FOOD MATERIALS SCIENCE:
EFFECTS OF POLYPHENOLS ON SUCROSE CRYSTALLIZATION AND CHARACTERIZATION AND CREATION OF ALTERNATIVE SALTS OF THIAMINE

by

Collin James Felten

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Food Science

West Lafayette, Indiana

December 2018

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Lisa Mauer, Chair
School of Agriculture
Dr. Lynne Taylor
School of Agriculture
Dr. Kee-Hong Kim
School of Agriculture

Approved by:

Dr. Buck Doe
Head of the Graduate Program

I would like to dedicate this work to my friends and family, without whom I would not have made it through the past two years. Particularly, I would like to thank my parents, brother, Joss, Ciera, and Emmett. I'm proud to have you all in my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: Felten, Collin, J. MS
Institution: Purdue University
Degree Received: December 2018
Title: Food Materials Science: Effects of Polyphenols on Sucrose Crystallization and Characterization and Creation of Alternative Salts of Thiamine
Committee Chair: Lisa Mauer

Proper understanding of materials science is critical in understanding the functionality of ingredients in food products, as well as their behavior in these products over time. Amorphous materials are metastable, eventually rearranging to the thermodynamically stable crystalline state. Amorphous materials have properties which are beneficial in some food products: they are softer in texture and dissolve more rapidly. The amorphous state of sucrose might provide an increase in quality in applications like powdered beverages where rapid dissolution is preferred. A number of classes of compounds have been shown to delay the crystallization of amorphous sucrose; however, polyphenols, particularly their glycosylated forms, have been little explored. Glycosylated polyphenols contain two distinct structural regions: a more hydrophilic sugar unit(s) and a more hydrophobic polyphenol backbone. While the sugar unit should be able to easily associate with sucrose molecules, the polyphenolic backbone may not and might provide hindrance to crystal nucleation and growth.

Thiamine is an essential nutrient that is found naturally in foods such as whole grains and pork. The processing of grains removes nearly the entirety of the natural thiamine content; thus, foods are often enriched with synthetic thiamine. Two salts of thiamine are used commercially: thiamine mononitrate and thiamine chloride hydrochloride. The two forms have specific applications driven by their specific properties, specifically their aqueous solubility and hygroscopicity. While these two salts provide adequate functionality, it is possible new salts may have properties beneficial in certain food applications. A method making use of silver nitrate was developed to produce new salt forms. An intermediate in this reaction, $TCl{\cdot}H_2O$, was characterized including measurements of stability in aqueous solutions and solid state properties.

CHAPTER 1. INTRODUCTION

1.1 Introduction

Solid materials are a part of nearly all foods, as both the final product as well as components. Understanding the properties and behavior of solid materials in different environments is thus critical to understanding the functionality of ingredients and the behavior of food products during production and storage. This work explores two kinds of solid state systems; first, the transition between the amorphous to crystalline state of sucrose was investigated. Various glycosylated polyphenols were used to affect the stability of the amorphous state and conclusions were drawn which may allow for greater product quality and shelf life for some applications. Then, different salt forms of thiamine (vitamin B1) were explored. A method was developed to exchange the anion associated with thiamine which has allowed for the production of never before reported salts. These salts may have favorable properties for some applications due to differences in hygroscopicity, solubility, and/or stability. In addition, the solution state degradation kinetics of a known but poorly characterized salt, thiamine chloride monohydrate, were studied.

1.2 Water-Solids Interactions

Water is a ubiquitous material present to some degree in all foods and environments. A driving force originating from differences in chemical potential will cause the migration of water from high to low activity. This leads to interactions with atmospheric water even for dry solids, resulting in a non-zero water content. Thus, understanding the interactions between water and solid materials is critical to understand the fundamental science behind the behavior of foods. Water is the primary solvent in foods and facilitates reactions in food systems leading to

increased degradation and crystallization rate, clumping of powders, and other physical and chemical changes (15). Furthermore, many of the properties of water are atypical compared to other fluids common in foods. Solid water can exist as a number of polymorphs, its free volume increases upon crystallization, and properties such as density, heat capacity, and viscosity change in non-linear and unpredictable manners as a function of temperature and pressure (16).

Interactions between water and solids are classified into five mechanisms; surface adsorption, capillary condensation, deliquescence, hydrate formation, and bulk absorption. In the context of this work, all mechanisms excluding deliquescence are important in some manner. For the work on sucrose crystallization, bulk absorption into the amorphous sucrose matrix is the mechanism by which the majority of water will be taken up by the solid. As the water content increases, the glass transition temperature (T_g) will be lowered and the rate of crystallization will increase. Eventually, the amorphous solid will crystallize and most of this absorbed water will be expelled (34). If the RH is increased further, the crystalline sucrose may deliquesce, however this will only occur at or above an RH of 85.7% at 25°C (17). In the work dealing with thiamine, surface adsorption, capillary condensation, and hydrate formation are the mechanisms of interest. While the reactions to produce new salt forms were solution mediated, the properties of the crystal forms in regard to these three mechanisms was the primary motivation for preparing new salts. Crystalline solids will take on less water than amorphous solids at a given RH and cannot undergo bulk absorption unless uncommonly high degrees of defects are present (18). Surface adsorption and capillary condensation can still account for a large difference in the way different crystals will interact with water in an environment. In vapor sorption studies performed for this work, TClHCl sorbed more than an order of magnitude more water than TMN at the same RH

and exposure time. Understanding these differences can help avoid issues such as clumping and caking and can guide the choice of ingredients based on the specific application (19, 20, 23).

1.3 Crystallization

Crystallization is the process by which a solid is formed wherein atoms are arranged in a consistent pattern. This solid is known as a crystal. Crystals can be formed through a number of ways: solvent removal, antisolvent addition, raising or lowering temperature, or directly from a gas (21). In this work, crystals were formed through antisolvent addition, lowering of solution temperature, and through water mediated transformation from the amorphous state.

As a specific arrangement of atoms is needed to form a crystal, defects are relatively uncommon. Defects may be introduced through sudden energy input or removal, such as through quenching, or through the presence of impurities. Recrystallization is the process by which a crystalline material with a high degree of deformity is allowed to form a more ordered crystal structure. Often the material is dissolved and then crystallized out of solution. This can allow impurities to remain dissolved while a purer material is selectively precipitated. Recrystallization is a common method used to purify crystalline materials.

Compared to amorphous solids, crystalline solids are harder, more brittle, slower to dissolve, and slower to react. They also have a well-defined melting point (22). In some food applications this is desirable and in others this is undesirable. For ingredient storage the low reactivity and reduced water holding capacity of crystalline solids is desired. Due to their higher moisture sorption for a given set of environmental conditions, amorphous materials suffer from issues with caking or clumping which can make transportation difficult (23). For beverages, where rapid dissolution is desired, amorphous solids would be preferred if storage of these ingredients were feasible. If an amorphous ingredient crystallizes over the time required for

storage and transportation this would negatively impact quality. Thus, the control of the nature of the solid state is critical in maintaining quality of foods.

1.4 Polyphenols

Polyphenolic compounds are widely present in natural foods and are often bioactive. In plants, polyphenols are classified as secondary metabolites and generally provide ultraviolet protection and reduced pathogen aggression (4, 29). While the category contains a wide range of structures, generally the backbone consists of an aromatic ring with two or more hydroxyl groups. Polyphenolic compounds are ubiquitous in the human diet and are intimately linked to food quality and human health (3, 6, 24-27). Enzymatic browning of polyphenols by polyphenol oxidase is linked to undesirable changes in color, flavor, and nutrient quality.

Polyphenols are common micronutrients in the human diet and numerous studies have demonstrated their benefits in the prevention of degenerative diseases like cancer and cardiovascular disease. Polyphenols act as antioxidants in the body and have been shown to affect the activity of a number of enzymes and cell receptors (3). Three main categories exist with subclasses of each: phenols/phenolic acids, derivatives of hydroxycinnamic acid, and flavonoids (5).

Anthocyanins are a subcategory of flavonoid. Chemically, they are polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium (Figure 1.1 A). Seventeen of these compounds are found abundantly in foods, specifically the fruits of blueberries, raspberries, and grapes. Significant quantities can also be found in the leaves of these fruits. Color is pH dependent and ranges from red to blue. The most prevalent anthocyanins are glycosides of cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (27).

Flavonols are the most common flavonoid in foods and are found in a wide variety of edible products. This subcategory of flavonoid contains derivatives of a 3-hydroxyflavone skeleton (Figure 1.1 B). Quercetin, kaempferol, and myricetin belong to this category (28).

Flavones are structurally similar to flavonols, containing a backbone of 2-phenylchromen-4-one (Figure 1.1 C). These compounds are most often found in grains and herbs. Apigenin, luteolin, and chrysoeriol all belong to this category (28).

Hydroxycinnamic acid derivatives are of interest due to their known ability to act as antioxidants. Glycosylated forms are less common than for other skeletal structures, though some glycosylated forms exist. Caffeic, ferulic, and vanillic acids are all representatives of hydroxycinnamic acids. Figure 1.1 D gives the structure of 4-hydroxycinnamic acid. The para isomer is the most common natural form and can be found in peanuts and tomatoes (10, 29).

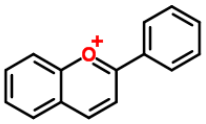
Flavanones are flavone derivatives found almost exclusively in citrus fruits. Natural sources of flavanones are typically glycosylated including naringin which is found in grapefruit. Hesperidin (hesperetin-7-rutinoside) is found in oranges. Flavanones contain a backbone of (2S)-Flavanone which can be seen in Figure 1.1 E (6).

Steroidal glycosides consist of a sugar attached to a steroid nucleus. The steroid nucleus consists of 17 carbon atoms: three six-membered rings and one five-membered ring. Gonane, the simplest steroidal nucleus can be seen in Figure 1.1 F. Medicinally they are used to block the outflow of sodium in cellular sodium-potassium ATP pumps causing a decreased rate of contraction and increased output force in the heart. Major natural sources include foxglove and the cane toad (30).

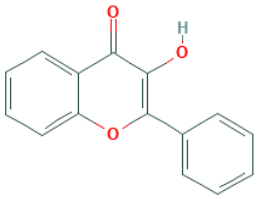
Steviol glycosides are extracted from *Stevia rebaudiana*, a South American plant. A small number of additional species also produce such compounds but the vast majority of steviol

glycosides used industrially are derived from *S. rebaudiana*. Dried extract is 200 - 300 times as sweet as table sugar and does not produce a glycemic response in humans. As a result, it is a popular alternative sweetener. Stevioside is the most abundant steviol glycoside and makes up 5-10 wt% of the dried leaf (31). The steviol backbone can be seen in Figure 1.1 G.

