to-drink teas presweetened with low calorie sweeteners, 60% in chewing gum, 30% in icing or glazes used on baked goods, 15% in hard candies, 10% in dietetic soft candies, 3 grams per serving in ready-to-eat cereals, 5 grams per serving in powdered products prepared with milk, 10% in low fat, reduced fat, diet, energy, or nutrient fortified bars, and 3% in light ice cream, frozen milk

desserts, low-fat and non-fat frozen yogurt and related frozen novelties (Rulis 2001). Tagatose has also been approved in Australia, New Zealand, and Korea without a specified acceptable daily intake (ADI) (Skytte 2006). In 2005, Brazil and South Africa approved tagatose as a food ingredient and the European Union approved tagatose as a Novel Food Ingredient (Skytte 2006). Tagatose approval is underway in Canada, Japan, and Mexico (IFT 2005; Skytte 2006). After a thorough evaluation process by the Joint FAO/WHO Expert Committee on Food Additives, tagatose was found to be safe as a food ingredient, and due to only minor negative side effects being associated with tagatose consumption, the committee did not specify an ADI for the sugar (WHO 2005).

Although the approval of tagatose by numerous regulatory committees has become fairly widespread throughout the world, the addition of tagatose to food products has been fairly small. Nutrilab, a subsidiary of the Belgian company Damhert, has recently begun using tagatose in chocolate, spreads, cookies, and jams (Damhert 2008). In addition, they are selling tagatose as a home sugar replacer under the name Tagatesse (Damhert 2008). Other products containing tagatose are 7-Eleven's® Diet Pepsi Slurpee, Miada® Chocolite®, Pasco Light and Tasty Juice, Shugr by Swiss Diet, SweetFiber by Dr. Murray Natural Living, and Therasweet™ by Living Fuel (Wise 2008).

Along with the introduction of tagatose into foods found on the retail market, researchers have begun replacing a portion of sucrose with tagatose in bakery products to determine consumer likeability of the products. Two of these studies were conducted by Taylor and others (2008) and Armstrong and others (2009).

In the first study, a cookie recipe was formulated with various levels of sucrose replaced by tagatose to determine the physical properties and consumer likeability of cookies containing tagatose (Taylor and others 2008). The amount of tagatose substituted for sucrose ranged from 25 to 100% in the cookies. When compared to the control cookies (made with 100% sucrose), it was found that the cookies containing tagatose were harder, darker in color, and had less spread. Evaluation of the cookies by 53 untrained panelists showed the panelists liked the color of the 100% tagatose cookies better than the color of the control cookies, but did not like the sweetness of the 100% tagatose cookies. When only half of the sucrose was replaced by tagatose, the overall likeness scores were the same between the experimental cookies and the control cookies. Not only were the physical properties acceptable in cookies where tagatose partially replaced sucrose, but the cookies containing 50% tagatose were liked by panelists (Taylor and others 2008).

A second study looked at adding tagatose to cinnamon muffins, lemon cookies, and chocolate cakes, at levels of 1 and 2%, to study the effect of tagatose on their flavors, and to evaluate consumer likeability of the baked goods (Armstrong and others 2009). In this study, each of the three baked goods mentioned above were prepared twice, once with 1% tagatose and once with 2% tagatose, and compared to products made without tagatose. The products were evaluated by untrained panelists, using triangle tests to study the flavor differences and a hedonic scale to measure how much the panelists liked the products. Upon the conclusion of the experiment, it was found that the panelists were unable to differentiate between baked goods made with and without tagatose, and the mean likeness scores were not significantly different between baked goods made with or

without tagatose. The results show the ability for low levels of tagatose to be added to bakery products without affecting their flavor (Armstrong and others 2009).

Tagatose Stability

Because tagatose is a reducing sugar, it is able to participate in the Maillard reaction in the presence of an amino acid or protein. The Maillard reaction is a type of non-enzymatic browning that occurs between the carbonyl group of an acyclic reducing sugar and the unprotonated amine group, found on either an amino acid or a protein. These complex series of reactions are responsible for the formation of a brown color, as well as changes in flavor and loss of nutritional value (Brands and others 2000). While a large portion of the Maillard reaction is not clearly understood, it is generally accepted that upon interaction between the reducing sugar and the unprotonated amine, a glycosylamine is formed, which then undergoes the Amadori rearrangement to produce a 1-amino-2-keto sugar. This product then participates in a series of reactions to ultimately produce melanoidin pigments, which are responsible for the brown appearance of foods (Daniel and others 2007). After participating in the Maillard reaction, the amino acid is no longer available as a nutrient, causing the product to lose some of its nutritional value (Daniel and others 2007).

There are many factors that influence the Maillard reaction, including temperature, pH, and reactant concentration (Brands and others 2002). While the Maillard reaction generally occurs at higher temperatures associated with thermal processing conditions (60-80°C), it has even been found to occur in products stored at room temperature. Also, the Maillard reaction occurs more rapidly at higher pH values, where more unprotonated amines are available. The reaction rate is also directly

proportional to the reactant concentration. As the reactant concentration increases, the Maillard reaction generally increases since more of the reactant is available to participate in the reaction, and there is a greater likelihood that the reactive form of the sugar or amino acid will be present (Daniel and others 2007). While the browning and flavors caused by the Maillard reaction are desirable in some products, such as baked bread and roasted peanuts, in other products, like dried milk, the reaction is undesirable. Understanding the Maillard reaction and the factors that affect it is important when studying the reactivity of a reducing sugar, such as tagatose.

Besides the Maillard reaction, monosaccharides are prone to degradation reactions influenced by the pH of the solution in which they are held. Under alkaline conditions, monosaccharides undergo a series of rearrangements, including ionization, mutarotation, enolization, and isomerization to form an enediol anion species. After undergoing several other reactions, the enediol anion species is ultimately degraded into carboxylic acids (De Bruijn and others 1986). The formation of carboxylic acids causes the pH of the solution to decrease, ultimately slowing down monosaccharide degradation reactions that had been taking place under the initial alkaline conditions.

Under acidic conditions, monosaccharides undergo a set of degradation reactions that differ from the reactions that are seen in alkaline solutions. When heated in a strongly acidic solution, dehydration occurs and furfural compounds are formed (Wong 1989). In dilute acid solutions, aldohexoses lose a water molecule to form anhydro products, most commonly the 1,6-anhydro sugar (Wong 1989). Although through different reaction pathways, both aqueous alkaline and acidic solutions can cause the degradation of monosaccharides. Since a large portion of tagatose needs to remain intact

in order to provide the prebiotic benefit, studying the stability of tagatose at different pH values under typical processing temperatures is critical.

Ryu and others (2003) conducted a study to observe the effects of pH and temperature on the non-enzymatic browning reaction between tagatose and glycine in aqueous solutions. To observe the temperature effect on non-enzymatic browning, solutions containing 0.2 M tagatose and 0.2 M glycine were heated without pH control in a water bath for 5 hours at 70, 80, 90, or 100°C. The effect of pH on non-enzymatic browning was determined by heating tagatose-glycine solutions with a pH of 3, 4, 5, 6 or 7 in a 100°C water bath for five hours. The acid stability of tagatose was studied by heating a 5% tagatose solution at 100°C for 5 hours, at pH 3, 4, and 5. The heat stability of tagatose was tested by heating a 10% tagatose solution for 5 hours at 100°C, with no pH control. For each test, one sample was pulled from the water bath each hour for analysis. The samples were analyzed using a spectrophomoter, Hunter color measurements, and HPLC. Overall, the researchers found that the rate of browning was dependent on the temperature (as the temperature increased, so did the browning). In regards to pH, they found that browning increased as the pH increased from 3-7, with a slight decrease in browning occurring at pH 6. They also found that there was no browning or decomposition of tagatose under any of the temperature or pH conditions studied in this experiment, unless glycine was present (Ryu and others 2003). Based on their data, they inferred the browning was caused by tagatose and glycine undergoing the Maillard reaction (Ryu and others 2003).

The storage stability of tagatose has recently been studied by Dobbs (2008). In this study, buffered solutions at either pH 3 or 7 were held at 20, 30, and 40°C for 265

days. It was found that the greatest tagatose loss occurred in solutions containing higher buffer concentrations (0.1 rather than 0.02 M) at higher pH levels (7 rather than 3). Also, phosphate buffer solutions promoted the breakdown of tagatose more than solutions buffered with citrate. When held at 20, 30, and 40°C, tagatose degradation increased with the storage temperature. After being held under the appropriate conditions for a period of 100 days, tagatose degradation ranged from 0.4 to 56.7% with the greatest loss taking place in 0.1 M phosphate solutions at pH 7 and 40°C. It was also found that at increased levels of tagatose loss, a greater extent of brown pigment formation was observed.

The study by Dobbs (2008) indicates that tagatose degradation does occur under storage conditions based on temperature, pH, buffer type, and buffer concentration. This leads to the possibility that tagatose degradation may also occur under processing conditions. The experiment carried out by Ryu and others (2003), provides a basic understanding of how tagatose might react under elevated temperatures, but there is a need for a more thorough investigation of this topic. More reliable kinetic data, regarding the breakdown of tagatose over a period of time, can be achieved by carrying out the experiments for a greater length of time than five hours. Also, in the study conducted by Ryu and others (2003), the researchers controlled the pH of their solutions by using hydrochloric acid or sodium hydroxide. However, most foods contain citrate or phosphate buffers to regulate pH, so it would be beneficial to look at the influence of buffer type and concentration on the stability of tagatose and the ability of tagatose to cause browning at elevated temperatures. More research in this area would be beneficial

in providing a better understanding of tagatose and its interactions under various conditions that may be encountered during food processing techniques.

The objective of this study was to determine the effect of pH, temperature, buffer concentration, and buffer type on the thermal stability and browning of tagatose. Corresponding to temperatures used in processing techniques such as pasteurization, solutions containing about 1% tagatose, with or without glycine, were held at 60, 70, and 80°C for a period of time ranging from 5-216 hours. In order to create an environment similar to that which is found in common foods, such as fruit juice and milk, pH 3 and 7 were used in this experiment. Citrate and phosphate buffers were also added to the solutions at levels of 0.02 M and 0.1 M, since they are commonly found in foods to maintain a desired pH. The results from this experiment will provide insight on the stability of tagatose at processing temperatures and the ability of tagatose to cause brown discoloration under various conditions.

CHAPTER 3: MATERIALS AND METHODS

Sample Preparation

A total of 16 different solutions were prepared for this experiment. Eight solutions contained 0.05 M tagatose and either citrate or phosphate buffer at levels of either 0.02 M or 0.1 M, and at pH 3 or 7. The other 8 solutions were similar to the previous 8 solutions, but also included 0.05 M glycine. The tagatose used for the experiment was donated by Arla Food Ingredients (Basking Ridge, N.J., USA) and represents the quality of tagatose available to food manufacturers. Product specifications indicated the purity of tagatose was greater than 99% with water, non-tagatose monosaccharides, and ash making up the balance. The sodium phosphate dibasic anhydrous, sodium phosphate monobasic monohydrate, sodium citrate, citric acid anhydrous, 85% phosphoric acid, and glycine were obtained from Fisher Scientific (Pittsburgh, PA).

The tagatose solutions containing 0.1 M phosphate buffer at pH 7 were prepared using the following procedure. First, a bulk buffer solution containing 3.45 g of sodium phosphate monobasic monohydrate and 2.25 g of tagatose was mixed in a 250 mL volumetric flask. The flask was filled with enough distilled water to reach 250 mL. The second bulk buffer solution was made by adding 3.55 g of sodium phosphate dibasic anhydrous and 2.25 g of tagatose to a 250 mL volumetric flask. Again, enough distilled water was added to bring the volume to 250 mL. Once the bulk buffer solutions were

mixed thoroughly, appropriate volumes of the 2 solutions were mixed together until the solution reached pH 7. The pH of the solutions were determined using a pH meter (model 920A, Orion Research Inc, Boston, MA). The solution was separated into 3, 50 mL aliquots and held in a -80°C freezer until use. The tagatose solutions containing 0.02 M phosphate buffer at pH 7 were prepared in a similar fashion to the 0.1 M phosphate solutions at pH 7, except only 0.690 g of sodium phosphate monobasic monohydrate and 0.710 g of sodium phosphate dibasic anhydrous were included in the bulk buffer solutions.

The tagatose solutions containing 0.1 M phosphate buffer at pH 3 were prepared by first adding 6.90 g of sodium phosphate monobasic monohydrate to a 500 mL volumetric flask, and filling the flask with distilled water to reach a volume of 500 mL. After mixing the solution thoroughly, 250 mL of the solution was transferred to a 250 mL volumetric flask containing 2.25 g of tagatose to create the first bulk buffer solution, which had a pH around 4.4. Next, 2.88 g of 85% phosphoric acid were added to distilled water in a 250 mL volumetric flask, and distilled water was added to the flask until the solution reached 250 mL. Part of the 85% phosphoric acid solution was then mixed with the remaining sodium phosphate monobasic monohydrate solution (the one without tagatose), to create a solution with a pH of 2.7. Once the desired pH was reached, 2.25 g of tagatose were added to 250 mL of this pH 2.7 solution to create the second bulk buffer solution. Portions of the two bulk buffer solutions at pH 2.7 and 4.4 were mixed together until pH 3 was achieved. The final solution was then divided into 3 separate 50 mL aliquots, which were stored in a -80°C freezer until use. The tagatose solutions containing 0.02 M phosphate buffer at pH 3 were prepared in a similar fashion to the 0.1

M phosphate solutions at pH 3, except only 0.576 g of 85% phosphoric acid and 1.38 g of sodium phosphate monobasic monohydrate were included in the bulk buffer solutions.