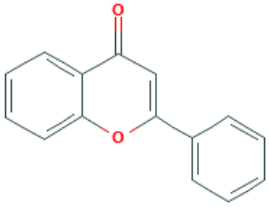
Saponins are surface active sterol or triterpene glycosides. The combination of a hydrophobic sapogenin (Figure 1.1 H) and a hydrophilic glycoside give these molecules soap-like properties. Chickpeas, alfalfa sprouts, and soybeans contain relatively high concentrations of



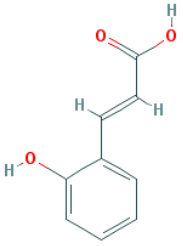
A: Flavyium: anthocyanin backbone (7)



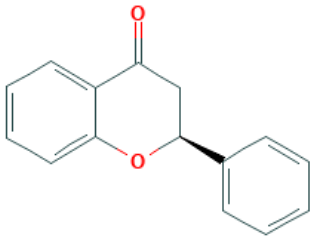
B: 3-Hydroxyflavone: flavonol backbone (8)



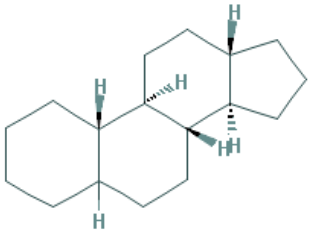
C: 2-phenylchromen-4-one: flavone backbone (9)



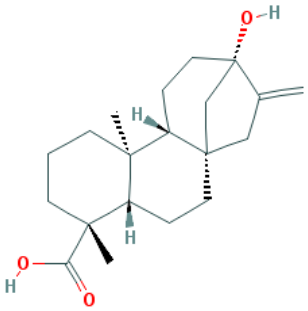
D: 2-Hydroxycinnamic Acid (10)



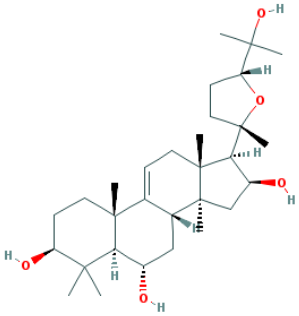
E: (2S)-Flavanone (11)



F: Gonane: steroid nucleus (12)



G: Steviol (13)



H: Sapogenin (14)

Figure 1.1 Structures of polyphenol backbones

saponins. Typically, a single plant will contain a range of saponin structures which can be difficult to isolate individually. In the human diet, saponins have been shown to lower plasma cholesterol levels and thus have the potential to reduce the risk of coronary heart disease (32).

While the structures discussed above can be classified into a range of categories, all contain the same basic components: a hydrophobic core containing ring structures and one or more hydrophilic oxygen containing functionalities. By definition, polyphenols do not contain nitrogen functionalities or any elements other than carbon, hydrogen, and oxygen. These backbones contain little structural similarity to sucrose. The hydrophilic functionalities may interact favorably with sucrose; however, the relatively large hydrophobic benzene groups should minimize the extent of this interaction. In contrast, the sugar side group of a glycosylated polyphenol should be able to interact much more favorably due to the high degree of structural similarity between sucrose and other sugar groups. It is this potential for interaction which has formed the foundation of the hypothesis explored in Chapter 2. If an association can be made between the sugar group of the polyphenol and sucrose, perhaps the polyphenol backbone may be able to limit association of this sucrose molecule with nearby sucrose molecules, thus delaying crystallization.

1.5 Properties of Commercial Forms of Thiamine

Presently, two salts of thiamine are widely available commercially: thiamine mononitrate (TMN) and thiamine chloride hydrochloride (TClHCl). TClHCl is often referred to as thiamine chloride; however, in this work neutralized TClHCl or TCl is used as an intermediate and thus the distinction between these two salts is important and the terms are not used interchangeably.

TMN is the choice for dry applications where maximum solid state stability and minimum hygroscopicity is desired. The solubility of TMN has been measured to be 27 mg/mL

(44). While this solubility is much lower than that of TClHCl or TCl, for fortification of flours this is more than adequate as the total quantity of thiamine needed is much less than this amount. TMN also sorbs much less water compared to TClHCl at a given relative humidity (RH).

TClHCl is the choice for beverage applications as its solubility is much greater than TMN (570 g/mL(44)) and its higher hygroscopicity is not of concern.

1.6 Salt Forming Reactions

The simplest definition of a salt is an ionic compound formed as the product of an acid-base neutralization. The salt can be broken up into two components: a positively charged cation and a negatively charged anion. The relative abundance of each species is such that the net charge is zero. Upon dissolution, these cations and anions will dissociate and move about freely in solution. A number of reactions produce salts as their products. Some of these include reactions between: an acid and base (Equation 1.1 A**)**, an acid and a metal (Equation 1.1 B), an acid and base anhydride (Equation 1.1 C), and between two different salts (Equation 1.1 D). Chapter 3 focuses on the forming new salts of thiamine through the fourth reaction.

$$A\colon\ NaOH + HCl \rightarrow NaCl + H_2O$$

$$B\colon\ Ca + H_2SO_4 \rightarrow CaSO_4 + H_2$$

$$C\colon\ 2HNO_3 + Na_2O \rightarrow NaNO_3 + H_2O$$

$$D\colon\ AgNO_3 + KCl \rightarrow KNO_3 + AgCl$$

Equation 1.1 Salt forming reactions

1.6.1 Salt Metathesis Reaction

Often called a double replacement reaction, salt metathesis reactions involve the exchange of anions between two salts, resulting in two new salts. The general reaction is presented in Equation 1.2.

$$AB + CD \leftrightarrow AD + CB$$ Equation 1.2 Salt metathesis reaction

The bonds between both the reactants and products may be ionic or covalent, and typically one of the products remains in solution while the other precipitates out of solution (33). In the context of this work, species "AB" would be thiamine chloride where "A" is the thiamine ion and "B" is the chloride ion. Species "CD" would be the salt or acid of the desired anion. Here "C" is a sodium or hydrogen ion and "D" is the desired anion. The product "AD" is the desired new thiamine salt, and "CB" is either sodium chloride or hydrochloric acid. Ideally, the solubility of "AD" is either much less or much more than that of sodium chloride, allowing for a simple selective precipitation of the desired thiamine product.

1.6.2 Silver Method

The double replacement reaction between silver nitrate and another salt to form a new salt and silver chloride is well known in introductory chemistry courses. Silver nitrate has a solubility of 256 g/100 mL water at 25°C while the solubility of silver chloride is only 0.19 mg/100 mL water (1). As silver nitrate represents a practical upper limit on the solubility of silver salts and silver chloride represents a lower limit, it is possible other silver salts will have intermediate solubilities. By first reacting silver nitrate with the sodium salt of the desired anion and then reacting these new silver-anion salts with thiamine chloride monohydrate ($TCl{\cdot}H_2O$), it should be possible to precipitate silver chloride, leaving thiamine and the other anion in solution.

Cramer and Carrie used this method to produce thiamine hydrochloride hemisulfate monohydrate (2). Silver sulfate (Ag_2SO_4) was reacted with TClHCl in a 2:1 stoichiometric ratio, immediately producing AgCl as a precipitate. After filtering and evaporating the supernatant, the product was recrystallized to form large, 2-D crystals of thiamine hydrochloride hemisulfate monohydrate.

In this work, silver nitrate was dissolved in ultrapure water to which the sodium salt of the desired anion was added. As the solubility of this new silver salt is less than silver nitrate, it precipitated. The precipitant was filtered, dried, and stored in the absence of light. $TCl{\cdot}H_2O$ was then dissolved in water and the silver-anion salt was added. The slurry was mixed using a vortex mixer and centrifuged. The supernatant was removed, filtered, and added dropwise to an antisolvent, usually acetone. The resulting precipitant was then analyzed to determine whether the chloride was successfully replaced by the desired anion.

1.7 Analytical Techniques

1.7.1 Dynamic Vapor Sorption

Dynamic vapor sorption is a gravimetric technique that measures the mass change of a sample over time as it is exposed to an environment of a specific vapor concentration. In the context of this work, water is the only solvent studied, though the technique can be applied to any volatile solvent. RH is changed over time and changes in the mass of amorphous sucrose samples are measured. By measuring mass change as a function of RH, estimates of water sorption isotherms can be developed. Though a true isotherm would achieve equilibrium at each RH step, by stating a minimum mass change per unit of time, a close approximation can be obtained.

Crystalline and amorphous solids will exhibit differently shaped isotherms as the modes of water-solid interactions are fundamentally different. Below their deliquescence point, crystalline materials will only take up water through surface adsorption, capillary condensation, and/or hydrate formation. Total water uptake through these modes is minimal and is usually limited to a few weight percent of the dry solid. Above the deliquescence point, the profile will exhibit a vertical increase in mass versus %RH as the solid dissolves. The solution will continue to take up water until the RH of the solution is equal to the RH of the environment. While a crystal exposed to any RH greater than or equal to its deliquescence point will eventually take up enough water to dissolve, at RH's very close to this point the time scale over which this occurs can be quite large. As the difference is increased, the kinetics of this uptake will increase. Thus, estimates of RH_0 are consistently higher than the true value when measured in desiccator studies. In contrast, amorphous solids will exhibit a sigmoidal moisture sorption isotherm. In addition to capillary condensation and surface adsorption, amorphous solids can absorb water. Absorption differs from adsorption in that it is not only a surface phenomenon, water can be absorbed internally. The lack of a tight, regular crystal structure is what allows for the penetration of water into the internal structure of amorphous solids. Crystalline materials with a high abundance of defects can also demonstrate this behavior, though to a lesser extent than amorphous solids (40).

1.7.2 X-Ray Crystallography

X-ray crystallography is an indispensable tool for the analysis of crystalline and amorphous materials. X-ray crystallography allows for the determination of the specific molecular arrangement of a crystal. In this work, two forms of X-ray crystallography were used, single crystal XRD and powder XRD. The primary indicator that a new thiamine salt has been formed was a unique diffractogram collected through scXRD. Since different anions occupy

different volumes in the crystal structure, different arrangements will be observed for different salts. pXRD is used to screen for potential new forms. If, based on the pXRD pattern, a new form is suspected, scXRD is used to confirm a new form has been isolated. Since only a single crystal of the material is used for this analysis, it is possible to accurately characterize the material in a near pure state, even if the bulk material is less pure and only a small amount is available. While pXRD may show a mix of two forms, scXRD should show the molecular arrangement of the phase pure material. scXRD is also used to determine whether the crystal is either a hydrate or anhydrous. This information is particularly relevant as knowing the molecular weight of the new form is critical to proper calculation of purity through analysis with HPLC.

The working principle behind XRD involves the elastic scattering of monochromatic X-ray light off of the surface of a solid material. X-ray light is used as it has a wavelength that spans the range of average spacing between atomic planes. When struck by X-rays, atoms will scatter light in an elastic and inelastic manner. Elastic scattering does not absorb any energy and thus the scattered light has the same wavelength as the incident light. Amorphous solids lack any long range order in their atomic arrangements and thus the scattered light will destructively interfere with itself, producing a signal that is devoid of any sharp peaks. When atoms are arranged in a consistent pattern, certain angles will produce scattering that adds constructively. This relationship between the angle and wavelength of the incident light and the spacing between atoms is given by Equation 1.3, known as Bragg's law. Here d is the spacing between atomic planes, θ is the angle of the incident light, n is an integer, and λ is the wavelength of the incident light (38).

$$2dsin(\theta) = n\lambda \qquad \text{Equation 1.3}$$

1.7.3 Fourier Transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is a spectroscopic technique used to obtain absorbance or emissions spectra of a material in the infrared range. Dispersive spectrometers can accomplish the same task however FTIR holds many advantages including a much more rapid analysis time and higher quality data collection.