A citrate buffer was also used in some of the tagatose solutions. Those solutions containing 0.1 M citrate buffer both at pH 3 and pH 7 were prepared by initially adding 7.35 g of sodium citrate and 2.25 g of tagatose to a 250 mL volumetric flask. The flask was then filled with distilled water until the volume reached 250 mL to create a bulk buffer solution. In a second flask, 4.80 g of citric acid and 2.25 g of tagatose were mixed thoroughly with distilled water to create 250 mL of this second bulk buffer solution. Once each of the solutions were mixed thoroughly, both solutions were combined in varying proportions to reach the desired pH (either pH 3 or pH 7). Three, 50 mL aliquots of the pH 3 solution, and 3, 50 mL aliquots of the pH 7 solution were held in a -80°C freezer until use. The tagatose solutions containing 0.02 M citrate buffer at pH 3 and pH 7 were prepared in a similar fashion to the 0.1 M citrate solutions, except only 1.47 g of sodium citrate and 0.961 g of citric acid were included in the bulk buffer solutions.

The 8 tagatose-glycine solutions were prepared using the same techniques as those described above for the tagatose solutions, except that each bulk buffer solution also contained 0.05 M glycine (0.938 g of glycine in each 250 mL flask of solution).

Experiments

The experiments were carried out in a water bath preheated to either 60, 70, or 80°C. While all of the 16 solutions (8 tagatose solutions and 8 tagatose-glycine solutions) were tested at 80°C, only 12 of the solutions were tested at 60 and 70°C. The tagatose and tagatose-glycine solutions with 0.02 M citrate and 0.02 M phosphate buffers at pH 3 were not tested at 60 and 70°C due to extremely low reactivity at 80°C. The duration of

the experiments ranged from 5 hours for the fastest reaction to a maximum of 216 hours for the slowest reaction (Tables 3.1 and 3.2). Each of the experiments were carried out under the following procedure.

For each experiment, the desired solution was thawed to room temperature and mixed thoroughly with a vortex. Using a 20 mL syringe fitted with a 25 mm, 0.2 μm nylon filter and needle, approximately 1.5 mL solution was distributed into eleven to fifteen 7 inch x 5 mm Kontes brand NMR sample tubes obtained from Fisher Scientific. Once filled, the NMR tubes were then fitted with push caps and placed into a rack sitting in the preheated water bath. After being placed in the water bath, it took the solutions inside the tubes approximately 1 minute to reach the temperature of the water bath. To measure the actual temperature of the samples, an NMR tube was filled with distilled water, and a thermocouple sensor (Type K, Fisher Scientific, Pittsburgh, PA) was placed in the tube to provide continuous temperature readings. At regular time intervals, individual tubes were removed from the water bath and immediately placed in an ice bath for approximately 1 minute to cool the solution to below room temperature in order to stop any reactions. For example, at 70°C the 0.1 M phosphate buffer solutions at pH 7 were pulled at 1 hour intervals for a total of 10 hours. The temperature of the water bath and the time at which samples were pulled were recorded. Upon cooling, the solution was transferred from the NMR tube to a 5 mL cryogenic vial using a plastic pipette.

Each cryogenic vial was labeled according to the sample it contained. The buffer concentration was denoted as either "1" meaning 0.1 M or "02" meaning 0.02 M. Buffer type was indicated by the first letter of the buffer: "P" meaning phosphate, "C" meaning

Table 3.1. Length (h) of Tagatose Experiments.

Sample	Temperature (°C)		
	60	**70**	**80**
pH 3			
0.02 M phosphate	ND	ND	10.0
0.02 M citrate	ND	ND	10.0
0.1 M phosphate	215.8	168.0	10.0
0.1 M citrate	215.8	168.0	10.0
pH 7			
0.02 M phosphate	215.8	56.0	10.0
0.02 M citrate	215.8	56.0	10.0
0.1 M phosphate	81.5	10.0	5.0
0.1 M citrate	81.5	10.0	7.5

ND = not determined

Table 3.2. Length (h) of Tagatose-Glycine Experiments.

Sample	Temperature (°C)		
	60	**70**	**80**
pH 3			
0.02 M phosphate	ND	ND	10.0
0.02 M citrate	ND	ND	10.0
0.1 M phosphate	216.0	156.2	10.0
0.1 M citrate	216.0	156.2	10.0
pH 7			
0.02 M phosphate	216.0	60.0	10.0
0.02 M citrate	216.0	60.0	10.0
0.1 M phosphate	82.0	10.0	7.0
0.1 M citrate	82.0	10.0	7.0

ND = not determined

citrate. The pH value of the solution, either 3 or 7, was placed on the label, along with whether the sample contained only tagatose or both tagatose and glycine ("T" or "TG", respectively). Temperature, in Celsius, was denoted on the label as "60", "70", or "80". For example, 1P7-T80 meant that the vial contained a 0.1 M phosphate solution with only tagatose, at pH 7 and held at 80°C. After placing the sample in the correctly labeled vial and securing the vial with a screw cap, it was placed in the refrigerator until analysis, which generally occurred 1-2 days after the experiment was completed. Stability data from Dobbs (2008) indicated additional tagatose loss during refrigerated storage was minimal (less than 0.4% in 0.1 M phosphate buffer at pH 7 held for 7 d).

Sample Analysis

Tagatose Degradation

Tagatose degradation was determined by analyzing the tagatose concentration in each sample using reverse-phase high performance liquid chromatography (HPLC). A 250 x 4.6 mm Luna 5μ amino column (Phenomenex, Torrance, CA) and a 91%/9% acetonitrile/water mobile phase having a flow rate of 3 mL/min were used for analysis. Tagatose detection occurred through the use of a refractive index detector (Shimadzu, Kyoto, Japan). A Hewlett-Packard Integrator was used to integrate the data. Figure 3.1 shows a typical chromatogram where tagatose elutes around 7 minutes. Under the parameters of HPLC analysis for this experiment, the glycine was retained on the column; therefore for those samples that contained glycine, only a tagatose peak was observed on the chromatogram.

Six standard solutions with a known concentration of tagatose were analyzed prior to the analysis of the study samples. The area and concentration of the standards

were used to create a linear standard curve, from which the concentration of the samples from each experiment could be determined. From the chromatogram, the area of the tagatose peak was recorded and used to determine the concentration of tagatose in each sample from a standard curve.

To evaluate the validity of the analytical methods, 10 solutions of tagatose in 0.1 M phosphate buffer at pH 7 were prepared, 5 containing 0.0514 M tagatose and 5 containing 0.0302 M tagatose. Solutions were filtered and analyzed by HPLC, as described previously. The average percent recovery ranged from 99.5-102.6% and the coefficient of variation was less than 2%, indicating a highly reliable method.

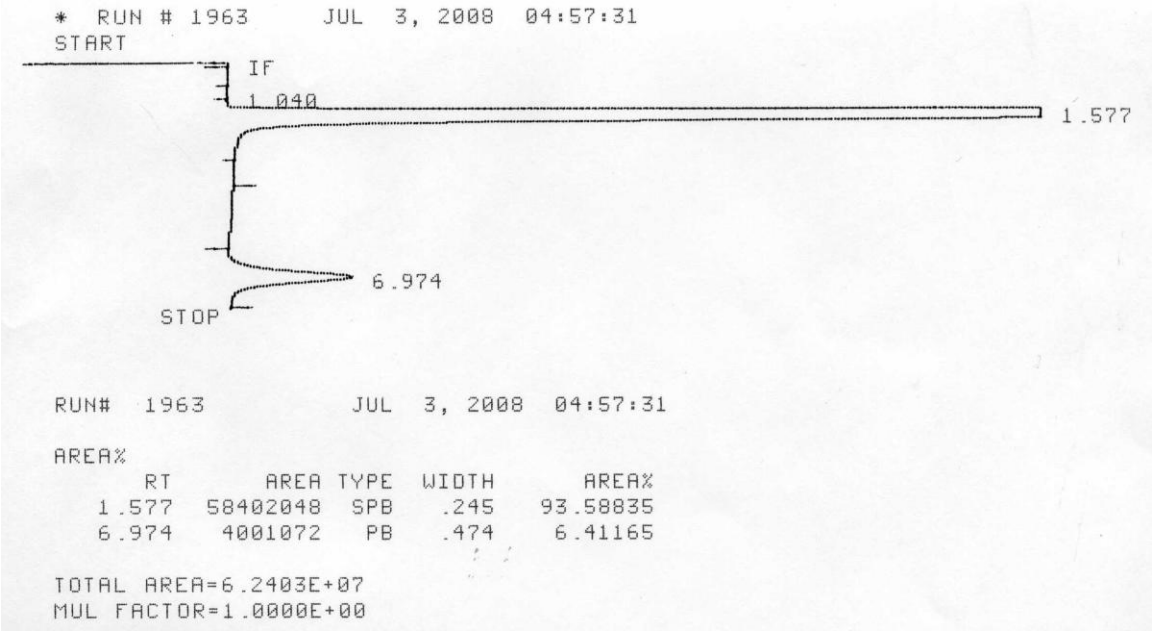

Figure 3.1. Sample HPLC Chromatogram for the Analysis of Tagatose in a 0.1 M Phosphate Buffer Solution at pH 3 containing 0.05 M Glycine. Tagatose Elutes at Approximately 7 minutes.

Brown Pigment Formation

The formation of brown pigment in the tagatose and tagatose-glycine samples was measured using a spectrophotometer (DU 640, Beckman Instrument Inc, Fullterton, CA). Approximately 1 mL aliquots of each sample were transferred to methacrylate semimicro style cuvettes. Absorption was read at 420 nm.

Data Analysis

Microsoft® Excel® was used to statistically analyze the data collected in this study. Monosaccharide degradation pathways have been studied using a first-order kinetic model (De Bruijn and others 1986; Kelly and Brown 1978/79). Therefore, tagatose degradation was modeled using first order kinetics (Eq. 3.1). The pseudo-first-order rate constants for tagatose loss were determined, along with 95% confidence intervals, using least squares analysis as described by Labuza and Kamman (1983). In the equation, "C" refers to the tagatose concentration (M) at a specific time, "C_0" is the initial tagatose concentration (M), "k" is the rate constant (h^{-1}), and "t" is the time (h).

$$\ln \frac{[C]}{[C_0]} = -kt \tag{3.1}$$

After plotting the data using pseudo-first-order kinetics, the tagatose concentrations appeared to level off in some of the experiments, deviating from typical first order behavior. Visual determination as well as comparison of the R^2 values with and without the "plateau" were used to establish its existence and magnitude for each experiment individually. For those experiments where a "plateau" was observed, only the initial data prior to the "plateau" were used when determining pseudo-first-order rate constants. For all other experiments, the complete data set was used in the kinetic model.

For solutions containing tagatose as well as glycine, the data would typically follow second order kinetics. However, since multiple reaction pathways are occurring simultaneously, it was determined to be more appropriate to model the data using pseudo-first-order kinetics (Eq. 3.1).

The formation of brown pigmentation has generally been modeled using pseudo-zero-order kinetics (Baisier and Labuza 1992; Karmas and others 1992; Warmbier and others 1976). Therefore, rate constants for brown pigment formation, as well as 95% confidence intervals, were determined using pseudo-zero-order kinetics (Labuza and Kamman 1983). In Eq. 3.2, "A" is the absorbance at time (h), "t", and "A_0" is the initial absorbance.

$$[A] = kt + [A]_0 \tag{3.2}$$

Upon plotting graphs of absorbance as a function of time for tagatose and tagatose-glycine samples, a lag phase was noticed in some of the experiments. For these experiments, the magnitude of the lag phase was determined individually, based on visual determination and comparison of the R^2 values with and without the lag phase. When determining pseudo-zero-order rate constants for those experiments, the lag phase was removed. All other experiments were void of any noticeable lag phase; therefore, all data were used to determine pseudo-zero-order rate constants.

Using the rate constants and average temperature for each experiment, activation energies, E_A, were determined using Eq. 3.3 (Labuza and Kamman 1983).

$$\ln k = \ln k_0 - \frac{E_A}{RT} \tag{3.3}$$

In equation 3.3, k is the rate constant (h^{-1}), k_0 is the pre-exponential factor (h^{-1}), R is the ideal gas constant (1.987 cal K^{-1} mol^{-1}), and T is the temperature in Kelvin. Plotting ln k versus $\frac{1}{T}$ gives a straight line, which has a slope equal to $-\frac{E_A}{R}$.

CHAPTER 4: RESULTS AND DISCUSSION

Tagatose Degradation

Tagatose degradation in solution is affected by many factors including temperature, pH, buffer type, and buffer concentration. Examples of the tagatose degradation profile and the pseudo-first-order plot are shown in Figures 4.1 and 4.2, respectively. As shown in Figure 4.2, the pseudo-first-order model did not fit the entire data set for tagatose degradation in 0.02 M phosphate buffer at pH 7 and 60°C. A leveling off of the tagatose degradation appeared after 120 h, which is likely due to a pH drop. For this particular model system, only the data up to 120 h were used for the kinetic model. This "plateau" was also noted by Dobbs (2008) for tagatose degradation at 20-40°C. Tagatose loss in the other buffer solutions followed pseudo-first-order kinetics over the duration of the experiment, without a clear "plateau". Pseudo-first-order rate constants and 95% confidence limits for tagatose degradation were calculated using least squares analysis and are listed along with the R^2 values in Table 4.1.

Effect of pH

In this study, solutions were held at pH 3 and pH 7 to determine the effect of pH on tagatose degradation. Tagatose degradation occurred faster at higher pH values (Table 4.1). This result is shown graphically in Figure 4.3, where the overall tagatose

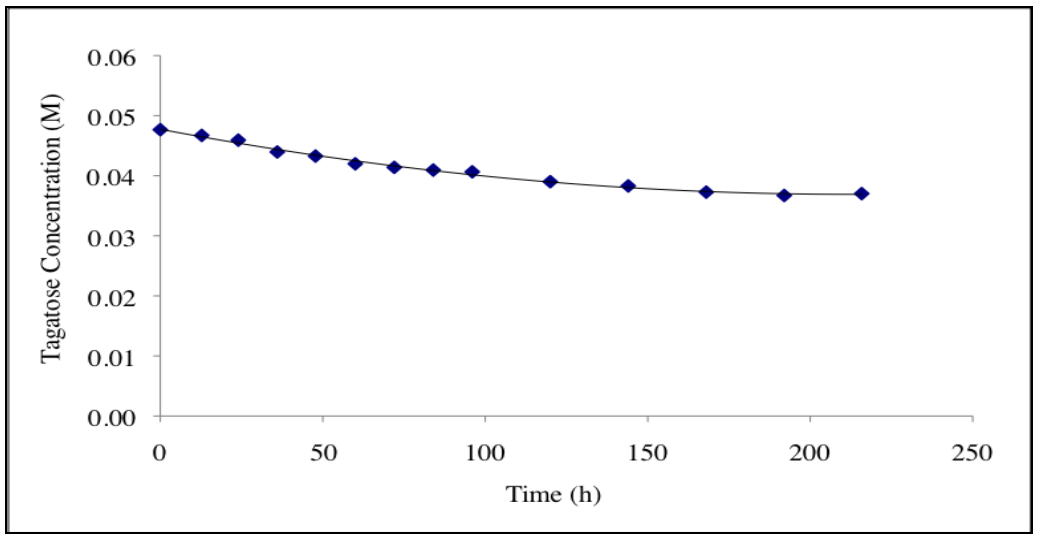

Figure 4.1. Tagatose Loss in 0.02 M Phosphate Buffer at pH 7 and 60°C.

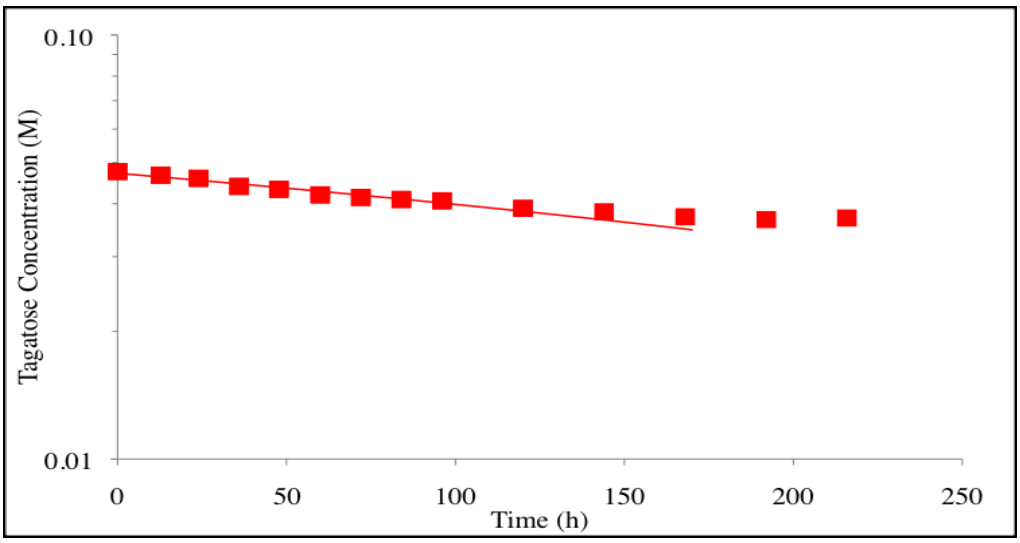

Figure 4.2. Pseudo-First-Order Plot of Tagatose Loss in 0.02 M Phosphate Buffer at pH 7 and 60°C.

Table 4.1. Pseudo-First-Order Rate Constants (h^{-1}) with 95% Confidence Limits for the Loss of Tagatose in Solution.

Sample	Temperature (°C)		
	60	**70**	**80**
pH 3			
0.02 M phosphate	ND	ND	0*
0.02 M citrate	ND	ND	0*
0.1 M phosphate	0.00021±0.00012 (R^2=0.55)	0.00059±0.00016 (R^2=0.82)	0*
0.1 M citrate	0.000092±0.00008 (R^2=0.32)	0.00063±0.00014 (R^2=0.88)	0*
pH 7			
0.02 M phosphate	0.00170±0.00024 (R^2=0.96)	0.00566±0.00071 (R^2=0.97)	0.0249±0.0020 (R^2=0.99)
0.02 M citrate	0.00082±0.00013 (R^2=0.94)	0.00345±0.00047 (R^2=0.96)	0.0192±0.0030 (R^2=0.96)
0.1 M phosphate	0.00661±0.00044 (R^2=0.99)	0.0301±0.0022 (R^2=0.99)	0.0756±0.0070 (R^2=0.98)
0.1 M citrate	0.00239±0.00071 (R^2=0.81)	0.0113±0.0036 (R^2=0.84)	0.0358±0.0046 (R^2=0.97)

ND = not determined due to extremely low reactivity at 80°C

*Not different from zero over the 10 h experiment duration based on the 95% confidence limit exceeding the value of the rate constant to encompass zero.

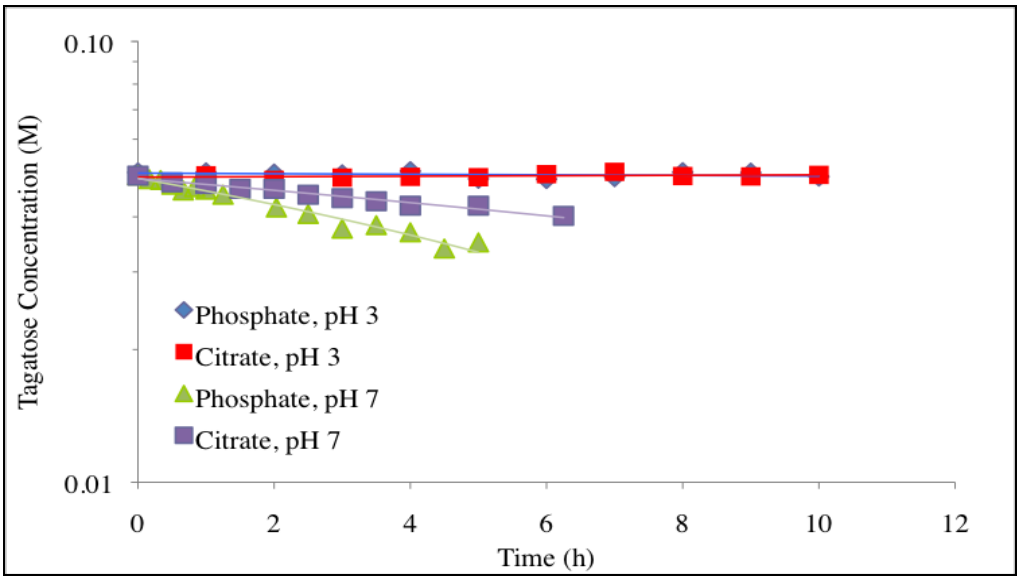

Figure 4.3. Tagatose Loss in 0.1 M Buffer Solutions at 80°C as Affected by pH.

concentration in 0.1 M citrate buffer at pH 7 and 80°C decreased by approximately 26% from the initial concentration in comparison to tagatose in 0.1 M citrate buffer at pH 3, which showed little to no change in tagatose concentration over the course of the experiment. Similar results were seen in the solutions containing phosphate buffer, in which greater tagatose degradation was also seen at pH 7 than pH 3.

Monosaccharides are able to undergo degradation reactions under both alkaline and acidic conditions. When monosaccharides are heated in a strongly acidic solution, dehydration occurs and furfural compounds are formed (Wong 1989). At pH 3, tagatose participated very minimally in the acidic degradation reaction (Table 4.1).

A greater extent of tagatose degradation was seen in tagatose solutions held at pH 7, with the fastest tagatose degradation occurring in 0.1 M phosphate buffer at 80°C. Since more tagatose degradation was seen at pH 7 than pH 3, it is apparent that tagatose more readily undergoes the alkaline degradation reaction than the degradation reaction

that occurs under acidic conditions. Under alkaline conditions, monosaccharides undergo a series of rearrangements, including ionization, mutarotation, enolization, and isomerization to form an enediol anion species. After undergoing several other reactions, the enediol anion species is ultimately degraded into carboxylic acids (De Bruijn and others 1986). The formation of carboxylic acids can cause the pH of the solution to decrease, which would slow down the rate of the alkaline degradation reaction. This effect was observed in 0.02 M phosphate buffer at pH 7 and 60°C, where tagatose degradation leveled off after 120 h. Dobbs (2008) noted a similar "plateau" in tagatose degradation after 100 d storage. As presented in Table 4.2, the pH of the tagatose solutions originally at pH 7 decreased over a period of 6-7 days at 60°C, indicating the formation of acidic degradation products. However, a definitive "plateau" in the degradation profile was only observed for tagatose degradation in 0.02 M phosphate buffer where the pH drop was greatest.

Table 4.2. Decrease in the pH of Tagatose Solutions During Storage at 60°C.

Sample	Initial pH	Final pH
0.02 M phosphate	6.93	5.07*
0.02 M citrate	7.04	6.19*
0.1 M phosphate	7.06	6.59**
0.1 M citrate	7.09	6.45**

* After 7 days of storage
**After 6 days of storage

The effect of pH on tagatose degradation, as determined by this study, is consistent with the findings of Dobbs (2008) in the respect that tagatose degradation was faster at pH 7 than pH 3. However, in the study by Dobbs (2008), the degradation of tagatose in most solutions appeared to level off at approximately 100 days when held at ambient storage temperatures (20, 30, and 40°C), especially in phosphate buffer at pH 7. As shown in Figure 4.3, tagatose degradation at high temperatures continued rather consistently without a "plateau" for all solutions. The only exception was degradation in 0.02 M phosphate buffer at pH 7 and 60°C, as mentioned earlier. The difference between the studies is likely due to differences in temperature and duration. Under the higher temperature conditions used in the current study, sufficient energy was available to compensate for the reduced pH, which maintained the reaction rates. Therefore, over the duration of most experiments in the current study, no "plateau" in tagatose degradation was seen. A "plateau" may have appeared if the duration of the experiment was increased, such as in 0.02 M phosphate buffer at pH 7 and 60°C.

Ryu and others (2003) also studied the effect of pH on tagatose degradation, however their results appear to conflict with those of the current study. In the study by Ryu and others (2003), unbuffered solutions containing tagatose were held at pH 3, 4, and 5 for a period of 5 hours at 100°C. No tagatose degradation occurred at any of the pH levels in their study. Even though the degradation of tagatose was not as dramatic as that which occurred at pH 7, tagatose degradation was seen in buffered tagatose solutions at pH 3 in the current study. One reason for the conflicting findings between the two studies was that Ryu and others (2003) did not use buffer salts in their solutions. As determined in this study and other studies (Bell and Wetzel 1995; Dobbs 2008;

Pachapurkar and Bell 2005), the presence of buffer salts affects the stability of molecules, including tagatose. Reaction rates tend to increase as buffer concentration increases. Another difference between the studies was the duration of the experiments. In the current study, the majority of the experiments were carried out for at least 10 hours, whereas the experiments conducted by Ryu and others (2003) only took place for 5 hours. The shorter experiment length may not have been sufficient for reactions leading to tagatose degradation to have taken place in detectable amounts, especially in the absence of buffer salts.

Effect of Buffer Type and Buffer Concentration

From the data presented in Table 4.1 and Figure 4.4, it is apparent that the tagatose solutions containing phosphate buffer generally had faster tagatose degradation than the solutions containing citrate buffer, with a more noticeable difference observed at the higher buffer concentration (0.1 M). As shown in Table 4.1, in the tagatose solutions with 0.1 M buffer at pH 7 and 80°C, the solution containing phosphate buffer had a rate constant twice as large as in the solution containing citrate buffer ($0.0756\pm0.0070\ h^{-1}$ and $0.0358\pm0.0046\ h^{-1}$, respectively). Under similar conditions, but at a lower buffer concentration (0.02 M), the rate constants for tagatose degradation in phosphate and citrate buffers were $0.0249\pm0.0020\ h^{-1}$ and $0.0192\pm0.0030\ h^{-1}$, respectively. Higher buffer concentrations also promoted faster tagatose degradation; tagatose loss in 0.1 M phosphate buffer at pH 7 and 70°C had rate constant of $0.0301\pm0.0022\ h^{-1}$ while that in 0.02 M phosphate buffer under the same conditions only had a rate constant of $0.00566\pm0.00071\ h^{-1}$. The effect of buffer type and concentration on tagatose degradation are in agreement with the findings of other researchers.

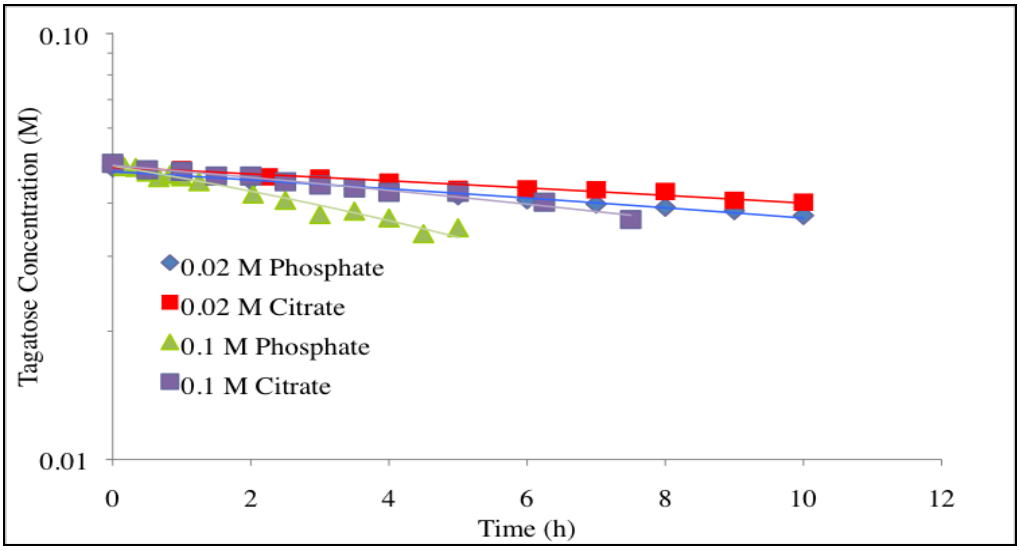

Figure 4.4. Tagatose Loss in Solution at pH 7 and 80°C as Affected by Buffer Type and Concentration.