An FTIR consists of a light source which is not monochromatic; it contains the entire range of wavelengths that is being examined. The incident light passes through an interferometer, a kind of mirror, which blocks certain wavelengths due to deconstructive interference. The light passes through the sample and some of the light is absorbed. The remaining light strikes a detector where intensity is recorded. As the raw data from an FTIR contains information about the entire range of wavelengths at each mirror position, it is necessary to use a computer to process the data. This has become a routine practice over the last 40 to 50 years.

Molecules can be excited in a number of different ways, each requiring a specific energy characteristic to that excitation for that compound. Infrared light is in the energy range that is similar to the energy required to cause bonds to resonate in this manner. Six common modes of vibration exist: symmetric stretching, asymmetric stretching, scissoring, rocking, wagging, and twisting. Regardless of the manner in which these excitations occur, they must produce a change in the dipole moment of the bond in order to be measurable through FTIR. Thus, stretching of a carbon-oxygen bond in CO_2 will not register through FTIR but scissoring will. For excitations where a change in dipole moment is not possible, Raman spectroscopy may be a relevant alternative (42).

In this work FTIR was used to rapidly screen precipitants produced in reactions to form new thiamine salts. All precipitants formed were first measured through FTIR and pXRD. If a unique spectrum and/or diffractogram were obtained, the samples were then measured by scXRD.

1.7.4 High-Performance Liquid Chromatography

High-performance liquid chromatography is a separation technique which utilizes a pressurized solvent and functionalized column to separate compounds which are dissolved together. It is a quantitative measurement and is often used to determine the purity of a material. The fundamental pieces of an HPLC are the solvent system, the column, and the detector. Each piece can be adapted to suit the particular system of interest.

Solvent choice and flow rates are dependent on the materials being analyzed, the possible impurities present, and the degree of quality of information needed. A more efficient method will better separate observed peaks and will allow for more accurate integration of peaks but will require more time spent on method development and will increase the duration of each experiment. The solvent system is either isocratic or gradient. Isocratic systems use one solvent, whereas a gradient system will vary between two or more solvents. It is important to note than solvents may not necessarily contain only one compound: 20:80, methanol:water is a common solvent used in HPLC systems. A method making use of only 20:80, methanol:water would be considered isocratic.

Columns used for HPLC are distinct from those used in gas chromatography but provide similar functionality. Columns are typically on the order of 10-100 cm in length and are filled with an adsorbent solid material. The difference in attraction between dissolved species and the column packing material will produce different retention times, allowing for the separation of

mixtures into individual fractions. Common packing materials include silica and a range of functionalized polymers. Typically, particle size is between 2-50 μm. A pressure drop over the length of the column during operation is typically in the range of 50 to 350 bar.

Multiple detector types are used: ultraviolet/visible spectrophotometers, photodiode array (PDA), and mass spectrometers are the most common. UV/Vis and PDA detectors rely on absorbance of light to quantify the concentration of a species. Detectors also exist which make use of other properties such as refractive index or conductivity, though these detectors are typically reserved for specific applications (43).

In this work, HPLC is used to quantify the purity of thiamine salts and to track the percent of initial thiamine remaining in degradation studies.

1.8 Summary

The goals of this work were twofold: first, to investigate the effect of glycosylated polyphenols on the stability of amorphous sucrose and, second, to prepare and characterize alternative salts of thiamine. In the first objective, it was hypothesized the sugar functional group of the glycosylated polyphenol would interact with a sucrose molecule while the polyphenol backbone would inhibit association between this sucrose molecule and others, thus delaying crystallization. In order to investigate this hypothesis, sucrose was lyophilized in the presence of both 1 and 5 wt% of 12 polyphenols and polyphenol containing food ingredients. Polyphenols were selected to include monoglycosylated, polyglycosylated, and aglycones containing a range of polyphenol backbones. Raw plant ingredients known to contain a high concentration of polyphenols were also included. Desiccator studies were performed to track the time required for amorphous sucrose to crystallize in three different RH's for the different formulations. A gravimetric moisture sorption instrument was used to track crystallization both by holding at a

set RH and by increasing RH stepwise. Karl Fischer and DSC were used to determine moisture content and T_g, respectively. SEM was used to compare observations of crystallization kinetics to surface morphology.

The second objective was to explore salts of thiamine which are not commercially available at this time. The hypothesis was that some of these salts may have properties which are favorable in certain applications, such as increased stability to environmental factors such as temperature or exposure to ultraviolet light. To prepare new salts of thiamine, two reaction routes were explored. Double replacement reactions between thiamine chloride and ten sodium salts and five weak acids were attempted, though no new forms produced in this manner have been successfully identified at this time. A second method was developed which makes use of silver nitrate and was used to successfully prepare five new salt forms. Research is ongoing to scale up this process and characterize these new forms. In addition, properties of $TCl{\cdot}H_2O$ were explored: solution state degradation kinetics and solubility were measured.

1.9 References

(1) *Seidell, Atherton; Linke, William F. (1919). Solubilities of Inorganic and Organic Compounds (2nd ed.).* New York City*: D. Van Nostrand Company. pp. 617–619*

(2) Cramer, R. E., & Carrie, M. J. J. (1989). Structure of thiamin hydrochloride hemisulfate monohydrate. Acta Crystallographica Section C Crystal Structure Communications, 45(12), 1899–1902. http://doi.org/10.1107/S0108270189003422

(3) Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52:673–751.

(4) Beckman CH. Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defense responses in plants? *Physiological Molecular Plant Pathology* 2000;57:101–110.

(5) Tuberoso, C. I. G., & Orrù, C. D. (2008). Phenolic compounds in food. *Progress in Food Chemistry*, (4), 1–45. http://doi.org/10.1007/s00394-008-2002-2

(6) Tomás-Navarro, M., Vallejo, F., & Tomás-Barberán, F. A. (2013). Bioavailability and Metabolism of Citrus Fruit Beverage Flavanones in Humans. *Polyphenols in Human Health and Disease*, 1, 537–551. http://doi.org/10.1016/B978-0-12-398456-2.00040-2

(7) "Flavylium." (R,R)-Stilbene Dibromide | C14H12Br2 | ChemSpider, Royal Society of Chemistry, www.chemspider.com/Chemical-Structure.128674.html.

(8) "Flavonol." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/3-Hydroxyflavone#section=Top.

(9) "Furan; 2-Phenylchromen-4-One." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/89705386#section=Top.

(10) "P-Coumaric acid." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/p-Coumaric%20acid#section=Top.

(11) "Flavanone, (-)-." PubChem Compound Database, U.S. National Library of Medicine, ubchem.ncbi.nlm.nih.gov/compound/_2S_-Flavanone#section=Top.

(12) "Gonane." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/gonane#section=Top.

(13) "Steviol." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Steviol#section=Top.

(14) "Sapogenin A." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/23265676#section=Top.

(15) Zografi, George. "States of Water Associated with Solids." *Drug Development and Industrial Pharmacy*, vol. 14, no. 14, 1988, pp. 1905–1926, doi:10.3109/03639048809151997. (16) Brovchenko, Ivan, and Alla Oleinikova. "Multiple Phases of Liquid Water." *ChemPhysChem*, vol. 9, no. 18, 2008, pp. 2660–2675., doi:10.1002/cphc.200800639. (17) Wei Yao, Xin Yu, Joo Won Lee, Xiaoda Yuan & Shelly J. Schmidt (2011) Measuring the Deliquescence Point of Crystalline Sucrose as a Function of Temperature Using a New Automatic Isotherm Generator, *International Journal of Food Properties*, 14:4, 882-893, DOI: 10.1080/10942910903474393

(18) Ritchie, Gary E. "General Chapters: Water-solid interactions in pharmaceutical systems." US Pharmacopeia, www.pharmacopeia.cn/v29240/usp29nf24s0_c1241.html.

(19) Aguilera, Josém., et al. "Caking Phenomena in Amorphous Food Powders." *Trends in Food Science & Technology*, vol. 6, no. 5, 1995, pp. 149–155., doi:10.1016/s0924-2244(00)89023-8.

(20) Zafar, U., et al. "A Review of Bulk Powder Caking." *Powder Technology*, vol. 313, 2017, pp. 389–401., doi:10.1016/j.powtec.2017.02.024.

(21) El-Yafi, Abdul Khaliq El-Zhry, and Hind El-Zein. "Technical Crystallization for Application in Pharmaceutical Material Engineering: Review Article." *Asian Journal of Pharmaceutical Sciences*, vol. 10, no. 4, 2015, pp. 283–291., doi:10.1016/j.ajps.2015.03.003.

(22) Libretexts. Crystalline and Amorphous Solids. Libretexts, 1 Feb. 2015, chem.libretexts.org/Textbook_Maps/General_Chemistry/Map:_Chemistry_(Averill_and_Eldredge)/12:_Solids/12.1:_Crystalline_and_Amorphous_Solids.

(23) Hartmann, Markus, and Stefan Palzer. "Caking of Amorphous Powders — Material Aspects, Modelling and Applications." *Powder Technology*, vol. 206, no. 1-2, 2011, pp. 112–121., doi:10.1016/j.powtec.2010.04.014.

(24) Khan, Naghma, and Hasan Mukhtar. "Tea Polyphenols for Health Promotion." *Life Sciences*, vol. 81, no. 7, 2007, pp. 519–533., doi:10.1016/j.lfs.2007.06.011.

(25) Scalbert, Augustin, et al. "Polyphenols: Antioxidants and Beyond." *The American Journal of Clinical Nutrition*, vol. 81, no. 1, Jan. 2005, doi:10.1093/ajcn/81.1.215s.

(26) Sies, Helmut. "Polyphenols and Health: Update and Perspectives." *Archives of Biochemistry and Biophysics*, vol. 501, no. 1, 2010, pp. 2–5., doi:10.1016/j.abb.2010.04.006.

(27) Pojer, Elisa, et al. "The Case for Anthocyanin Consumption to Promote Human Health: A Review." *Comprehensive Reviews in Food Science and Food Safety*, vol. 12, no. 5, 2013, pp. 483–508., doi:10.1111/1541-4337.12024.

(28) Herrmann, K. "Flavonols and Flavones in Food Plants: A Review." *International Journal of Food Science & Technology*, vol. 11, no. 5, 1976, pp. 433–448., doi:10.1111/j.1365-2621.1976.tb00743.x.

(29) Taofiq, Oludemi, et al. "Hydroxycinnamic Acids and Their Derivatives: Cosmeceutical Significance, Challenges and Future Perspectives, a Review." *Molecules*, vol. 22, no. 2, 2017, p. 281., doi:10.3390/molecules22020281.

(30) Jr, John P. Munafo, and Thomas J. Gianfagna. "Chemistry and Biological Activity of Steroidal Glycosides from the Lilium Genus." *Natural Product Reports*, vol. 32, no. 3, 2015, pp. 454–477., doi:10.1039/c4np00063c.