Bell and Wetzel (1995) studied aspartame degradation in citrate and phosphate buffer solutions and found that the reaction rates for aspartame degradation were significantly greater in phosphate buffer than citrate buffer at pH 7, with less dramatic differences between the buffer types seen at pH 3. They also found that aspartame degradation increased as the buffer concentration increased.

Pachapurkar and Bell (2005) found that thiamin degradation occurred faster in phosphate buffer rather than citrate buffer under similar conditions. Also, a decrease in thiamin stability was observed when the buffer concentration increased.

Dobbs (2008), who studied the effect of buffer type and concentration on tagatose degradation at storage temperatures, also found that tagatose degradation increased when held in phosphate buffer rather than citrate buffer. Tagatose degradation rates were also

found to increase as the buffer concentration increased. The fastest tagatose degradation was seen in 0.1 M phosphate buffer at pH 7, which is consistent with the data from the current study at high temperatures. As mentioned previously, Ryu and others (2003) found no tagatose loss in unbuffered solutions at 100°C for 5 h, which also indicates the importance of buffer salts on tagatose degradation.

The results from this study are consistent with the findings from the literature (Bell and Wetzel 1995; Pachapurkar and Bell 2005; Dobbs 2008). The dibasic anion (HPO_4^{-2}), which is the primary component in the phosphate buffer at pH 7, appears to be much more efficient at the required proton transfers for the degradation reaction than citrate anions. Bell and Wetzel (1995) discussed the ability of the small phosphate anions to act as bifunctional catalysts, meaning they can simultaneously donate and accept protons. Bifunctional catalysis by phosphate anions allows for faster proton transfers than by citrate anions, which are not bifunctional catalysts. Proton transfers are required in the alkaline degradation reaction of tagatose in order to form the enediol species, including the removal of a proton from one hydroxyl group during ionization and the transfer of a proton during mutarotation (Wong 1989). Since the phosphate dibasic anion is more efficient at these proton transfers than the citrate anion, tagatose degradation can occur faster in phosphate buffer solutions, as seen in this study.

Effect of Temperature

The effect of temperature on tagatose degradation is shown in Figure 4.5 and Table 4.1. A proportional relationship exists between temperature and tagatose degradation; as the temperature increased from 60 to 80°C, the rate constants for tagatose degradation also increased. Due to an increase in molecular movement at higher

temperatures, it is expected for reactions to occur faster when the temperature is raised. As shown in Table 4.1, the rate constants for tagatose loss in 0.1 M phosphate buffer at pH 7 increased from 0.00661±0.00044 h^{-1} at 60°C to 0.0756±0.0070 h^{-1} at 80°C. The results from this study are in agreement with the findings of Dobbs (2008). The study by Dobbs (2008) looked at the stability of tagatose under storage conditions (20, 30, and 40°C), and it was found that the higher temperatures enhanced tagatose degradation.

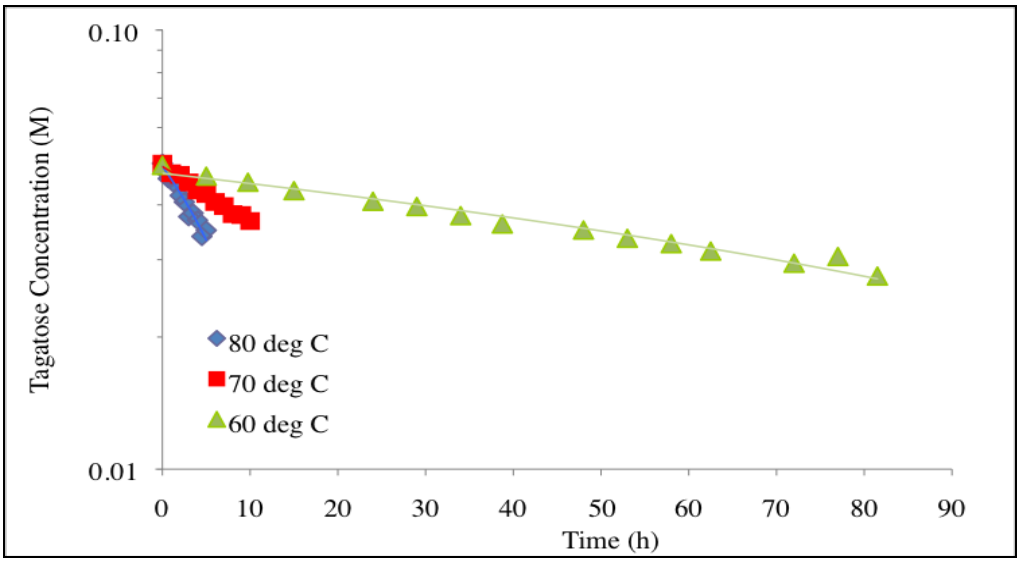

Figure 4.5. Tagatose Loss in 0.1 M Phosphate Buffer at pH 7 as Affected by Temperature.

The activation energies were calculated and are shown in Table 4.3. A higher activation energy indicates the reaction rate is more sensitive to changes in temperature, whereas a lower activation energy indicates the reaction rate is less sensitive to temperature changes. At pH 3, activation energies were unable to be determined due to

only having reliable rate constants at 2 temperatures (Table 4.1). At pH 7, the activation energies ranged from 31.4 to 38.7 kcal/mol. Dobbs (2008) calculated activation energies for tagatose degradation in 0.02 and 0.1 M phosphate and citrate buffers at pH 7 held at storage temperatures (20, 30, and 40°C). The activation energies for tagatose degradation in the solutions held at storage temperatures ranged from 6.4 to 14.8 kcal/mol, which are much lower than the activation energies for the same solutions held under thermal processing temperatures (Table 4.3). An explanation for the difference in the activation energies between the two temperature ranges is not clear, but may be due to different reaction pathways predominating at the lower storage temperatures as compared to the higher processing temperatures. Also, in the experiment conducted by Dobbs (2008), a "plateau" was seen in the data; therefore only the initial 100 days of storage data were used when determining the rate constants and activation energies. The portion of data containing the "plateau" in tagatose degradation was not included in the calculations. In the current study, the entire data set was used in calculating the activation energies for all experiments, except 0.02 M phosphate at pH 7 and 60°C. The utilization of initial rates by Dobbs (2008) may have contributed to some of the variation between the activation energies at the different temperature ranges.

The activation energies were slightly lower for tagatose degradation in phosphate buffer than citrate buffer (Table 4.3). The catalytic effect of the phosphate buffer, discussed previously, reduced the energy needed for the reaction as compared to citrate buffer.

Using the activation energies, the extent of tagatose degradation that may occur under actual thermal processing conditions can be mathematically predicted. Because the

Table 4.3. Activation Energies (E_A) for Tagatose Degradation at pH 7.

Sample	E_A (kcal/mol)
0.02 M phosphate	33.2
0.02 M citrate	38.7
0.1 M phosphate	31.4
0.1 M citrate	33.9

fastest tagatose degradation was seen in 0.1 M phosphate buffer at pH 7, the activation energy for this solution was used to predict the amount of tagatose degradation that would occur during commonly used pasteurization techniques in the food industry. Under vat pasteurization, liquids are subjected to a temperature of 63°C for 30 minutes, and high temperature short time (HTST) pasteurization consists of heating products for 15 seconds at 72°C. Using the activation energy of 31.4 kcal/mol for 0.1 M phosphate buffer at pH 7, it was calculated that less than 0.5 and 0.02% tagatose would be lost under basic vat and HTST pasteurization conditions, respectively. Under conditions where tagatose is more stable, even less tagatose loss would be expected.

Tagatose and the Maillard Reaction

Tagatose solutions also containing 0.05 M glycine were observed at the same temperatures, pH values, and buffer types and concentrations as the tagatose solutions without glycine in order to determine the participation of tagatose in the Maillard reaction. As explained previously, typically a solution containing two reactants would follow second order kinetics, however, in this case multiple reactions are occurring simultaneously. When plotted, the data fit well into a first order model, so pseudo-first-order kinetics were appropriate to use. A "plateau" in tagatose degradation was observed

in five tagatose-glycine solutions: 0.02 M phosphate and citrate buffers at pH 7 and 60°C, 0.02 M phosphate buffer at pH 7 and 70°C, and 0.1 M citrate buffer at 60 and 80°C at pH 7. For these solutions only the initial data prior to the "plateau" were used for the kinetic model. A graphical example of the data modeled by pseudo-first-order kinetics is shown in Figure 4.6. The pseudo-first-order rate constants with 95% confidence limits are presented in Table 4.4.

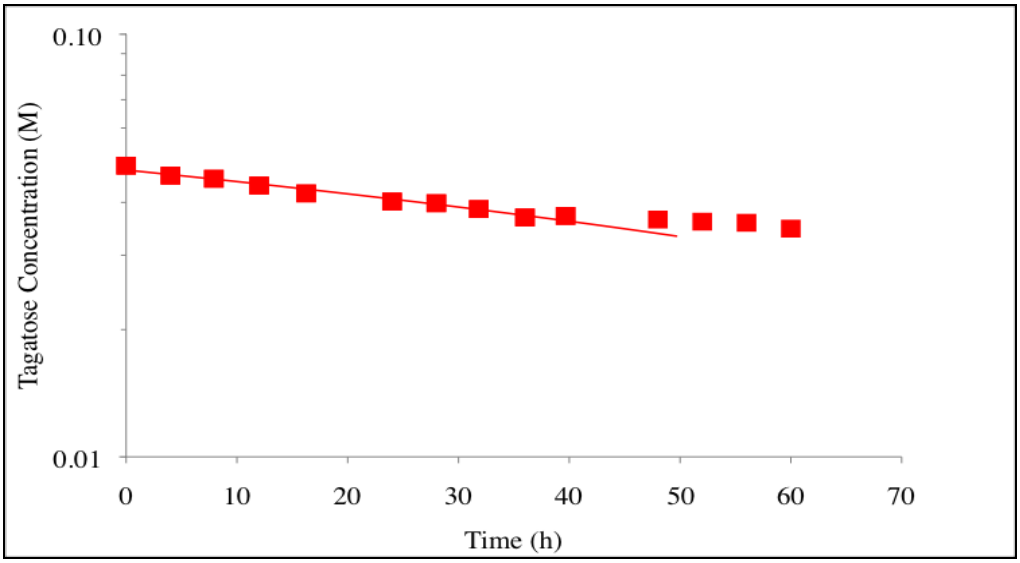

Figure 4.6. Pseudo-First-Order Plot of Tagatose Loss in 0.02 M Phosphate Buffer with 0.05 M Glycine at pH 7 and 70°C.

Effect of pH

The effect of pH on tagatose degradation in solutions containing 0.05 M glycine is shown in Table 4.4 and Figure 4.7. At pH 7, considerably faster tagatose loss occurred than at pH 3. In the 0.1 M phosphate buffer at pH 3 and 80°C, the rate constant for

tagatose degradation was 0.0031±0.0028 h^{-1}, but under the same conditions at pH 7, the rate constant increased to 0.0790±0.0085 h^{-1}. As discussed earlier, greater tagatose degradation at pH 7 could be attributed to tagatose more readily participating in the alkaline degradation reaction than the degradation reaction that can occur under acidic conditions. Also, because these solutions contain glycine, the Maillard reaction is likely taking place between tagatose and glycine. At pH 7, a greater amount of glycine will contain an amine group in the unprotonated form. The unprotonated form of the amine is more reactive in the Maillard reaction than the protonated form, which is in a greater concentration at lower pH values, thus explaining why greater tagatose degradation was seen in the solutions held at higher pH. Dobbs (2008) found similar results when looking at tagatose degradation at storage temperatures.

Effect of Buffer Type and Buffer Concentration

The buffer type and concentration also played a role in tagatose degradation when glycine was present. The effect is shown in Table 4.4 and Figure 4.8. Tagatose degradation was faster in phosphate buffer than citrate buffer, and the difference between the two buffer types was much more noticeable at pH 7 than pH 3. For example, the rate constant for tagatose loss in 0.02 M phosphate buffer at pH 7 and 80°C was 0.0260±0.0037 h^{-1}, whereas the rate constant for tagatose loss was 0.0183±0.0020 h^{-1} in the citrate buffer under the same conditions. Also, under similar conditions, the higher buffer concentration (0.1 M) resulted in greater tagatose loss than the lower buffer concentration (0.02 M); the rate constant for tagatose degradation in 0.02 M citrate buffer at pH 7 and 80°C was 0.0183±0.0020 h^{-1}, while the rate constant for tagatose degradation

in 0.1 M citrate buffer at pH 7 and 80°C was 0.0381±0.0050 h^{-1}. These results are consistent with the findings of Dobbs (2008) and Bell (1997).

Table 4.4. Pseudo-First-Order Rate Constants (h^{-1}) with 95% Confidence Limits for the Loss of Tagatose in Solution Containing 0.05 M Glycine.

Sample	**Temperature (°C)**		
	60	**70**	**80**
pH 3			
0.02 M phosphate	ND	ND	0*
0.02 M citrate	ND	ND	0.0032±0.0022 (R^2=0.54)
0.1 M phosphate	0.00014±0.00011 (R^2=0.37)	0.00062±0.00009 (R^2=0.94)	0.0031±0.0028 (R^2=0.41)
0.1 M citrate	0.00015±0.00014 (R^2=0.31)	0.00106±0.00026 (R^2=0.87)	0*
pH 7			
0.02 M phosphate	0.00177±0.00020 (R^2=0.97)	0.00689±0.00068 (R^2=0.98)	0.0260±0.0037 (R^2=0.97)
0.02 M citrate	0.00099±0.00014 (R^2=0.95)	0.00404±0.00051 (R^2=0.96)	0.0183±0.0020 (R^2=0.98)
0.1 M phosphate	0.00659±0.0013 (R^2=0.91)	0.0280±0.0066 (R^2=0.91)	0.0790±0.0085 (R^2=0.97)
0.1 M citrate	0.00359±0.00087 (R^2=0.88)	0.0144±0.0050 (R^2=0.83)	0.0381±0.0050 (R^2=0.97)

ND = not determined due to extremely low reactivity at 80°C

*Not different from zero over the 10 h experiment duration based on the 95% confidence limit exceeding the value of the rate constant to encompass zero.

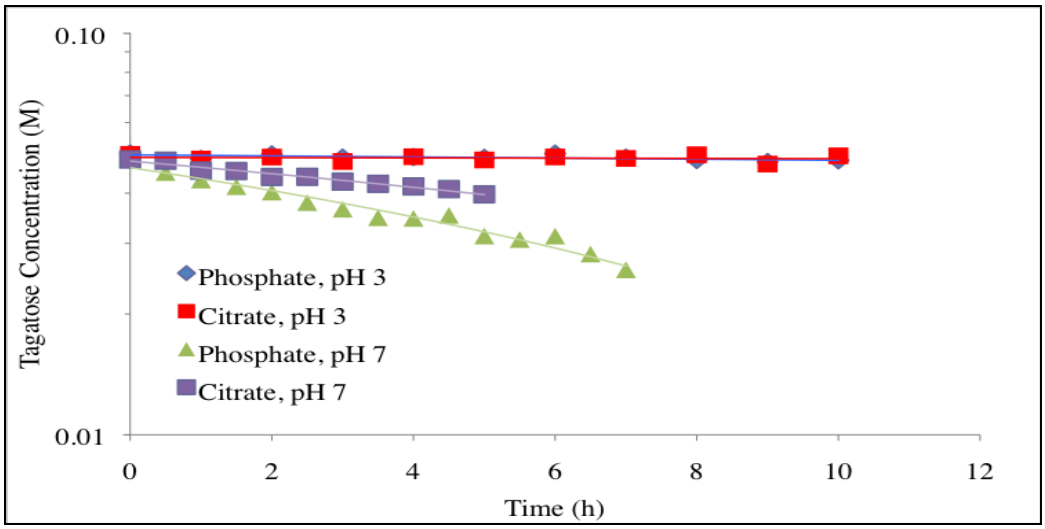

Figure 4.7. Tagatose Loss in 0.1 M Buffer Solutions Containing Tagatose and Glycine at 80°C as Affected by pH.

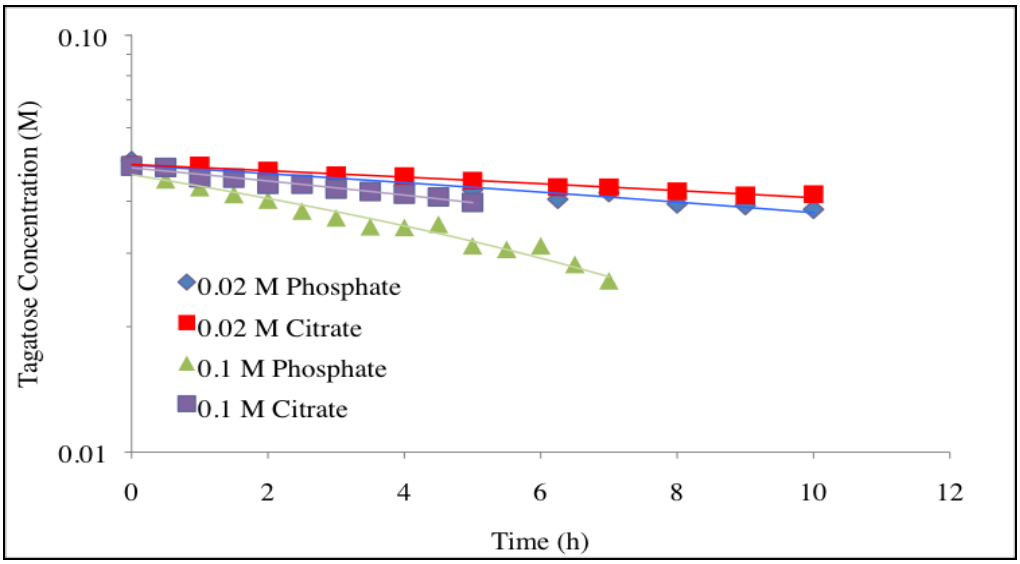

Figure 4.8. Tagatose Loss in Solution Containing Tagatose and Glycine at pH 7 and 80°C as Affected by Buffer Type and Concentration.

As mentioned earlier, phosphate dibasic anions are better than citrate anions at the proton transfers that are required for certain reactions to take place. In the Maillard reaction, a glycosylamine is formed after a number of proton transfers. Since the phosphate anions are more efficient at these proton transfers, tagatose degradation occurs at a faster rate, resulting in an overall greater tagatose loss in phosphate buffer solutions as compared to in citrate buffer solutions. Also, the increased buffer concentration means there are more buffer anions available to transfer protons; therefore, more tagatose degradation is seen in the solutions containing a higher buffer concentration.

Effect of Temperature

As shown in Table 4.4 and Figure 4.9, the increase in temperature from 60 to 80°C resulted in greater tagatose degradation in the presence of glycine. The rate constant for tagatose loss in 0.1 M citrate buffer at pH 7 was 0.00359±0.00087 h^{-1} at 60°C, while the rate constant for tagatose loss in this same solution at 80°C was 0.0381±0.0050 h^{-1}. Due to greater molecular movement at higher temperatures, reactions are expected to occur at a greater rate as the temperature increases, as seen in this study. These results are consistent with the study conducted by Dobbs (2008).

Activation energies were also calculated for the tagatose solutions that contained glycine (Table 4.5). At pH 3, activation energies were only calculated for tagatose loss in 0.1 M phosphate buffer because the other solutions only had reliable rate constants at 2 temperatures (Table 4.4). At pH 7, the activation energies for tagatose degradation in the presence of glycine range from 27.6 to 33.0 kcal/mol, with lower activation energies occurring in the solutions containing the higher buffer concentration. At pH 3, the activation energy value was 36.0 kcal/mol in 0.1 M phosphate buffer. The slightly higher

activation energy for degradation at pH 3 may indicate these pathways require more energy than those at pH 7.

For the samples containing 0.05 M glycine, the activation energies calculated in this experiment are considerably higher than those reported under storage conditions by Dobbs (2008). The activation energies for tagatose degradation in 0.02 and 0.1 M phosphate and citrate buffer solutions containing tagatose and 0.05 M glycine held at 20-40°C ranged from 16.2 to 17.1 kcal/mol (Dobbs 2008). As for the results for tagatose alone, these results suggest that the effect of temperature on various reaction pathways leading to tagatose degradation could be dependent on the temperature ranges.

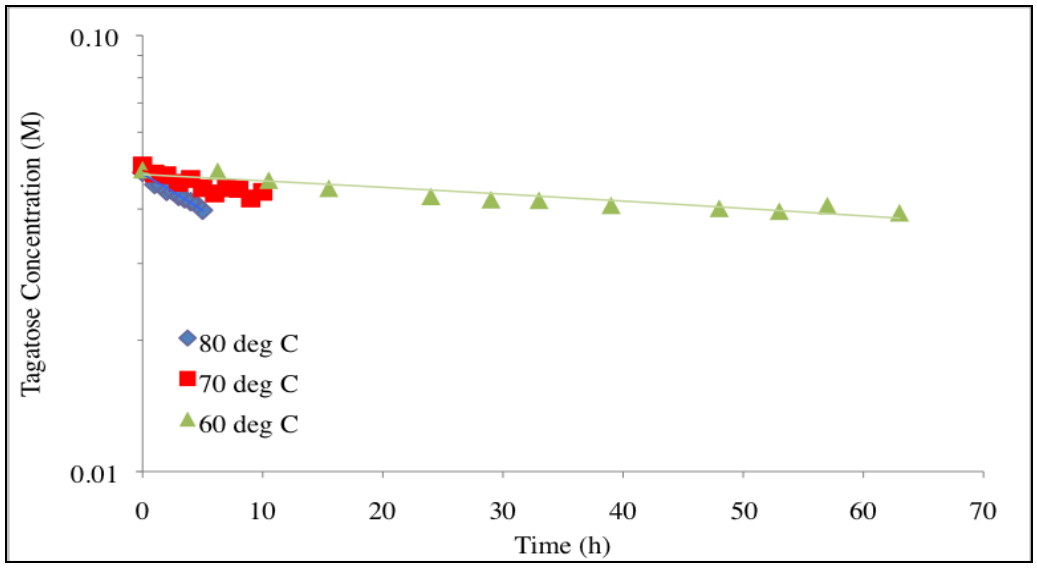

Figure 4.9. Tagatose Loss in 0.1 M Citrate Buffer Solutions Containing Tagatose and Glycine at pH 7 as Affected by Temperature.

Table 4.5. Activation Energies (E_A) for Tagatose Degradation in Solutions Containing 0.05 M Glycine.

Sample	E_A (kcal/mol)
0.02 M phosphate, pH 7	30.4
0.02 M citrate, pH 7	33.0
0.1 M phosphate, pH 7	29.0
0.1 M citrate, pH 7	27.6
0.1 M phosphate, pH 3	36.0

Using the activation energies, tagatose degradation under vat and HTST pasteurization conditions were able to be predicted. Since tagatose degradation in the presence of glycine occurred the fastest in 0.1 M phosphate buffer at pH 7, the activation energy of 29.0 kcal/mol was used in the calculations. Under vat pasteurization conditions, less than 0.5% tagatose degradation is predicted, and under HTST conditions the amount of tagatose degradation that would be expected is approximately 0.01%.

Brown Pigment Formation

The majority of solutions containing tagatose alone or tagatose with glycine browned to varying extents depending upon the solution composition and pH. The absorbance at 420 nm was measured for each sample, and these data were used to calculate pseudo-zero-order rate constants and 95% confidence limits for each experiment. The majority of experiments contained a lag phase (Figure 4.10) and based on visual determination as well as comparison of the R^2 values, the lag phase for each experiment was determined individually and removed from the rate constant calculations. Browning as affected by buffer type and pH is shown graphically in Figures 4.11 and

4.12. Tables 4.6 and 4.7 present the rate constants and 95% confidence limits for brown pigment formation ignoring the initial lag phase. From these tables and figures, the effect of temperature, pH, buffer type, and buffer concentration on the formation of brown pigment can be seen.

For both sets of experiments, those solutions containing only tagatose and those containing tagatose and glycine, it is apparent that the amount of brown pigment formed increased as the temperature increased. Also, the browning was faster at pH 7 than pH 3. Tagatose in phosphate buffer resulted in more browning than in citrate buffer solutions, and more browning was seen at the 0.1 M buffer concentration than the 0.02 M buffer concentration. Browning was also generally faster in the presence of glycine.

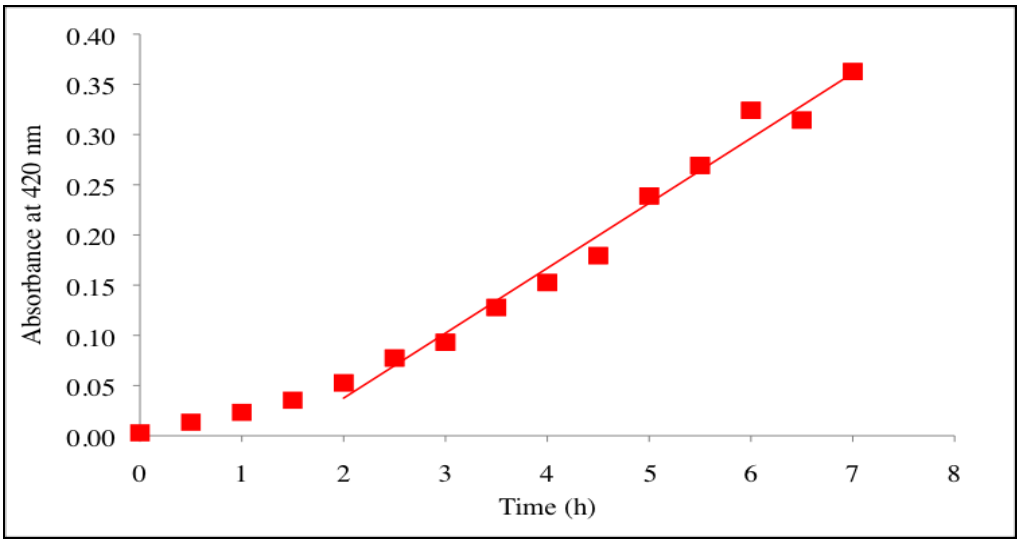

Figure 4.10. Browning in 0.1 M Phosphate Buffer Containing Tagatose and Glycine at pH 7 and 80°C.

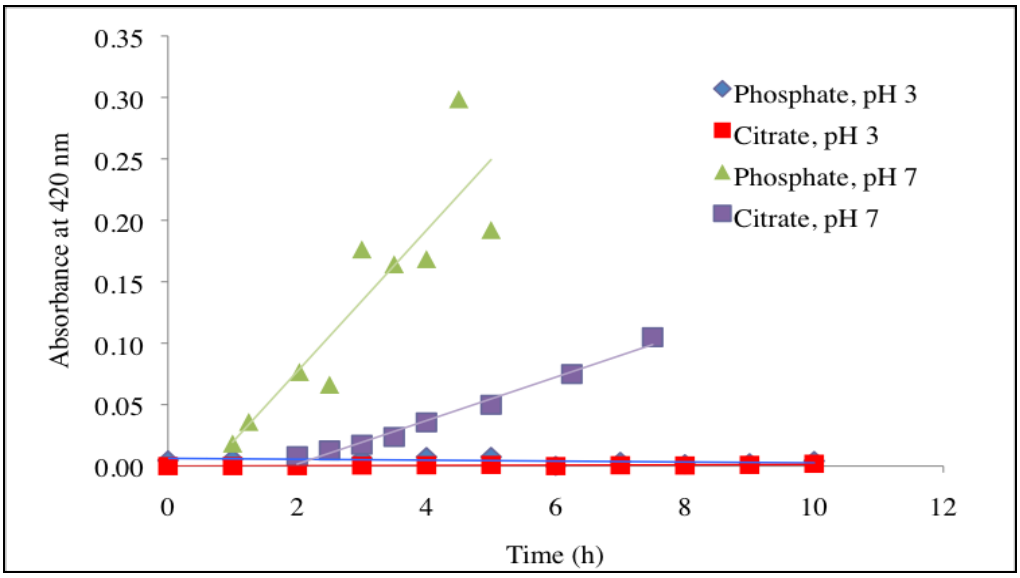

Figure 4.11. Brown Pigment Formation in 0.1 M Buffer Solutions Containing Tagatose at 80°C.