(31) Ceunen, Stijn, and Jan M. C. Geuns. "Steviol Glycosides: Chemical Diversity, Metabolism, and Function." *Journal of Natural Products*, vol. 76, no. 6, 2013, pp. 1201–1228., doi:10.1021/np400203b.

(32) Oakenfull, David. "Saponins in Food—A Review." *Food Chemistry*, vol. 7, no. 1, 1981, pp. 19–40., doi:10.1016/0308-8146(81)90019-4.

(33) McNaught, Alan D., and Andrew Wilkinson. *IUPAC Compendium of Chemical Terminology*. Royal Society of Chemistry, 2000.

(34) Makower, Benjamin, and W. B. Dye. "Sugar Crystallization, Equilibrium Moisture Content and Crystallization of Amorphous Sucrose and Glucose." *Journal of Agricultural and Food Chemistry*, vol. 4, no. 1, 1956, pp. 72–77., doi:10.1021/jf60059a010.

(35) Todd, A. R., and F. Bergel. "Aneurin. Part VII. A Synthesis of Aneurin." *Journal of the Chemical Society (Resumed)*, 1937, p. 364., doi:10.1039/jr9370000364.

(36) R. R. Williams, J. K. Kline, *Journal of the American Chemical Society*, 1936, p. 58

(37) Contant, P., et al. "ChemInform Abstract: A New Convergent Synthesis of Thiamine Hydrochloride." *ChemInform*, vol. 21, no. 45, June 1990, doi:10.1002/chin.199045320.

(38) Nave, R. "Bragg's Law." *Centripetal Force*, Georgia State University, hyperphysics.phy-astr.gsu.edu/hbase/quantum/bragg.html.

(39) Labuza, T. P., and J. F. Kamman. "Comparison of Stability of Thiamin Salts at High Temperature and Water Activity." *Journal of Food Science*, vol. 47, no. 2, 1982, pp. 664–665., doi:10.1111/j.1365-2621.1982.tb10146.x.

(40) Ritchie, Gary E. "General Chapters: WATER-SOLID INTERACTIONS IN PHARMACEUTICAL SYSTEMS." US Pharmacopeia, www.pharmacopeia.cn/v29240/usp29nf24s0_c1241.html.

(41) Different Types of SEM Imaging – BSE and Secondary Electron Imaging. Azom Materials, www.azom.com/article.aspx?ArticleID=14309.

(42) Doyle, Walter M. Principles and applications of Fourier transform infrared (FTIR) process analysis. Technical note AN-906 Rev. C, Hellma Axiom, Tustin, Ca. http://www.hellma-axiom.com/pdf/technical_notes/AN-906.pdf

(43) Belanger, Jacqueline M. R., et al. "High Performance Liquid Chromatography (HPLC): Principles and Applications." *Instrumental Methods in Food Analysis*, 1997.

(44) Voelker, Adrienne L., et al. "Chemical Stability and Reaction Kinetics of Two Thiamine Salts (Thiamine Mononitrate and Thiamine Chloride Hydrochloride) in Solution." *Food Research International*, vol. 112, 2018, pp. 443–456., doi:10.1016/j.foodres.2018.06.056.

CHAPTER 2. THE EFFECTS OF 12 POLYPHENOL AND POLYPHENOL CONTAINING INGREDIENTS ON THE STABILITY OF AMORPHOUS SUCROSE

2.1 Abstract

When some materials are rapidly cooled, or moisture is removed suddenly, a disordered, metastable solid form can be obtained. These materials are referred to as amorphous solids and have a unique set of properties intermediate between those typical of solid and liquid states. These properties can be detrimental (higher degradation rate) or beneficial (higher dissolution rate) depending on the system they exist in. While crystalline materials are quite hard, amorphous materials exhibit a temperature dependent range of hardness. Below the glass transition temperature amorphous solids are brittle, though less brittle than crystalline solids. Above this temperature they are ductile and are often referred to as rubbery. The "crunch" of crystalline sucrose is often undesirable and rates of dissolution can be slow. Consequently, amorphous forms are preferred, especially in powdered beverage applications. As amorphous materials are not thermodynamically stable, crystallization inevitably occurs and causes loss of quality. It is known that interactions between amorphous sucrose and other classes of compounds such as sugars and salts can affect crystallization; however, less is known about the effects of polyphenols. Polyphenols are widely present in foods and often contain sugar side groups. These side groups might interact with crystalline sucrose while the polyphenol backbone inhibits further association of sucrose molecules, much like an emulsifier can help prevent oil droplets in water from agglomerating. The aim of this study was to investigate the rate of crystallization of amorphous sucrose lyophilized with polyphenols that either contained zero, one, or more than one sugar side group. Amorphous sucrose was prepared by freeze drying solutions of sucrose

and a polyphenol. XRD was used to determine the physical state of the material. Samples were stored in desiccators of either 11%, 23%, or 33% RH at 25°C and were tested weekly over the period of one month. Some plant based ingredients with high polyphenol contents were also investigated. At both 1 and 5 wt% relative to sucrose, these plant based ingredients were found to increase the stability of amorphous sucrose most substantially, extending the life of amorphous sucrose by a factor of 2 at 1 wt% and greater than 6.4 times at 5 wt%. Monoglycosylated polyphenols were also quite effective, increasing the time to crystallize by a factor of 2 to 3.3 Polyglycosylated and aglycone forms also provided a slight decrease in crystallization rate, increasing longevity of the amorphous form by 25-50%. Understanding the effects of these materials on amorphous sucrose stability may lead to new formulations of existing products; allowing for the use of amorphous sucrose where finely powdered crystalline sucrose is presently used. Some of these products include: hot cocoa mix, sweetened instant coffee, and matcha smoothie mixes.

2.2 Introduction

2.2.1 Crystal Nucleation

Crystallization involves two separate steps: first nucleation, then growth. Encouraging crystallization thus involves increasing the rate of nucleation, growth, or both. In supersaturated solutions there is a driving force for molecules to come together. While this force exists for any solution of a concentration greater than the saturation, nuclei don't necessarily form on their own and often require a catalyst. This catalyst is typically small defects in the container the solution is held in. At these defects, molecules can come together and combine into nuclei. This process is referred to as heterogeneous nucleation. Crystallization without the presence of such a surface is referred to as homogeneous nucleation. As for any catalytic process, the need for the catalyst is

to reduce the activation energy of the process. An activation energy exists which must be overcome for a nucleus to be stable. Nuclei which are very small are probabilistically favored to disassociate before gaining enough molecules to reach a point of stability. Thus, the presence of a rough surface decreases this minimum number (23).

The origin of this activation energy can be explained by thermodynamics. The formation of a nucleus requires molecules come together out of solution. This introduces surface tension between the nucleus surface and the solution which is proportional to the surface area. Conversely, there is energy release when molecules come together that is proportional to the number of molecules. Since the surface area scales with the square of the radius and the number scales with the cube of the radius, there will be a critical radius where the two energies are equal. This equates to an equal probability of collapse or growth; at radii greater than this it is more probable growth will occur and the nucleus is said to be stable. From a thermodynamic standpoint, any factor which encourages crystallization must decrease this activation energy (23). Lower temperature and a higher degree of supersaturation both serve this purpose. Another means of accomplishing this change is by reducing the surface tension between the nucleus and the solution. In this work, this is the mechanism by which any change in crystallization rate, positive or negative, is accomplished. Any molecule which is also in solution with the species which is crystallizing has the potential to associate at the surface. Once associated, the surface tension must change. The magnitude and sign of this change is dependent on the nature of the solution and the additive and is highly dependent on the extent of association. In previous work, which will be discussed in further detail in Section 2.2.3, various additives are shown to either decrease or increase the rate of crystallization. Thus, it can be inferred that additives which

increase the rate of crystallization decrease the surface tension between nuclei and solution, and those which decrease the rate of crystallization increase the surface tension. (23)

The hypothesis driving the research presented in chapter two is formed based on these principles. Since a delay of the crystallization rate is desired, an ideal additive would both increase the surface tension and associate frequently to nuclei. Since both the sucrose and water are hydrophilic, a molecule which is purely hydrophilic or hydrophobic would not be ideal. Hydrophilic molecules would have the potential to associate with sucrose effectively but would not increase surface tension. Hydrophobic molecules would increase the surface tension but would not associate well with the sucrose. This effect is essentially the same principle as selecting an emulsifier but in reverse. Molecules with two discrete regions, one hydrophobic and one hydrophilic, are thus ideal candidates for accomplishing these goals. Polyphenols are mostly hydrophobic while sugars are hydrophilic. Glycosylated polyphenols, containing both sugar functionalities and a polyphenol backbone, fit these requirements well.

2.2.2 Crystal Growth

In the second phase of crystallization, nuclei acquire additional molecules and grow in size. In order for these molecules to add to the nucleus, two factors must be satisfied. Molecules must be able to reach the surface of the crystal and there must be a thermodynamic driving force for association to occur. Factors influencing the rate at which new molecules are delivered to the crystal surface include the nature of the crystallizing species (molecular weight, sweeping volume, etc.), the viscosity of the solution, the number of molecules in solution, temperature, and external energy input through forces such as mixing (23). Increasing the rate at which new molecules reach the surface, such as through increasing temperature or decreasing viscosity, will increase the rate of crystal growth. Even if molecules are rapidly delivered to the

crystal surface, if no driving force exists for them to add to the crystal, they will not bond to the surface. This driving force is maintained by the degree of supersaturation of the solution. As molecules add to the nuclei, this degree will decrease but as long as the solution is above saturation the force still encourages growth. If the solution becomes undersaturated the sign of the driving force will change, and molecules will dissolve until the crystals are either fully dissolved or saturation has been reached (23).

2.2.3 Previous Research on Amorphous Sucrose Stabilization

A number of classes of compounds have been shown to inhibit the crystallization of amorphous sucrose, including polymers, sugars, inorganic salts, and a limited number of polyphenols. Of these materials, polyphenols may represent an ideal choice to be used as a food additive as they are already widely present in natural food sources and are associated with numerous health benefits.

In "Effects of Mono-, Di-, and Tri-saccharides on the Stability and Crystallization of Amorphous Sucrose" (10), nine saccharides were lyophilized with sucrose. Mono- and disaccharides were added at 10 mol% relative to sucrose, and trisaccharides were added at a similar wt% as monosaccharides. The general trends observed regarding stability were as follows: control < fructose < glucose < raffinose < trehalose = isomaltulose < lactose = maltose = cellobiose = maltotriose. Thus, all sugars provided some degree of stabilization, and di- and trisaccharides provided more stabilization than monosaccharides.

Corn syrup as an inhibitor of sucrose crystallization is well known in home cooking in the context of confectionary products (30). Research by P. Gabarra and R. Hartel explored this effect by fractionating corn syrup into products with a range of molecular weights. These fractions all delayed sucrose crystallization as low as 10 wt% relative to sucrose, the lowest

concentration studied, as evidenced by an increase in the crystallization temperature and an increase in T_g (31).

S. Shamblin, et al. studied the effects of two polymeric additives, Ficoll and polyvinylpyrrolidone, on the crystallization of amorphous sucrose. They did not observe changes in T_g until a polymer concentration of 20 wt% relative to sucrose was added; however, the time to crystallize for sucrose treated with even 1 wt% of either polymer was found to be increased when held at temperatures in the range of 95-155°C. PVP affected this time more significantly than did Ficoll (20).

Chloride and sulfate salts have also been investigated as potential stability enhancers for amorphous sucrose. In "Effects of Chloride and Sulfate Salts on the Inhibition or Promotion of Sucrose Crystallization in Initially Amorphous Sucrose−Salt Blends" (18), nine chloride and six sulfate salts were colyophilized with sucrose. Mono-, di-, and trivalent salts for each anion were selected and added at a 0.1:1 molar ratio relative to sucrose. RH and temperature were controlled in a manner similar to that described in Section 2.3. For chloride salts, the trend in stability was observed as follows: LiCl < KCl < NaCl = sucrose < $MgCl_2$ < $CuCl_2$ = $FeCl_2$ < $CaCl_2$ < $AlCl_3$ < $FeCl_3$. For sulfate salts, the trend was: $MgSO_4$ < K_2SO_4 < Na_2SO_4 < sucrose < $CuSO_4$ < Fe-II-SO_4 < Fe-III-SO_4. With some exceptions, the anion did not affect the stability, indicating the observed effect was a result of the cation. As a general trend, trivalent cations provided the greatest enhancement of amorphous sucrose stability followed by divalent cations. Monovalent cations were found to decrease stability.

Previous research into the effects of polyphenols on sucrose crystallization has investigated the effects of two glycosides, naringin and glycyrrhizic acid, on the crystallization of amorphous sucrose. (19) Naringin is a flavanone found in citrus fruit. Glycyrrhizic acid is found in liquorice. Its aglycone is a pentacyclic triterpenoid which is similar to the backbone of saponins. Again, RH and temperature were controlled in the same manner as described in Section 2.3.4. The results from this study are presented in Table 2.1.

Table 2.1 Effects of naringin and glycyrrhizic acid on sucrose crystallization, adapted from (19)

Sample Name	Storage Conditions (RH and Temperature)					
	11% RH		23% RH		33% RH	
	25°C	40°C	25°C	40°C	25°C	40°C
Sucrose Control	A	A	A	1 Day	7 Days	1 Day
1 wt% Naringin	A	A	A	A	15 Days	1 Day
5 wt% Naringin	A	A	A	A	A	1 Day
1 wt% Glycyrrhizic Acid	A	A	30 Days	30 Days	21 Days	1 Day
5 wt% Gllycyrrhizic Acid	A	A	A	A	A	1 Day

When stored at 33% RH and 25°C, both glycyrrhizic acid and naringin delayed crystallization at 1 wt% by a factor of 2.1 and 3 respectively. These effects were more substantial at 5%; both naringin and glycyrrhizic acid increased the stability of amorphous sucrose from seven to greater than 30 days. The effectiveness of these additives and the relative lack of study into effects on amorphous sucrose stability by similar compounds motivated the desire to further investigate such compounds.

Together, these studies indicate a broad range of compounds can affect the stability of sucrose in its amorphous state to a meaningful degree. At 33% RH and 25°C, amorphous pure sucrose crystallizes in seven days. Many formulations remained amorphous even after a 30 day

period in these conditions. While this information may be interesting from a scientific perspective, many of these compounds would not make acceptable additives in the kind of products where a high stability of amorphous sucrose would be desired as they would contribute off flavors. Polyphenols represent potentially viable additives to these products as they are already naturally present in foods, often in the exact products for which amorphous sucrose is desirable. Tea, coffee, and chocolate are often used as the primary flavor in many powdered beverages and all contain relatively high quantities of polyphenols. They also have been strongly associated with health benefits. If it were possible to enhance amorphous sucrose stability merely by adding ingredients which are already present, gains may be made in improving product quality, shelf life, and healthfulness with a minimal negative impact on flavor. Therefore, the objective of this study was to investigate the ability of these compounds to delay sucrose crystallization and to draw conclusions on the potential mechanisms behind these stabilizing effects.

2.2.4 Polyphenols in Natural Foods

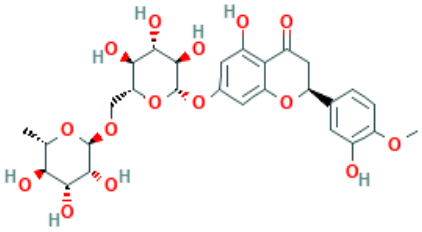
Polyphenolic compounds are widely present in natural foods and are often bioactive. Polyphenolic compounds are ubiquitous in the human diet and are intimately linked to food quality and human health. While the category contains a wide range of structures, generally the backbone consists of an aromatic ring with two or more hydroxyl groups. Polyphenols chosen in this study were selected to represent a variety of aglycone structures as well **as a range of numb**

Flavonols are the most common flavonoid in foods and are found in a wide variety of edible products. This subcategory of flavonoid contains derivatives of a 3-hydroxyflavone skeleton. Flavonols explored in this chapter include quercetin and rutin.

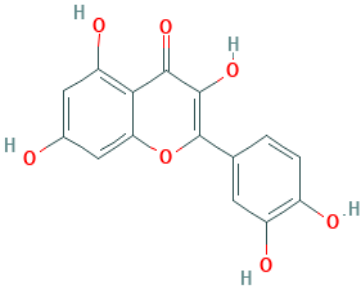
Flavones are structurally similar to flavonols, containing a backbone of 2-phenylchromen-4-one. These compounds are most often found in grains and herbs. Apigenin is the flavone explored in this work. Isoflavones are similar to flavones but differ in the location of the phenol ring. Isoflavones explored in this chapter include daidzein and puerarin.

Flavanones are flavone derivatives found almost exclusively in citrus fruits. Natural sources of flavanones are typically glycosylated, including naringin which is found in grapefruit. Hesperidin (hesperetin-7-rutinoside) is found in oranges. The flavanone explored in this chapter is hesperidin.

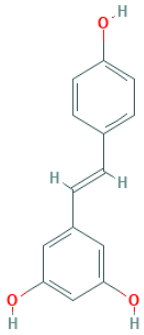
Other isolated compounds included in this chapter include: phenyl β-D-glucopyranoside, resveratrol, and α-arbutin. Phenyl β-D-glucopyranoside and α-arbutin are both glycosylated phenols and were selected as their backbone is structuraly similar to polyphenols, containing a benzene ring and no nitrogen functionalities, but is of a lower molecular weight. Resveratrol was included as it has a structure similar to polyphenols and has been used commonly in supplements in recent years, evidence of its benefits for health in humans is limited (40). Cocoa powder, matcha, green tea extract, and green coffee are also included in this work (29). Cocoa beans contain 6-8 wt% polyphenols on a dry basis. Catechins, flavonol glycosides, anthocyanins, and procyanins make up the majority of the polyphenol content (14). Matcha, or powdered green tea, contains primarily catechins, which can make up 30% of the dry weight of leaves, and flavonol glycosides (15,16). The green tea extact used in this study is labeled as 100% polyphenols with epigallocatechin gallate making up 51 wt% and caffeine <0.4 wt%. Green coffee contains approximately 9 wt% polyphenols. Chlorogenic acid makes up approximately 90% of this content, with caffeic acid and ferulic acid making up most of the remainder (17).



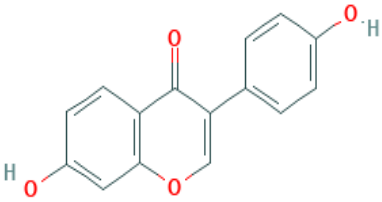
A: Hesperidin (6)



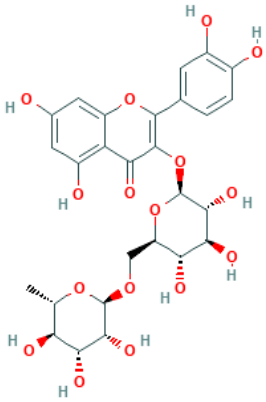
B: Quercetin (7)



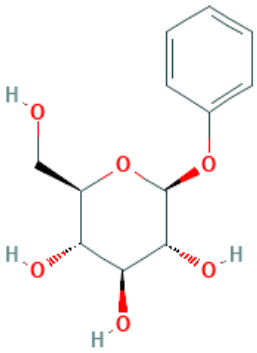
C: Resveratrol (27)



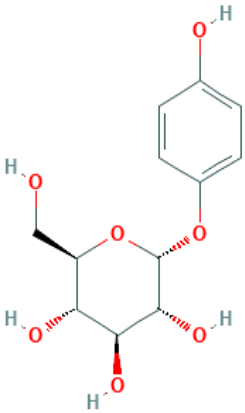
D: Daidzein (8)



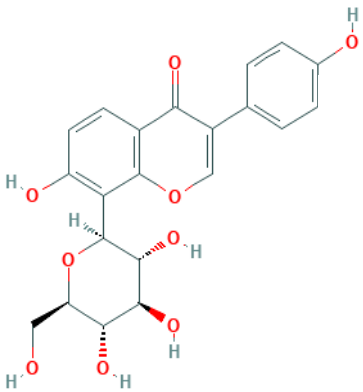
E: Rutin (9)



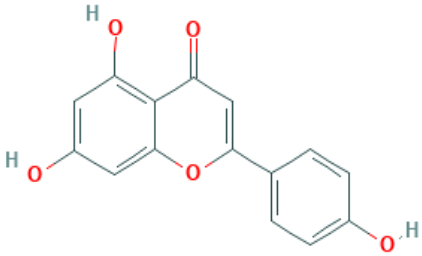
F: Phenyl β-D-glucopyranoside (11)



G: α-Arbutin (12)



H: Puerarin (13)



I: Apigenin (26)

Figure 2.1 Structures of polyphenols studied in this work

2.3 Materials and Methods

2.3.1 Compounds

Sucrose was purchased from Mallinckrodt (UK). Isolated polyphenols were selected to cover a range of backbone types and number of sugar units. Common food ingredients known to contain an abundance of polyphenols were also included. Examples of nonglycosylated polyphenols include quercetin dihydrate (bulksupplements.com, Henderson, Nevada USA), apigenin (Sigma Aldrich, St. Louis, Missouri USA), resveratrol (bulksupplements.com, Henderson, Nevada USA), and daidzein (Sigma Aldrich, St. Louis, MO USA). Monoglycosylated polyphenols include puerarin (Sigma Aldrich, St. Louis, Missouri USA), phenyl beta-d-glucopyranoside (Sigma Aldrich, St. Louis, Missouri USA), and α-arbutin (L'eternal World). Polyglycosylated polyphenols include hesperidin (Santa Cruz Biotechnology Inc., Dallas, Texas USA) and rutin (bulksupplements.com, Henderson, Nevada USA). Raw food ingredients include cocoa powder (nuts.com, Cranford, New Jersey USA), matcha (Encha, ceremonial-grade, San Jose, CA USA), green tea extract (Nutrients Scientific, Diamond Bar, CA USA), and green coffee (bulksupplements.com, Henderson, Nevada USA).

Salts used to control the relative humidity in desiccators were purchased from the following sources: lithium chloride (Acros Organics, New Jersey USA), potassium acetate (Fisher Scientific, Hampton, New Hampshire USA), and magnesium chloride (Fisher Scientific, Hampton, New Hampshire USA). Phosphorous pentoxide was purchased from Acros Organics (New Jersey USA).