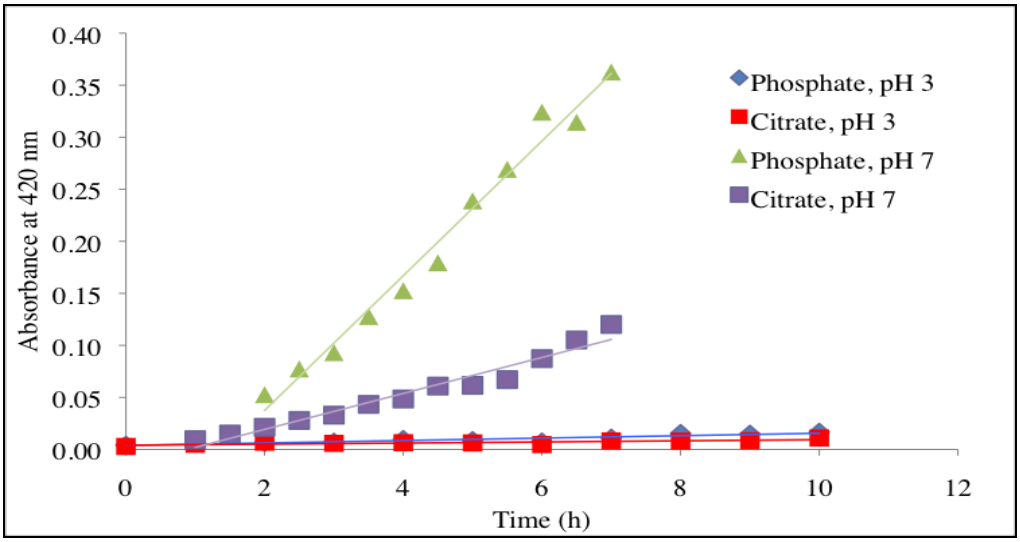

Figure 4.12. Brown Pigment Formation in 0.1 M Buffer Solutions Containing Tagatose and Glycine at 80°C.

Table 4.6. Pseudo-Zero-Order Rate Constants (OD/h) with 95% Confidence Limits for the Formation of Brown Pigment in Tagatose Solutions.

Sample	Temperature (°C)		
	60	**70**	**80**
pH 3			
0.02 M phosphate	ND	ND	0*
0.02 M citrate	ND	ND	0*
0.1 M phosphate	0.000053±0.000010 (R^2=0.92)	0.00030±0.00006 (R^2=0.92)	0*
0.1 M citrate	0.000027±0.000008 (R^2=0.81)	0.00029±0.00008 (R^2=0.89)	0.00014±0.00010 (R^2=0.53)
pH 7			
0.02 M phosphate	0.00051±0.00011 (R^2=0.92)	0.00192±0.00041 (R^2=0.91)	0.00774±0.0016 (R^2=0.94)
0.02 M citrate	0.000053±0.000035 (R^2=0.48)	0.00084±0.00037 (R^2=0.77)	0.00374±0.00073 (R^2=0.96)
0.1 M phosphate	0.00137±0.00038 (R^2=0.88)	0.00215±0.00031 (R^2=0.97)	0.055±0.018 (R^2=0.80)
0.1 M citrate	0.00028±0.00018 (R^2=0.53)	0.00099±0.00028 (R^2=0.87)	0.0193±0.0022 (R^2=0.99)

ND = not determined due to extremely low reactivity at 80°C

*Not different from zero over the 10 h experiment duration based on the 95% confidence limit exceeding the value of the rate constant to encompass zero.

Table 4.7. Pseudo-Zero-Order Rate Constants (OD/h) with 95% Confidence Limits for the Formation of Brown Pigment in Solutions Containing Tagatose and Glycine.

Sample	Temperature (°C)		
	60	**70**	**80**
pH 3			
0.02 M phosphate	ND	ND	0*
0.02 M citrate	ND	ND	0.00042±0.00019 (R^2=0.74)
0.1 M phosphate	0.00019±0.00003 (R^2=0.96)	0.00120±0.00011 (R^2=0.98)	0.00118±0.00036 (R^2=0.86)
0.1 M citrate	0.00012±0.00002 (R^2=0.93)	0.00098±0.00026 (R^2=0.90)	0.00054±0.00028 (R^2=0.69)
pH 7			
0.02 M phosphate	0.00200±0.00037 (R^2=0.94)	0.0064±0.0011 (R^2=0.94)	0.02402±0.0055 (R^2=0.94)
0.02 M citrate	0.00086±0.00041 (R^2=0.74)	0.00378±0.00032 (R^2=0.99)	0.01182±0.0046 (R^2=0.84)
0.1 M phosphate	0.0078±0.0014 (R^2=0.94)	0.0138±0.0022 (R^2=0.97)	0.0647±0.0065 (R^2=0.98)
0.1 M citrate	0.00141±0.00023 (R^2=0.95)	0.00304±0.00051 (R^2=0.97)	0.0187±0.0033 (R^2=0.95)

ND = not determined due to extremely low reactivity at 80°C
*Not different from zero over the 10 h experiment duration based on the 95% confidence limit exceeding the value of the rate constant to encompass zero.

In the study by Dobbs (2008), it was found that at the storage temperature of 40°C, the 0.1 M phosphate buffer at pH 7 that contains only tagatose showed a steep increase in browning followed by a decrease and then a leveling off. When glycine was added to this solution, the browning increased dramatically before somewhat leveling off. This browning pattern was not seen under the thermal processing conditions tested in this experiment. Instead, the formation of brown pigment increased steadily over the course of the experiment without any leveling off occurring (Figures 4.11 and 4.12). Other than

the differences at 0.1 M phosphate buffer at pH 7 and 80°C, the results from this study are consistent with the findings from the study conducted by Dobbs (2008).

Ryu and others (2003) conducted a study looking at the effect of temperature and pH on the non-enzymatic browning reaction that occurs between tagatose and glycine. They found that more brown pigment formed as the temperature increased from 70°C to 100°C, and browning increased as the pH increased from 3 to 7. These results are in agreement with the findings of the current study.

When looking at the effect of buffer type and concentration on the Maillard reaction, Bell (1997) found that solutions containing glucose and glycine at pH 7 and held at 25°C participated more readily in the Maillard reaction in phosphate buffer than citrate buffer. While higher phosphate buffer concentrations resulted in faster brown pigment formation, no browning was seen in the solutions containing the citrate buffer. After further investigation, Bell (1997) determined that citrate did not act as an inhibitor to the Maillard reaction, but rather citrate only has minimal effects on the non-enzymatic browning reaction. The higher temperatures in the current study are likely responsible for the presence of brown pigment formation in not only the phosphate buffers but also the citrate buffers. However, the study by Bell (1997) reiterates the point that phosphate anions are more efficient at proton transfers than citrate anions, as explained earlier.

Activation energies for the brown pigment formation were also calculated (Table 4.8). At pH 3, activation energies were not calculated due to extremely small and inconsistent rate constants (Tables 4.6 and 4.7). At pH 7, those solutions containing only tagatose had activation energies ranging from 33.6 to 52.1 kcal/mol, while those solutions containing tagatose and glycine had activation energies ranging from 24.7 to 30.1

kcal/mol. For both the solutions containing only tagatose, as well as those containing tagatose and glycine, the citrate buffer solutions had higher activation energies than the phosphate buffer solutions at pH 7. Also, the activation energies for brown pigment formation were higher in the solutions containing only tagatose as compared to the solutions containing tagatose and glycine. The contribution of the Maillard reaction pathways to browning is apparently less sensitive to temperature than the browning association with the alkaline degradation reaction of monosaccharides.

Table 4.8. Activation Energies (kcal/mol) for Brown Pigment Formation in Tagatose and Tagatose-Glycine Solutions at pH 7.

Sample	Tagatose	Tagatose-Glycine
0.02 M phosphate	33.6	28.1
0.02 M citrate	51.8	29.7
0.1 M phosphate	45.5	24.7
0.1 M citrate	52.1	30.1

Effect of Glycine on Tagatose Degradation and Brown Pigment Formation

The addition of an amino acid such as glycine to a solution containing a reducing sugar, like tagatose, would be expected to increase tagatose degradation and brown pigment formation due to the participation of glycine and tagatose in the Maillard reaction. Looking at the results, the addition of glycine to tagatose solutions did enhance the browning (Figure 4.13), but there was not much of an effect on tagatose degradation between the solutions with and without glycine (Tables 4.1 and 4.4).

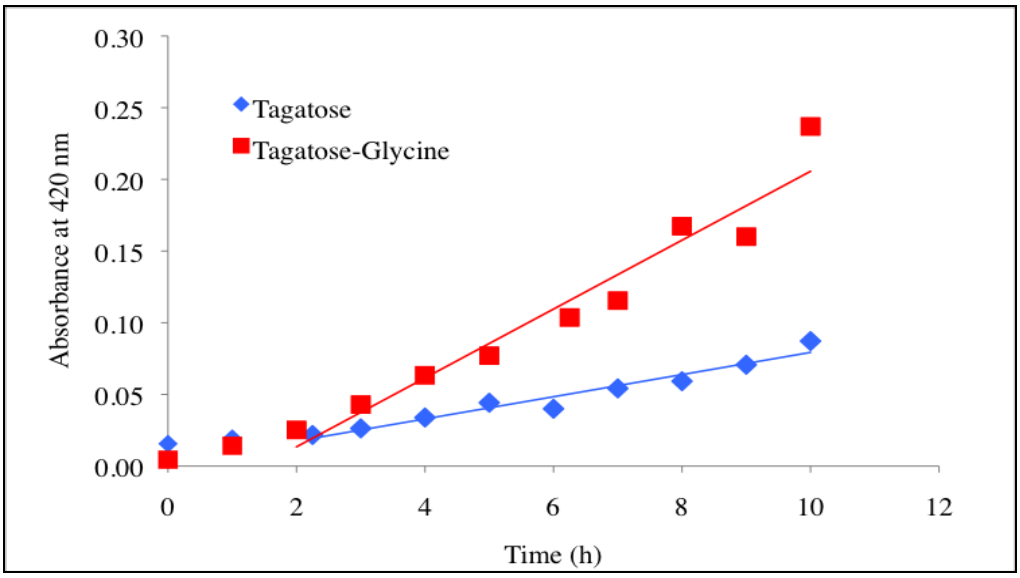

Figure 4.13. Brown Pigment Formation in 0.02 M Phosphate Buffer at pH 7 and 80°C.

When comparing the browning seen in solutions containing only tagatose to the solutions which contained tagatose and glycine, it is noticeable from the rate constants that more browning occurred in the solutions containing glycine. In the 0.02 M citrate buffer at pH 7 and 80°C, the pseudo-zero-order rate constant with the 95% confidence limit for brown pigment formation in the solution only containing tagatose was 0.00374±0.00073 OD/h, but the addition of glycine increased the rate constant to 0.0118±0.0046 OD/h. This result is expected since tagatose can interact with glycine to form brown pigment through the Maillard reaction.

With an increase in browning in the solutions containing glycine, a correlating increase in tagatose degradation would be expected. However, the rate constants for tagatose degradation are very similar between the samples with and without glycine. This same observation was noted when tagatose degradation was studied under storage

conditions by Dobbs (2008). While the reason as to why the addition of glycine would cause an increase in browning but not an increase in tagatose degradation is unclear, Dobbs (2008) suggests that glycine may disturb the equilibrium positioning of reversible steps involved in the alkaline degradation reaction and Maillard reaction: formation of the enediol in the alkaline degradation reaction and the formation of the glycosylamine in the Maillard reaction. The addition of glycine could change the equilibrium and direct some of the tagatose towards the Maillard reaction. The combination of re-established equilibrium occurring in competing reaction pathways may result in similar or slower tagatose loss when glycine is present (Dobbs 2008).

This study shows that the addition of glycine to tagatose solutions containing either a phosphate or citrate buffer causes an increase in brown pigment formation, but the tagatose degradation remains relatively unchanged from solutions without glycine. Further investigation is needed in order to determine the exact mechanism of the reactions causing this phenomenon.

CHAPTER 5: SUMMARY AND CONCLUSIONS

From this study, it was determined that the thermal stability of tagatose, with and without the presence of glycine, was influenced by temperature, pH, buffer concentration, and buffer type. Tagatose degradation was found to increase as the temperature increased, with the greatest tagatose loss seen at 80°C. Also, at pH 7 more tagatose degradation was seen than at pH 3. Buffer type and concentration also affected tagatose degradation, with higher levels of tagatose degradation occurring in phosphate buffer than citrate buffer, and an increase in tagatose loss seen at higher buffer concentrations. Overall, the most reactive solution observed was 0.1 M phosphate buffer at pH 7 and 80°C.

Brown pigment formation was found to be higher in the solutions containing glycine than those only containing tagatose. As seen with the tagatose degradation, the brown pigment formation increased as the temperature and pH increased. Also, more browning occurred in the phosphate buffer than the citrate buffer, and the higher buffer concentration resulted in an increase in brown pigment formation. Browning occurred the fastest in the 0.1 M phosphate buffer at pH 7 and 80°C.

Based on the results from this study, it was determined that although tagatose does breakdown at elevated temperatures, the amount of tagatose lost during the times and temperatures associated with typical thermal processing conditions would be virtually negligible. Processing under vat and HTST pasteurization conditions would

result in less than 0.5% and 0.02% tagatose degradation, respectively. Due to this minimal tagatose degradation from typical thermal processes, the majority of tagatose would remain present in a beverage after pasteurization, allowing its presence to provide the consumer with prebiotic benefits.

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APPENDIX A

TAGATOSE LOSS IN BUFFER SOLUTIONS WITHOUT GLYCINE

Table A1. Tagatose Degradation in 0.02 M Phosphate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
ND*	ND*	ND*	ND*	0.0	0.05047
				1.0	0.04897
				2.0	0.04950
				3.0	0.05066
				4.0	0.05046
				5.0	0.05153
				6.0	0.04906
				7.0	0.04890
				8.0	0.05003
				9.0	0.04960
				10.0	0.04898

*ND = Not determined due to low reactivity at 80°C.