All water used in this work was classified as ultrapure. House water supply was treated using a Barnstead E-Pure Ultrapure water purification system. According to manufacturer supplied specifications, resistivity of the water was less than 18.2 MΩ-cm, and total organic

carbon concentrations were less than 10 ppb. A 0.2 µm filter eliminated possible bacterial and particulate contamination. (2)

Karl Fischer reagents were all purchased from Honeywell (Mexico City, Mexico). HYDRANAL – Composite 2 was used as a titrant and HYDRANAL – Methanol Rapid was used as a solvent. The solvent bottle was kept dry using Drierite™ (Xenia, OH USA).

2.3.2 Sample Preparation and Freeze Drying Parameters

Freeze drying conditions were adapted from Yu, et al. (35). The work presented in this chapter makes use of HarvestRight's Scientific model. This model does not reach the minimum temperature achieved by Yu, et al. which led to changes in the temperature profile. Prior to lyophilization of samples used in this study a test batch of material was lyophilized and analyzed by XRD to confirm the sucrose was successfully made amorphous. All samples prepared in that test run were found to be amorphous and thus this method was judged as sufficient. Pressure was held at 300 mTorr for the duration of the freeze-drying process. The temperature profile is given in Figure 2.2.

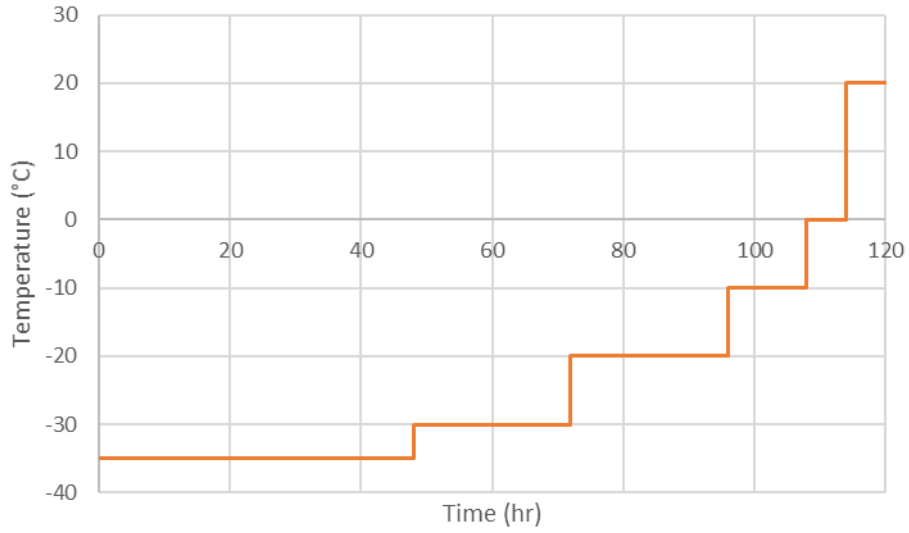


Figure 2.2 Temperature profile for lyophilization process

In order to prepare samples for lyophilization, sucrose was first dissolved in UP water at a concentration of 1.00g/10.0 mL. Polyphenols were added as needed at either 1 or 5 wt% based on the mass of sucrose and were mixed to dissolve. A control was included containing no phenolic additive. For some materials, particularly cocoa powder, a fraction of the additive remains undissolved even after vigorous mixing. This is likely due to two factors: starch and hydrophobic compounds. While starch could be gelatinized though the addition of heat, this was undesirable as increased temperatures may degrade the polyphenols. For hydrophobic compounds nothing may be done to increase their solubility apart from changing the solvent or heating, neither of which is possible. A conscious decision was made to leave these undissolved portions as they were.

Solutions of sucrose, additive, and UP water were frozen in a -8°C freezer and the freeze drier was cooled to -30°C prior to initiating the protocol. This removal of heat prior to initiating the freeze drying protocol reduces the cooling load on the freeze drier and helps ensures all of the solution was at a temperature less than its T_g' by the time lyophilization occurs which is -32°C (34). Once the freeze drier reaches -35°C the protocol begins and follows the aforementioned pattern. After the experiment concluded, a plastic weigh boat containing approximately 5 grams of phosphorous pentoxide was added to each container holding the amorphous solids and airtight lids were added. The phosphorous pentoxide ensures the headspace in the container has the minimum possible water activity.

Containers of amorphous solids were stored in a 25°C room until use. An initial diffractogram was collected using pXRD prior to use in any other experiment. This ensures the freeze-drying process was successful and a fully amorphous solid material had been obtained.

2.3.3 Dynamic Vapor Sorption Method Parameters

A SPSx1 μ Dynamic Vapor Sorption Analyzer manufactured by Projekt Messtechnik was used in this work. In this study two sets of conditions were examined. For both sets of conditions, temperature was controlled at 25°C. For one experiment, the RH profile was as follows: hold at 0% RH for 96 hours followed by a hold at 40% RH for 96 hours. The initial step at 0% RH acts to dry the material of any water taken up during sample preparation and handling. The purpose of this experiment is to identify the exact time crystallization occurs at a specific RH. For the second experiment the RH profile was as follows: RH was increased stepwise in 5% increments from 0 to 80% RH. A minimum step time was set to 50 minutes and a maximum was set to 12 hours. If a rate of mass change of less than 0.001% weight change in a period of 30 minutes for all samples was observed for a given step before 12 hours had been reached and after 50 minutes had elapsed, the next RH step was triggered. The purpose of this experiment was to approximate the critical RH at which enough moisture has been sorbed to lower T_g to room temperature and trigger crystallization (33).

Samples of approximately 100 mg of each material were tested and samples were run in duplicate, other than the control (no additive), which was run as a singlet. Drierite™ was used to dry ambient air and a water basin filled with UP water was used to produce a headspace with the desired RH.

2.3.4 Desiccator Study Method Parameters

Traditionally, desiccator studies are performed in large containers with a large volume of saturated salt solution and a large headspace, at least 10% of which is filled with saturated salt solution (32). Since opening the desiccator introduces air of a different RH than the desiccator, frequent sampling can lead to results which do not accurately represent reported conditions.

Thus, the time scale of equilibration must be much less than the time in between sampling. In order to avoid this issue, small desiccators were prepared using 870 mL plastic boxes. These boxes are airtight and can be prepared for each day of sampling, eliminating any introduction of ambient air through periodic sampling. In each box were placed two cups of saturated salt solution with a total volume of approximately 100 mL. Amorphous materials were then placed individually in small cups and the box was sealed and dated. On the day of analysis, the box was opened, the samples were analyzed, and the samples were discarded.

Three RHs and one temperature were used in this work. An 11% RH environment was maintained using a saturated solution of lithium chloride. A 23% RH environment was maintained using a saturated solution of potassium acetate. A 33% RH environment was maintained using a saturated solution of magnesium chloride. Water activity of these solutions was measured using an AquaLab 4TE water activity meter prior to the start of the experiment in order to ensure the solutions were prepared properly and the solution had reached saturation. Temperature for all samples was controlled at 25°C through storage of desiccator chambers in a temperature controlled room.

Samples of approximately 200 mg of "dry", amorphous solids were placed in Decagon brand water activity cups and were labeled appropriately. Plastic cups with a volume of approximately two ounces each were filled with approximately one ounce of saturated salt solution and additional salt was added to ensure the solution remained saturated over the duration of the experiment. Two cups of saturated salt solution and six cups of sample were placed in each Lock and Lock brand airtight container. The empty volume of each container, not including sample and saturated salt solution volume, was 870 mL, leaving approximately 600 mL of headspace.

Samples were tested using XRD first at day "0" or the day they were packed into desiccators, followed by days 7, 14, 21, and 30. All samples were amorphous as measured by pXRD at day 0. For samples which demonstrated crystallinity on day 7, a second round of samples were prepared. These samples were analyzed at days 1, 2, and 4.

2.3.5 Karl-Fischer Method Parameters

Karl-Fischer reagents were all purchased from Honeywell. HYDRANAL – Composite 2 was used as a titrant and HYDRANAL – Methanol Rapid was used as a solvent. The solvent bottle was kept dry using Drierite™. Between 35 to 100 mg of sample was used per analysis. This equates to approximately 1 mg of water per sample. As the samples were readily soluble, the homogenizer was not needed. This method was adapted from Thorat, et al. (10).

2.3.6 Differential Scanning Calorimetry Method Parameters

Calorimetric data presented in this work were collected using a Perkin Elmer DSC 4000 equipped with a Perkin Elmer Intracooler SP set to -85°C. Nitrogen of 99.995% purity was used as a purge gas. Samples were weighed and sealed hermetically in aluminum Perkin Elmer 50 µL pans. Sample mass ranged from 3.0 to 10 mg. Pans were pierced with a 20-gauge syringe in order to vent any water content during the first heating step and ensure a water was not influencing the measured T_g. The second heating step, where T_g was measured from, was then of the dry sample. Samples were prepared in triplicate. The temperature profile was as follows: heat from 10 to 100°C at a rate of 20°C/minute, cool to 10°C at a rate of 50°C/minute, hold for 5 minutes, heat to 100°C at a rate of 20°C/minute. Onset of T_g is defined as the temperature at which an endothermic baseline shift (glass transition) is observed in the second heating step. This method was also adapted from Thorat, et al. (10).

2.3.7 X-Ray Crystallography Method Parameters

XRD data collected for Chapter 2 was collected on two devices. A Shimadzu LabX XRD-6000 and a Rigaku Smartlab diffractometer were used, with the majority of data being collected using the Shimadzu device. The instruments were equipped with a Cu-Kα radiation source. A range for 2θ of 5 to 35 was used with a scan rate of 4° per minute and a step size of 0.04°. Daily calibration was performed prior to analysis when using the Shimadzu device. A silicon standard (111) peak was confirmed to be measured between 28.423° and 28.463° 2θ. An observed diffractogram showing a diffuse halo was labeled amorphous and diffractograms containing one or more sharp peaks were labeled as at least partially crystalline. These peaks must have had an amplitude of greater than two times the standard deviation to be considered significant. Thus, an otherwise amorphous material which exhibited statistically insignificant peaks were labeled as amorphous.

2.3.8 Statistical Analysis

Statistical analysis was performed using single-variable ANOVA through the use of SAS 9.4 (SAS Institute, Cary, NC USA). Tukey's post hoc test was used to determine differences using a significance level of $\alpha=.05$.

2.4 Results and Discussion

2.4.1 Moisture Sorption in an Active Headspace – Hold at 40% RH

A statistically significant enhancement in the stability of amorphous sucrose for samples containing both 1 and 5 wt% additive for many polyphenolic materials in the moisture sorption experiments. When considered as a full data set, groupings emerge: polyglycosylated and nonglycosylated polyphenols provided modest enhancements while monoglycosylated

polyphenols and raw sources provided substantial enhancement of amorphous sucrose stability compared to sucrose controls. In this work, crystallization is defined as the event where % mass change suddenly drops. This corresponds to an expulsion of absorbed water as the material transitions from the amorphous to crystalline state.

Comparing additives at 1 wt%, when held at 40% RH, monoglycosylated polyphenols provided an extension in the time to crystallize that ranged from a factor of 1.8 to a factor of 2.4. Raw sources provided an enhancement which ranged from a factor of 1.4 to 2.5. In comparison, the polyglycosylated polyphenols extended the time to crystallize by a factor of 1.1 to 1.2. Similarly, nonglycosylated polyphenols enhanced stability by a factor of 1.1 to 1.3.

At 5 wt% addition of polyphenol the contrast was greater. For polyglycosylated polyphenols, hesperidin treated samples had no significant change in sucrose crystallization, while rutin provided a 50% increase in the time to for sucrose to crystallize. Nonglycosylated polyphenols had no significant effect on the crystallization rate of sucrose. Monoglycosylated polyphenols again performed quite well: phenyl β-D-glucopyranoside doubled the time for sucrose to crystallize and puerarin increased the time to crystallize by a factor of 2.9. Of all the materials, green coffee and green tea extract provided the most substantial increase; green coffee increased the time to crystallize by 4.5 times and sucrose lyophilized with 5 wt% green tea extract failed to exhibit any weight loss characteristic of crystallization by the end of the experiment (>96 hours or >6.4 times).

Variations are observed in the maximum weight gain and the final percent weight gain post crystallization. Maximum weight gain is defined as the difference in mass change between the end of the drying step and the peak mass change prior to crystallization. Weight loss after crystallization is the difference between the mass change at the end of the drying step and the

equilibrium mass change following crystallization. For samples which remained amorphous at the end of the experiment, this value is not quantifiable. It is reasonable to assume additives may affect these values; however, a number of factors unrelated to the formulation may affect these values as well. Two primary factors are incomplete crystallization and case hardening. If part of the material remains amorphous, less water will be lost. Similarly, if the outer portion of the sample crystallizes before the inside, diffusion of water out of the internal material will be limited, trapping this water inside a crystalline shell. It was found that differences in the weight loss after crystallization were not significant. Significant differences were observed for the maximum weight gain though these differences were not consistent between samples treated with 1 and 5 wt% of a specific polyphenol. For example, quercetin added to sucrose at 1 wt% lost statistically the least amount of water upon crystallization and at 5 wt% lost the most. Further, the pure sucrose control showed nearly a 1.2% difference between experiments, indicating there were significant differences in the behavior of identical samples within experiments which are not the result of formulation. As a result, no conclusions may be made from this data.

Table 2.2 Moisture loss upon crystallization and maximum moisture gain for sucrose lyophilized with polyphenols at 1 and 5 wt%

Polyphenol Name	Polyphenol Type	Weight Loss After Crystallization		Maximum Weight Gain	
		1 wt%	5 wt%	1 wt%	5 wt%
Control	None	0.351 +/- 0.000 (A)	1.159 +/- 0.000 (A)	6.008 +/- 0.000 (AB)	7.174 +/- 0.000 (AB)
Apigenin	Nonglycosylated	1.770 +/- 0.259 (A)	No Data	6.114 +/- 0.118 (A)	No Data
Quercetin	Nonglycosylated	1.585 +/- 0.196 (A)	0.583 +/- 1.242 (A)	6.141 +/- 0.257 (A)	6.608 +/- 0.032 (D)
Daidzein	Nonglycosylated	No Data	0.457 +/- 0.393 (A)	No Data	6.417 +/- 0.015 (D)
α-Arbutin	Monoglycosylated	1.445 +/- 0.762 (A)	2.294. +/- 0.777 (A)	5.784 +/- 0.019 (AB)	6.924 +/- 0.050 (C)
Puerarin	Monoglycosylated	0.208 +/- 0.041 (A)	1.155 +/- 0.447 (A)	5.930 +/- 0.055 (AB)	6.972 +/- 0.068 (BC)
Phenyl β-D-glucopyranoside	Monoglycosylated	1.121 +/- 0.046 (A)	2.209 +/- 0.431 (A)	6.130 +/- 0.035 (A)	6.880 +/- 0.036 (C)
Hesperidin	Polyglycosylated	1.340 +/- 1.080 (A)	1.418 +/- 0.305 (A)	5.486 +/- 0.097 (C)	6.886 +/- 0.082 (C)
Rutin	Polyglycosylated	0.678 +/- 0.212 (A)	1.761 +/- 0.466 (A)	6.138 +/- 0.064 (A)	6.831 +/- 0.045 (C)
Cocoa Powder	Bulk Food Ingredient	0.650 +/- 0.374 (A)	1.029 +/- 0.185 (A)	5.755 +/- 0.009 (ABC)	7.030 +/- 0.084 (ABC)
Green Tea Extract	Bulk Food Ingredient	0.624 +/- 0.515(A)	Amorphous	6.151 +/- 0.191 (A)	6.997 +/- 0.044 (ABC)
Matcha	Bulk Food Ingredient	0.875 +/- 0.681 (A)	1.160 +/- 0.081 (A)	5.953 +/- 0.082 (AB)	7.199 +/- 0.031 (A)
Green Coffee	Bulk Food Ingredient	0.149 +/- 0.315 (A)	Amorphous	5.569 +/- 0.113 (BC)	7.024 +/- 0.009 (ABC)

Table 2.3 Time at moisture loss for amorphous sucrose treated with polyphenols held at 40% RH

Polyphenol Name	Polyphenol Type	1 wt%		5 wt%	
		Time to Crystallize (hrs)	Enhancement	Time to Crystallize (hrs)	Enhancement
Control	None	13.4 +/- 0 (h)	1	15 +/- 0 (f)	1
Apigenin	Nonglycosylated	15.0 +/- 1.6 (fgh)	1.1	No Data	No Data
Quercetin	Nonglycosylated	17.4 +/- 1.3 (fg)	1.3	16.4 +/- 0.2 (f)	1.1
Daidzein	Nonglycosylated	No Data	No Data	14.2 +/- 0.0 (f)	0.9
α-Arbutin	Monoglycosylated	23.8 +/- 1.1 (cd)	1.8	39.8 +/- 6.1 (cd)	2.7
Puerarin	Monoglycosylated	31.9 +/- (b)	2.4	43.3 +/- 6.1 (c)	2.9
Phenyl β-D-glucopyranoside	Monoglycosylated	32.0 +/-0.8 (ab)	2.4	30.3 +/- 1.3 (de)	2.0
Hesperidin	Polyglycosylated	14.7 +/- 0.4 (gh)	1.1	14.9 +/- 0.3 (f)	1.0
Rutin	Polyglycosylated	16.3 +/- 0.5 (fgh)	1.2	22.8 +/- 1.7 (ef)	1.5
Cocoa Powder	Bulk Food Ingredient	19.2 +/- 0.1 (fe)	1.4	30.6 +/- 0.6 (de)	2.0
Green Tea Extract	Bulk Food Ingredient	28.8 +/-2.3 (bc)	2.1	>96 +/- 0.0 (a)	>6.4
Matcha	Bulk Food Ingredient	22.2 +/-1.6 (de)	1.7	38.7 +/- 1.6 (cd)	2.6
Green Coffee	Bulk Food Ingredient	33.9 +/- 1.1 (a)	2.5	67.7 +/- 0.1 (b)	4.5

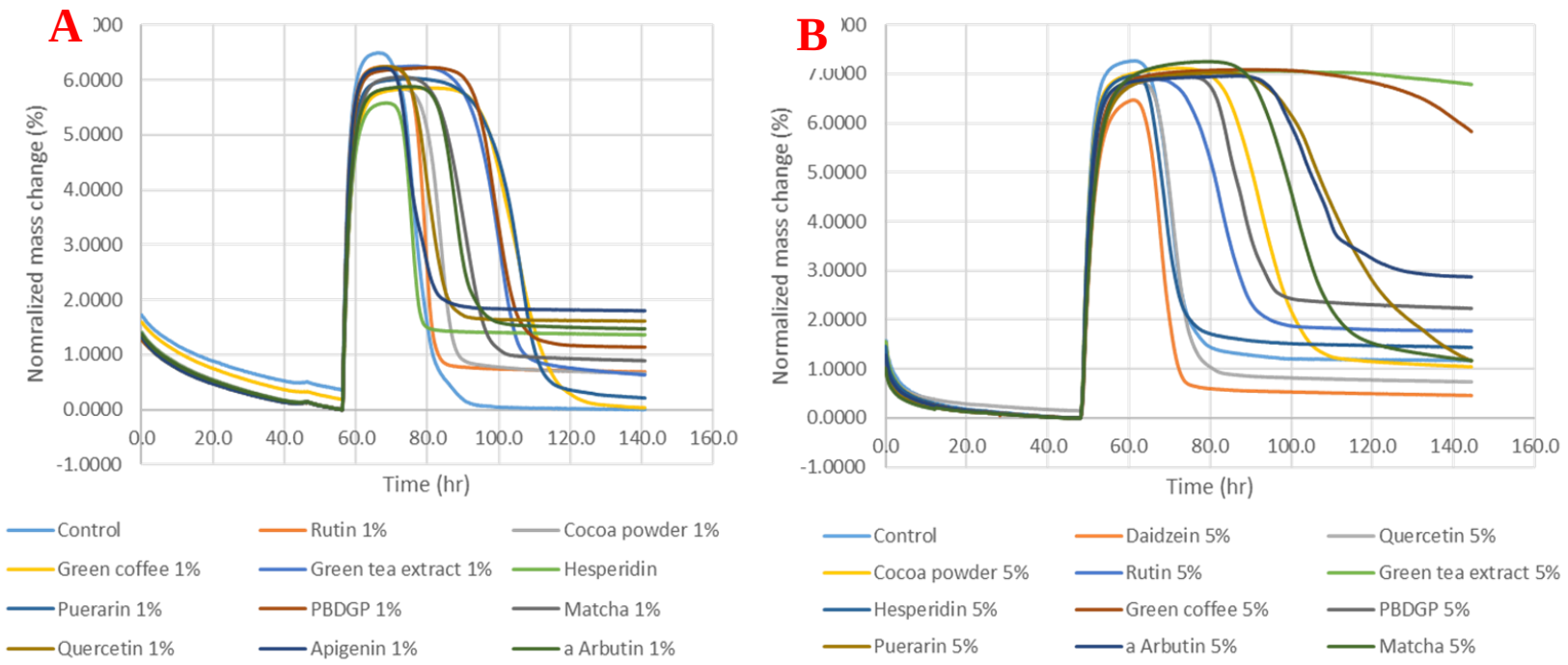


Figure 2.3 Weight change as a function of time for pure sucrose and sucrose treated with polyphenol additives when held at 40% RH:
A: Samples formulated with 1 wt% of additive held at 40% RH for 96 hours after a 48 hour drying step
B: Samples formulated with 5 wt% of additive held at 40% RH for 96 hours after a 48 hour drying step

2.4.2 Moisture Sorption in an Active Headspace – RH Increased Stepwise from 0 to 80%

Considering the experiment where RH was increased stepwise, many formulations showed a significant delay in the onset of sucrose crystallization relative to the pure sucrose control. At 1 wt%, three of eight samples (duplicates of four formulations) treated with poly or nonglycosylated polyphenols increased stability relative to the control. Apigenin and hesperidin produced no change relative to the control. Rutin delayed the RH at crystallization by 5% and quercetin delayed the crystallization event by 5% in one of the two samples tested. All monoglycosylated polyphenols and raw sources demonstrated higher stability relative to the control, increasing the RH at crystallization by 5%. Effects were more substantial at 5 wt%. Poly and nonglycosylated polyphenols all crystallized at the same RH as the control while all monoglycosylated polyphenols and raw sources crystallized at an RH of 5 to 15% greater than the control. As in the previously discussed vapor sorption experiment, green coffee and green tea extract performed most effectively, enhancing the RH at crystallization from 40 to 55%. These results are indicative of the maximum RH at which the sucrose formulations may be exposed to temporarily without initiating crystallization. At this RH, a sufficient quantity of water has been absorbed to lower the T_g to room temperature and thus induce crystallization (33). On a practical level, this RH is the RH at which sucrose may be kept in storage in a temperature, but not RH, controlled room for either an entire day or night. While the differences observed would not have any practical effect on sucrose stored in Indiana, in Arizona, only three months of the year average RHs less than 35% while all months average less than 55% (36, 37).