Table A2. Tagatose Degradation in 0.02 M Phosphate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04766	0.0	0.04890	0.0	0.04840
12.8	0.04671	4.0	0.04615	1.0	0.04650
24.0	0.04592	8.0	0.04576	2.0	0.04510
36.0	0.04395	12.0	0.04404	3.0	0.04420
47.8	0.04327	16.0	0.04232	4.0	0.04260
60.0	0.04198	28.0	0.04026	5.0	0.04160
72.0	0.04139	32.0	0.03876	6.0	0.04070
84.0	0.04094	36.0	0.03890	7.0	0.03980
96.0	0.04065	40.0	0.03829	8.0	0.03910
120.0	0.03901	48.0	0.03501	9.0	0.03840
144.0	0.03830	52.0	0.03587	10.0	0.03740
168.0	0.03727	56.0	0.03543		
192.0	0.03671				
215.8	0.03702				

Table A3. Tagatose Degradation in 0.1 M Phosphate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04984	0.0	0.05118	0.0	0.05045
12.8	0.04872	12.0	0.04969	1.0	0.05046
24.0	0.05015	24.0	0.04893	2.0	0.05015
36.0	0.04784	35.5	0.04796	3.0	0.04995
47.8	0.04960	47.8	0.04780	4.0	0.05079
60.0	0.04920	59.3	0.04903	5.0	0.04880
72.0	0.04943	71.8	0.04707	6.0	0.04872
84.0	0.04828	84.0	0.04730	7.0	0.04922
96.0	0.04884	96.0	0.04792	8.0	0.05051
120.0	0.04892	107.8	0.04744	9.0	0.05039
144.0	0.04737	120.0	0.04592	10.0	0.04937
168.0	0.04769	132.0	0.04547		
192.0	0.04754	144.0	0.04654		
215.8	0.04768	156.0	0.04519		
		168.0	0.04574		

Table A4. Tagatose Degradation in 0.1 M Phosphate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04920	0.0	0.04964	0.0	0.04970
5.0	0.04649	1.0	0.04720	0.2	0.04870
9.8	0.04504	2.0	0.04681	0.3	0.04850
15.0	0.04309	3.0	0.04498	0.5	0.04730
24.0	0.04075	4.0	0.04313	0.7	0.04590
29.0	0.03965	5.0	0.04234	0.8	0.04670
34.0	0.03780	6.0	0.04060	1.0	0.04610
38.8	0.03618	7.0	0.03973	1.3	0.04490
48.0	0.03505	8.0	0.03810	2.0	0.04200
53.0	0.03356	9.0	0.03792	2.5	0.04060
58.0	0.03264	10.0	0.03674	3.0	0.03760
62.5	0.03139			3.5	0.03830
72.0	0.02945			4.0	0.03690
77.0	0.03051			4.5	0.03390
81.5	0.02754			5.0	0.03500

Table A5. Tagatose Degradation in 0.02 M Citrate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
ND*	ND*	ND*	ND*	0.0	0.04905
				1.0	0.04890
				2.0	0.04963
				3.0	0.04961
				4.0	0.04978
				5.0	0.04849
				6.0	0.04881
				7.0	0.04915
				8.0	0.04919
				9.0	0.04838
				10.0	0.04941

*ND = Not determined due to low reactivity at 80°C.

Table A6. Tagatose Degradation in 0.02 M Citrate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04860	0.0	0.04964	0.0	0.04940
12.8	0.04721	4.0	0.04844	1.0	0.04795
24.0	0.04695	8.0	0.04707	2.3	0.04610
36.0	0.04645	12.0	0.04662	3.0	0.04580
47.8	0.04646	16.0	0.04541	4.0	0.04480
60.0	0.04569	24.0	0.04421	5.0	0.04310
72.0	0.04574	28.0	0.04451	6.0	0.04320
84.0	0.04459	32.0	0.04245	7.0	0.04299
96.0	0.04321	36.0	0.04277	8.0	0.04260
120.0	0.04218	40.0	0.04221	9.0	0.04060
144.0	0.04233	48.0	0.04144	10.0	0.04020
168.0	0.04155	52.0	0.04059		
192.0	0.04201	56.0	0.04092		
215.8	0.04034				

Table A7. Tagatose Degradation in 0.1 M Citrate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.05011	0.0	0.05158	0.0	0.04960
12.8	0.04917	12.0	0.04956	1.0	0.04970
24.0	0.04965	24.0	0.04907	2.0	0.04850
36.0	0.04955	35.5	0.04810	3.0	0.04910
47.8	0.04848	47.8	0.04905	4.0	0.04930
60.0	0.04891	59.3	0.04738	5.0	0.04920
72.0	0.04864	71.8	0.04774	6.0	0.05000
84.0	0.04842	84.0	0.04725	7.0	0.05060
96.0	0.04873	96.0	0.04716	8.0	0.04950
120.0	0.04807	107.8	0.04636	9.0	0.04940
144.0	0.04891	120.0	0.04691	10.0	0.04980
168.0	0.04878	132.0	0.04595		
192.0	0.04876	144.0	0.04644		
215.8	0.04861	156.0	0.04532		
		168.0	0.04512		

Table A8. Tagatose Degradation in 0.1 M Citrate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.05131	0.0	0.05047	0.0	0.04960
5.0	0.04989	1.0	0.04934	0.5	0.04790
9.8	0.04851	2.0	0.04928	1.0	0.04750
15.0	0.04793	3.0	0.04804	1.5	0.04630
24.0	0.04579	4.0	0.04795	2.0	0.04630
29.0	0.04600	5.0	0.04799	2.5	0.04490
34.0	0.04438	6.0	0.04838	3.0	0.04410
38.8	0.04578	7.0	0.04518	3.5	0.04340
48.0	0.04566	8.0	0.04698	4.0	0.04240
53.0	0.04207	9.0	0.04512	5.0	0.04240
58.0	0.04400	10.0	0.04451	6.3	0.04020
62.5	0.04341			7.5	0.03660
72.0	0.04518				
77.0	0.03952				
81.5	0.04093				

APPENDIX B

TAGATOSE LOSS IN BUFFER SOLUTIONS WITH GLYCINE

Table B1. Tagatose Degradation in 0.02 M Phosphate Buffer (with 0.05 M Glycine) at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
ND*	ND*	ND*	ND*	0.0	0.04954
				1.0	0.05022
				2.0	0.04948
				3.0	0.05033
				4.0	0.04848
				5.0	0.05008
				6.0	0.04948
				7.0	0.04861
				8.0	0.04902
				9.0	0.04680
				10.0	0.04933

*ND = Not determined due to low reactivity at 80°C.

Table B2. Tagatose Degradation in 0.02 M Phosphate Buffer (with 0.05 M Glycine) at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04851	0.0	0.04887	0.0	0.05003
12.0	0.04625	4.0	0.04629	1.0	0.04747
24.0	0.04518	7.9	0.04551	2.0	0.04635
35.8	0.04411	12.0	0.04386	3.0	0.04553
48.0	0.04371	16.3	0.04202	4.0	0.04368
60.0	0.04219	24.0	0.04020	5.0	0.04258
72.0	0.04164	28.0	0.03983	6.3	0.04042
83.7	0.04099	31.8	0.03858	7.0	0.04193
95.9	0.03972	36.0	0.03685	8.0	0.03940
120.0	0.03897	39.7	0.03716	9.0	0.03904
144.0	0.03864	48.0	0.03644	10.0	0.03823
168.0	0.03756	52.0	0.03602		
192.3	0.03612	56.0	0.03580		
216.0	0.03654	60.0	0.03468		

Table B3. Tagatose Degradation in 0.1 M Phosphate Buffer (with 0.05 M Glycine) at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04974	0.0	0.04665	0.0	0.05023
12.0	0.04955	12.6	0.04687	1.0	0.04866
24.0	0.04930	23.9	0.04647	2.0	0.04996
35.8	0.04936	36.0	0.04570	3.0	0.04904
48.0	0.04916	48.0	0.04560	4.0	0.04922
60.0	0.05027	60.0	0.04543	5.0	0.04898
72.0	0.04966	72.0	0.04481	6.0	0.05020
83.7	0.04944	84.0	0.04430	7.0	0.04903
95.9	0.04944	96.0	0.04375	8.0	0.04838
120.0	0.04827	108.2	0.04404	9.0	0.04784
144.0	0.05010	120.0	0.04356	10.0	0.04830
168.0	0.04938	132.0	0.04405		
192.3	0.04831	144.0	0.04252		
216.0	0.04740	156.2	0.04261		

Table B4. Tagatose Degradation in 0.1 M Phosphate Buffer (with 0.05 M Glycine) at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04908	0.0	0.04728	0.0	0.04886
6.3	0.04429	1.0	0.04594	0.5	0.04501
10.5	0.04647	2.0	0.04340	1.0	0.04312
15.5	0.04517	3.0	0.04420	1.5	0.04151
24.0	0.03815	4.0	0.04296	2.0	0.04025
29.0	0.03526	5.0	0.04053	2.5	0.03788
33.0	0.03539	6.0	0.03836	3.0	0.03649
39.0	0.03397	7.0	0.03758	3.5	0.03474
48.0	0.03531	8.0	0.03567	4.0	0.03461
53.0	0.03330	9.0	0.03751	4.5	0.03521
57.0	0.02916	10.0	0.03689	5.0	0.03128
63.0	0.03182			5.5	0.03066
72.0	0.02934			6.0	0.03127
77.0	0.02963			6.5	0.02821
82.0	0.02798			7.0	0.02577

Table B5. Tagatose Degradation in 0.02 M Citrate Buffer (with 0.05 M Glycine) at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
ND*	ND*	ND*	ND*	0.0	0.05005
				1.0	0.04997
				2.0	0.04978
				3.0	0.04979
				4.0	0.04950
				5.0	0.04896
				6.0	0.04944
				7.0	0.04848
				8.0	0.04800
				9.0	0.04972
				10.0	0.04842

*ND = Not determined due to low reactivity at 80°C.

Table B6. Tagatose Degradation in 0.02 M Citrate Buffer (with 0.05 M Glycine) at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04954	0.0	0.04895	0.0	0.04888
12.0	0.04764	4.0	0.04817	1.0	0.04872
24.0	0.04670	7.9	0.04619	2.0	0.04737
35.8	0.04670	12.0	0.04609	3.0	0.04615
48.0	0.04568	16.3	0.04485	4.0	0.04593
60.0	0.04490	24.0	0.04341	5.0	0.04479
72.0	0.04427	28.0	0.04183	6.3	0.04324
83.7	0.04371	31.8	0.04172	7.0	0.04317
95.9	0.04380	36.0	0.04087	8.0	0.04224
120.0	0.04294	39.7	0.04077	9.0	0.04130
144.0	0.04288	48.0	0.03887	10.0	0.04157
168.0	0.04084	52.0	0.03913		
192.3	0.03975	56.0	0.03881		
216.0	0.04150	60.0	0.03887		

Table B7. Tagatose Degradation in 0.1 M Citrate Buffer (with 0.05 M Glycine) at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.05161	0.0	0.04817	0.0	0.04998
12.0	0.04948	12.6	0.04909	1.0	0.04866
24.0	0.05033	23.9	0.04795	2.0	0.04921
35.8	0.04849	36.0	0.04769	3.0	0.04798
48.0	0.04958	48.0	0.04638	4.0	0.04930
60.0	0.04836	60.0	0.04783	5.0	0.04840
72.0	0.04995	72.0	0.04679	6.0	0.04924
83.7	0.04841	84.0	0.04665	7.0	0.04882
95.9	0.04923	96.0	0.04449	8.0	0.04986
120.0	0.04993	108.2	0.04309	9.0	0.04727
144.0	0.04895	120.0	0.04302	10.0	0.04956
168.0	0.04896	132.0	0.04431		
192.3	0.04836	144.0	0.04122		
216.0	0.04847	156.2	0.04185		

Table B8. Tagatose Degradation in 0.1 M Citrate Buffer (with 0.05 M Glycine) at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.04925	0.0	0.05035	0.0	0.04853
6.3	0.04887	1.0	0.04825	0.5	0.04819
10.5	0.04658	2.0	0.04782	1.0	0.04552
15.5	0.04464	3.0	0.04593	1.5	0.04542
24.0	0.04277	4.0	0.04687	2.0	0.04390
29.0	0.04203	5.0	0.04465	2.5	0.04393
33.0	0.04190	6.0	0.04341	3.0	0.04275
39.0	0.04080	7.0	0.04463	3.5	0.04220
48.0	0.04015	8.0	0.04447	4.0	0.04158
53.0	0.03955	9.0	0.04228	4.5	0.04095
57.0	0.04075	10.0	0.04379	5.0	0.03973
63.0	0.03923			5.5	0.04150
72.0	0.04230			6.0	0.04171
77.0	0.04032			6.5	0.04182
82.0	0.03896			7.0	0.04201

APPENDIX C

BROWNING OF TAGATOSE IN BUFFER SOLUTIONS WITHOUT GLYCINE

Table C1. Browning of 0.05 M Tagatose in 0.02 M Phosphate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm
ND*	ND*	ND*	ND*	0.0	0.0154
				1.0	0.0165
				2.0	0.0160
				3.0	0.0179
				4.0	0.0167
				5.0	0.0167
				6.0	ND**
				7.0	ND**
				8.0	0.0156
				9.0	0.0168
				10.0	0.0171

*ND = Not determined due to low reactivity at 80°C.
**ND = Not determined due to insufficient sample volume.

Table C2. Browning of 0.05 M Tagatose in 0.02 M Phosphate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm
0.0	0.0020	0.0	-0.0001	0.0	0.0156
12.8	0.0076	4.0	0.0068	1.0	0.0194
24.0	0.0115	8.0	0.0266	2.3	0.0217
36.0	0.0288	12.0	0.0184	3.0	0.0263
47.8	0.0390	16.0	0.0230	4.0	0.0339
60.0	0.0519	24.0	0.0268	5.0	0.0442
72.0	0.0630	28.0	0.0460	6.0	0.0400
84.0	0.0466	32.0	0.0607	7.0	0.0542
96.0	0.0754	36.0	0.0636	8.0	0.0592
120.0	0.0830	40.0	0.0857	9.0	0.0707
144.0	0.0848	48.0	0.1168	10.0	0.0872
168.0	0.0890	52.0	0.0858		
192.0	0.1212	56.0	0.0989		
215.8	0.1154				

Table C3. Browning of 0.05 M Tagatose in 0.1 M Phosphate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm
0.0	-0.0006	0.0	0.0025	0.0	0.0047
12.8	-0.0010	12.0	0.0050	1.0	0.0058
24.0	-0.0012	24.0	0.0060	2.0	0.0058
36.0	-0.0006	35.5	0.0077	3.0	0.0064
47.8	0.0008	47.8	0.0112	4.0	0.0074
60.0	0.0000	59.3	0.0139	5.0	0.0075
72.0	-0.0001	71.8	0.0164	6.0	0.0002
84.0	0.0018	84.0	0.0186	7.0	0.0035
96.0	0.0007	96.0	0.0205	8.0	0.0016
120.0	0.0050	107.8	0.0266	9.0	0.0024
144.0	0.0048	120.0	0.0390	10.0	0.0042
168.0	0.0064	132.0	0.0367		
192.0	0.0093	144.0	0.0350		
215.8	0.0093	156.0	0.0495		
		168.0	0.0423		

Table C4. Browning of 0.05 M Tagatose in 0.1 M Phosphate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm
0.0	0.0014	0.0	0.0001	0.0	0.0000
5.0	0.0027	1.0	0.0029	0.2	0.0058
9.8	0.0070	2.0	0.0040	0.3	0.0063
15.0	0.0110	3.0	0.0052	0.5	0.0154
24.0	0.0179	4.0	0.0065	0.7	0.0243
29.0	0.0226	5.0	0.0114	0.8	0.0246
34.0	0.0299	6.0	0.0098	1.0	0.0184
38.8	0.0474	7.0	0.0137	1.3	0.0360
48.0	0.0438	8.0	0.0161	2.0	0.0766
53.0	0.0560	9.0	0.0193	2.5	0.0663
58.0	0.0585	10.0	0.0230	3.0	0.1764
62.5	0.0572			3.5	0.1643
72.0	0.0683			4.0	0.1685
77.0	0.1115			4.5	0.2986
81.5	0.0953			5.0	0.1922

Table C5. Browning of 0.05 M Tagatose in 0.02 M Citrate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm
ND*	ND*	ND*	ND*	0.0	0.0151
				1.0	0.0167
				2.0	0.0167
				3.0	0.0166
				4.0	0.0165
				5.0	0.0168
				6.0	0.0149
				7.0	0.0163
				8.0	0.0165
				9.0	0.0172
				10.0	0.0171

*ND = Not determined due to low reactivity at 80°C.

Table C6. Browning of 0.05 M Tagatose in 0.02 M Citrate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm
0.0	0.0027	0.0	0.0001	0.0	0.0160
12.8	0.0043	4.0	0.0017	1.0	0.0176
24.0	0.0045	8.0	0.0033	2.3	0.0195
36.0	0.0063	12.0	0.0039	3.0	0.0203
47.8	0.0066	16.0	0.0066	4.0	0.0230
60.0	0.0081	24.0	0.0121	5.0	0.0258
72.0	0.0081	28.0	0.0149	6.0	0.0279
84.0	0.0071	32.0	0.0264	7.0	0.0318
96.0	0.0081	36.0	0.0271	8.0	0.0352
120.0	0.0185	40.0	0.0105	9.0	0.0412
144.0	0.0139	48.0	0.0267	10.0	0.0476
168.0	0.0116	52.0	0.0419		
192.0	0.0049	56.0	0.0446		
215.8	0.0196				

Table C7. Browning of 0.05 M Tagatose in 0.1 M Citrate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm
0.0	-0.0002	0.0	0.0008	0.0	-0.0001
12.8	-0.0007	12.0	0.0006	1.0	0.0000
24.0	-0.0006	24.0	0.0012	2.0	-0.0001
36.0	0.0002	35.5	0.0020	3.0	0.0005
47.8	0.0017	47.8	0.0050	4.0	0.0007
60.0	0.0021	59.3	0.0049	5.0	0.0010
72.0	-0.0006	71.8	0.0080	6.0	-0.0002
84.0	0.0013	84.0	0.0072	7.0	0.0007
96.0	0.0002	96.0	0.0105	8.0	0.0005
120.0	0.0025	107.8	0.0155	9.0	0.0010
144.0	0.0037	120.0	0.0164	10.0	0.0019
168.0	0.0036	132.0	0.0277		
192.0	0.0042	144.0	0.0214		
215.8	0.0056	156.0	0.0363		
		168.0	0.0336		

Table C8. Browning of 0.05 M Tagatose in 0.1 M Citrate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm	Time (h)	Absorbance at 420 nm
0.0	0.0013	0.0	0.0003	0.0	-0.0009
5.0	0.0029	1.0	0.0024	0.5	-0.0006
9.8	0.0019	2.0	0.0022	1.0	0.0050
15.0	0.0021	3.0	0.0028	1.5	0.0040
24.0	0.0085	4.0	0.0040	2.0	0.0081
29.0	0.0038	5.0	0.0060	2.5	0.0128
34.0	0.0028	6.0	0.0038	3.0	0.0174
38.8	0.0114	7.0	0.0066	3.5	0.0237
48.0	0.0021	8.0	0.0069	4.0	0.0356
53.0	0.0119	9.0	0.0115	5.0	0.0499
58.0	0.0279	10.0	0.0106	6.3	0.0749
62.5	0.0112			7.5	0.1049
72.0	0.0149				
77.0	0.0187				
81.5	0.0209				

APPENDIX D

BROWNING OF TAGATOSE AND GLYCINE IN BUFFER SOLUTIONS

Table D1. Browning of 0.05 M Tagatose and 0.05 M Glycine in 0.02 M Phosphate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
ND*	ND*	ND*	ND*	0.0	0.0030
				1.0	0.0049
				2.0	0.0046
				3.0	0.0049
				4.0	0.0081
				5.0	0.0055
				6.0	0.0046
				7.0	0.0061
				8.0	0.0057
				9.0	0.0064
				10.0	0.0066

*ND = Not determined due to low reactivity at 80°C.

Table D2. Browning of 0.05 M Tagatose and 0.05 M Glycine in 0.02 M Phosphate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.0006	0.0	-0.0009	0.0	0.0045
12.0	0.0134	4.0	0.0115	1.0	0.0142
24.0	0.0301	7.9	0.0277	2.0	0.0252
35.8	0.0480	12.0	0.0514	3.0	0.0429
48.0	0.0621	16.3	0.0730	4.0	0.0634
60.0	0.0800	24.0	0.1024	5.0	0.0770
72.0	0.1518	28.0	0.1327	6.3	0.1037
83.7	0.1161	31.8	0.1587	7.0	0.1154
95.9	0.1518	36.0	0.1975	8.0	0.1673
120.0	0.2321	39.7	0.2288	9.0	0.1600
144.0	0.2218	48.0	0.3481	10.0	0.2369
168.0	0.2539	52.0	0.2604		
192.3	0.4280	56.0	0.3143		
216.0	0.3969	60.0	0.3466		

Table D3. Browning of 0.05 M Tagatose and 0.05 M Glycine in 0.1 M Phosphate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	-0.0008	0.0	-0.0007	0.0	0.0042
12.0	-0.0009	12.6	0.0022	1.0	0.0062
24.0	-0.0002	23.9	0.0052	2.0	0.0065
35.8	0.0020	36.0	0.0120	3.0	0.0071
48.0	0.0047	48.0	0.0199	4.0	0.0097
60.0	0.0038	60.0	0.0298	5.0	0.0087
72.0	0.0042	72.0	0.0385	6.0	0.0073
83.7	0.0072	84.0	0.0456	7.0	0.0110
95.9	0.0076	96.0	0.0683	8.0	0.0155
120.0	0.0137	108.2	0.0834	9.0	0.0148
144.0	0.0172	120.0	0.1026	10.0	0.0167
168.0	0.0278	132.0	0.1156		
192.3	0.0299	144.0	0.1304		
216.0	0.0352	156.2	0.1420		

Table D4. Browning of 0.05 M Tagatose and 0.05 M Glycine in 0.1 M Phosphate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.0012	0.0	-0.0006	0.0	0.0029
6.3	0.0184	1.0	0.0085	0.5	0.0135
10.5	0.0362	2.0	0.0151	1.0	0.0233
15.5	0.0522	3.0	0.0216	1.5	0.0354
24.0	0.1041	4.0	0.0350	2.0	0.0527
29.0	0.1234	5.0	0.0409	2.5	0.0774
33.0	0.1406	6.0	0.0502	3.0	0.0931
39.0	0.2085	7.0	0.0684	3.5	0.1278
48.0	0.3696	8.0	0.0810	4.0	0.1526
53.0	0.3161	9.0	0.1008	4.5	0.1794
57.0	0.3051	10.0	0.1206	5.0	0.2389
63.0	0.3760			5.5	0.2692
72.0	0.5581			6.0	0.3241
77.0	0.4819			6.5	0.3144
82.0	0.5445			7.0	0.3627

Table D5. Browning of 0.05 M Tagatose and 0.05 M Glycine in 0.02 M Citrate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
ND*	ND*	ND*	ND*	0.0	0.0026
				1.0	0.0044
				2.0	0.0044
				3.0	0.0047
				4.0	0.0055
				5.0	0.0052
				6.0	0.0068
				7.0	0.0054
				8.0	0.0055
				9.0	0.0086
				10.0	0.0072

*ND = Not determined due to low reactivity at 80°C.

Table D6. Browning of 0.05 M Tagatose and 0.05 M Glycine in 0.02 M Citrate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.0017	0.0	-0.0002	0.0	0.0047
12.0	0.0037	4.0	0.0025	1.0	0.0088
24.0	0.0075	7.9	0.0079	2.0	0.0110
35.8	0.0144	12.0	0.0159	3.0	0.0235
48.0	0.0223	16.3	0.0280	4.0	0.0260
60.0	0.0279	24.0	0.0523	5.0	0.0318
72.0	0.0196	28.0	0.0655	6.3	0.0513
83.7	0.0480	31.8	0.0770	7.0	0.0457
95.9	0.0641	36.0	0.0922	8.0	0.0589
120.0	0.0603	39.7	0.1173	9.0	0.1152
144.0	0.0792	48.0	0.1530	10.0	0.0998
168.0	0.1193	52.0	0.1543		
192.3	0.2065	56.0	0.1681		
216.0	0.1134	60.0	0.2014		

Table D7. Browning of 0.05 M Tagatose and 0.05 M Glycine in 0.1 M Citrate Buffer at pH 3 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	-0.0007	0.0	-0.0002	0.0	0.0029
12.0	0.0001	12.6	0.0026	1.0	0.0050
24.0	-0.0002	23.9	0.0026	2.0	0.0070
35.8	0.0007	36.0	0.0076	3.0	0.0059
48.0	0.0014	48.0	0.0163	4.0	0.0065
60.0	0.0035	60.0	0.0183	5.0	0.0063
72.0	0.0021	72.0	0.0260	6.0	0.0047
83.7	0.0026	84.0	0.0250	7.0	0.0082
95.9	0.0038	96.0	0.0776	8.0	0.0080
120.0	0.0068	108.2	0.0564	9.0	0.0084
144.0	0.0099	120.0	0.0713	10.0	0.0110
168.0	0.0123	132.0	0.0944		
192.3	0.0198	144.0	0.0946		
216.0	0.0193	156.2	0.1220		

Table D8. Browning of 0.05 M Tagatose and 0.05 M Glycine in 0.1 M Citrate Buffer at pH 7 as Affected by Temperature

60°C		70°C		80°C	
Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)	Time (h)	Tagatose Concentration (M)
0.0	0.0014	0.0	0.0002	0.0	0.0013
6.3	0.0035	1.0	0.0020	0.5	0.0050
10.5	0.0055	2.0	0.0023	1.0	0.0094
15.5	0.0097	3.0	0.0049	1.5	0.0148
24.0	0.0170	4.0	0.0058	2.0	0.0213
29.0	0.0217	5.0	0.0104	2.5	0.0279
33.0	0.0206	6.0	0.0128	3.0	0.0330
39.0	0.0333	7.0	0.0164	3.5	0.0433
48.0	0.0364	8.0	0.0165	4.0	0.0485
53.0	0.0502	9.0	0.0250	4.5	0.0609
57.0	0.0616	10.0	0.0260	5.0	0.0616
63.0	0.0771			5.5	0.0671
72.0	0.0857			6.0	0.0873
77.0	0.0907			6.5	0.1052
82.0	0.0854			7.0	0.1201

APPENDIX E

AVERAGE TEMPERATURES OF TAGATOSE AND

TAGATOSE-GLYCINE BUFFER SOLUTIONS

Table E1. Average Temperature of Tagatose Buffer Solutions

Sample	Temperature (°C)		
pH 3			
0.02 M phosphate	ND	ND	81.37
0.02 M citrate	ND	ND	81.35
0.1 M phosphate	62.26	71.05	81.30
0.1 M citrate	62.26	71.05	80.58
pH 7			
0.02 M phosphate	62.26	70.95	81.35
0.02 M citrate	62.26	70.95	81.50
0.1 M phosphate	61.06	71.03	79.39
0.1 M citrate	61.06	71.03	79.82

Table E2. Average Temperatures of Tagatose-Glycine Buffer Solutions

Sample	Temperature (°C)		
pH 3			
0.02 M phosphate	ND	ND	81.33
0.02 M citrate	ND	ND	81.33
0.1 M phosphate	61.32	71.21	81.67
0.1 M citrate	61.32	71.21	81.67
pH 7			
0.02 M phosphate	61.32	71.42	82.16
0.02 M citrate	61.32	71.42	82.16
0.1 M phosphate	61.51	71.35	81.72
0.1 M citrate	61.51	71.35	81.72