Table 2.4 Time at moisture loss for amorphous sucrose treated with polyphenols held at RHs increasing from 0 to 80%

Polyphenol Name	Polyphenol Type	RH at Crystallization (%)	
		1%	5%
Control	None	35 +/- 0 (b)	40 +/- 0 (d)
Apigenin	Nonglycosylated	35 +/- 0 (b)	No Data
Quercetin	Nonglycosylated	37.5 +/- 3.8 (ab)	40 +/- 0 (d)
Daidzein	Nonglycosylated	No Data	40 +/- 0 (d)
α-Arbutin	Monoglycosylated	40 +/- 0 (a)	45 +/- 0 (c)
Puerarin	Monoglycosylated	40 +/- 0 (a)	50 +/- 0 (b)
Phenyl β-D-glucopyranoside	Monoglycosylated	40 +/- 0 (a)	45 +/- 0 (c)
Hesperidin	Polyglycosylated	35 +/- 0 (b)	40 +/- 0 (d)
Rutin	Polyglycosylated	40 +/- 0 (a)	40 +/- 0 (d)
Cocoa Powder	Bulk Food Ingredient	40 +/- 0 (a)	45 +/- 0 (c)
Green Tea Extract	Bulk Food Ingredient	40 +/- 0 (a)	55 +/- 0 (a)
Matcha	Bulk Food Ingredient	40 +/- 0 (a)	50 +/- 0 (b)
Green Coffee	Bulk Food Ingredient	40 +/- 0 (a)	55 +/- 0 (a)

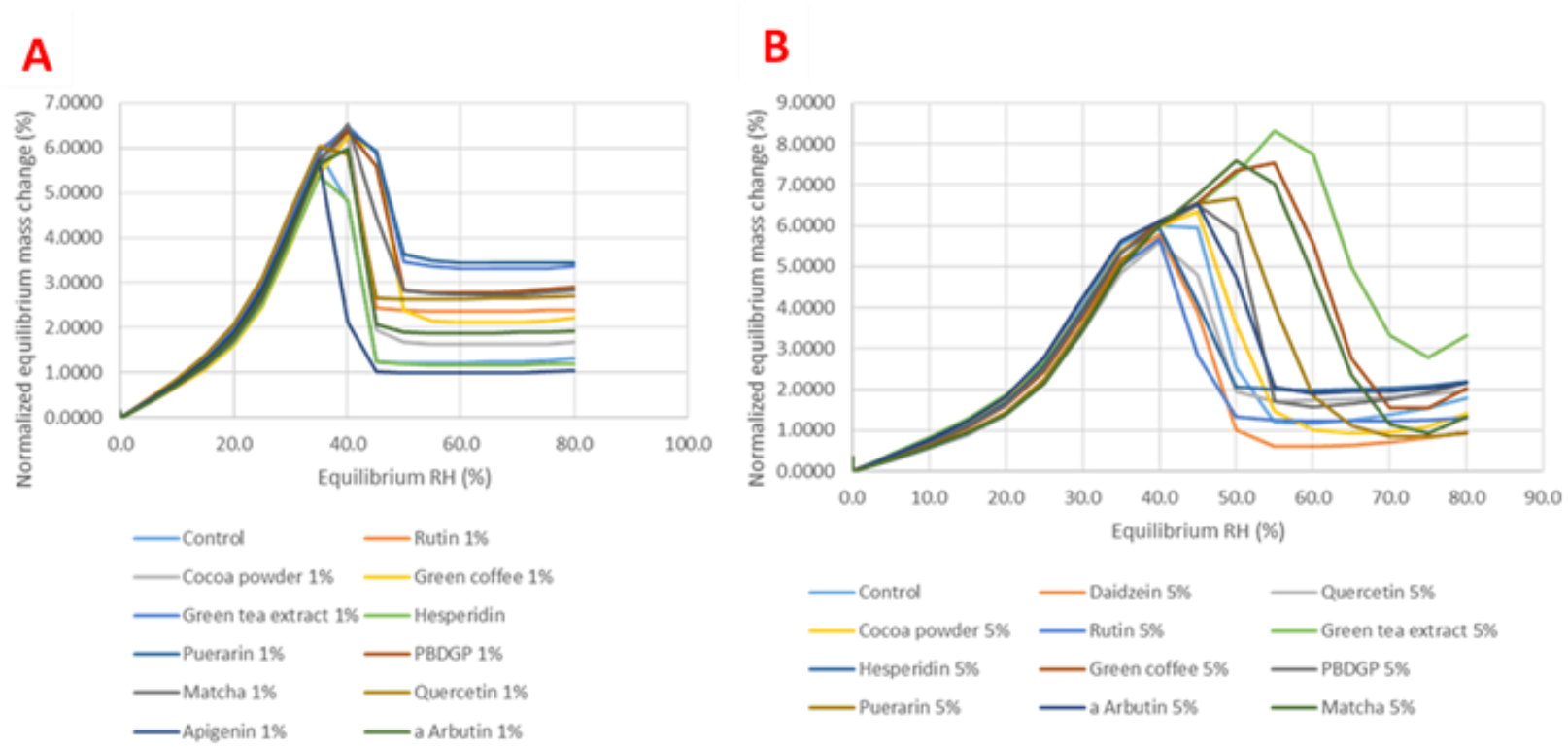


Figure 2.4 Weight change as a function of RH for pure sucrose and treated with polyphenol additives when RH is increased from 0 to 80% stepwise:
A: Samples formulated with 1 wt% of additive exposed to RH's ranging from 0 to 80% in 5% increments
B: Samples formulated with 5 wt% of additive exposed to RH's ranging from 0 to 80% in 5% increments

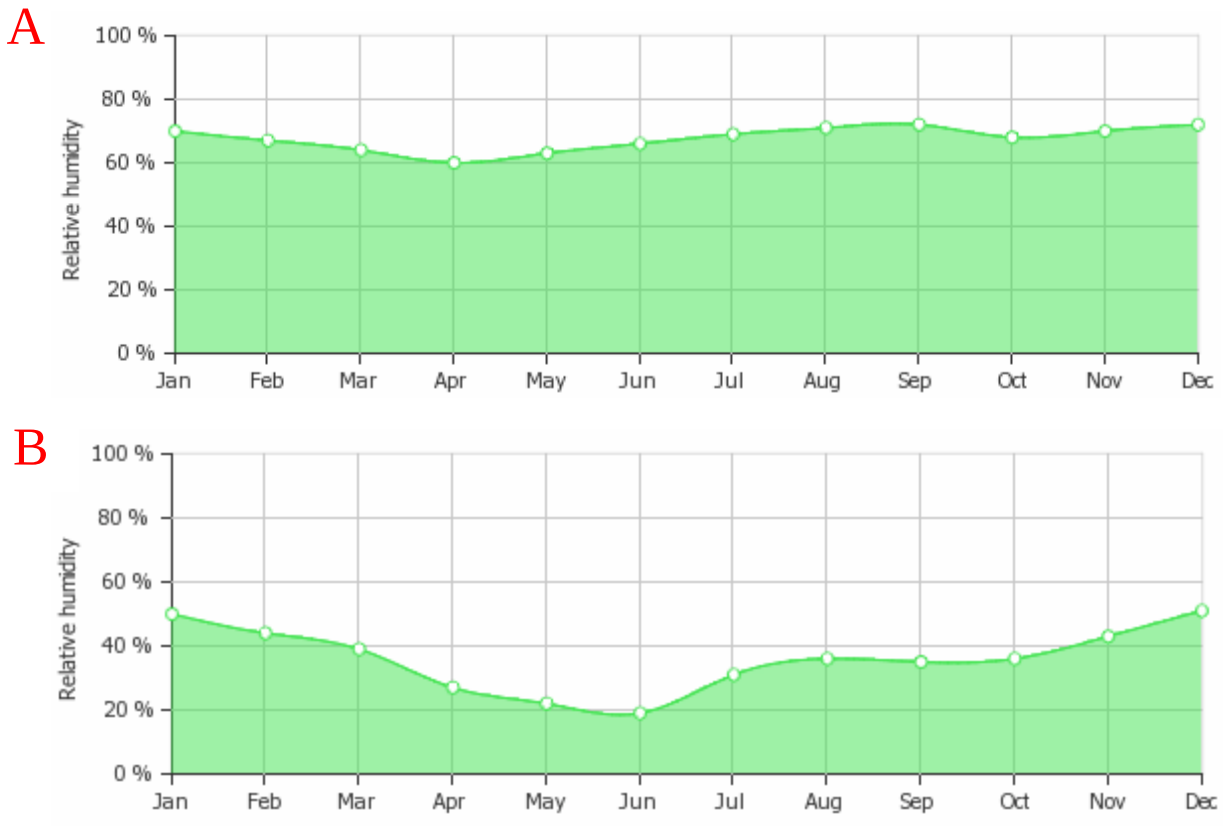


Figure 2.5 Average ambient RH of in two US states
A: Indiana, USA (36)
B: Arizona, USA (37)

2.4.3 Investigation of Onset of Sucrose Crystallization for Samples Stored in Desiccators

For all samples treated with 1 or 5 wt% additive as well as for the control, no evidence of sucrose crystallization was observed for samples held at either 11% or 23% RH over a 30-day period, as monitored by pXRD. At 33% RH differences emerged which mirrored those observed in the vapor sorption study. Pure sucrose control crystallized after a period of seven days. Sucrose containing nonglycosylated polyphenols crystallized over a range of four to 14 days depending on the specific polyphenol used. Quercetin and apigenin added at 5 wt% relative to sucrose produced the most substantial delay in sucrose crystallization out of this class of compounds, delaying crystallization of sucrose to 14 days. This delay in sucrose crystallization

was not observed in SPS studies. While no data is available for sucrose treated with 5 wt% apigenin, sucrose treated with 5 wt% quercetin showed no significant delay in crystallization in either SPS study. Without additional data, it is hard to draw conclusions on what might be causing this difference other than the difference in storage RH (33 versus 40% RH).

For the samples of sucrose treated with polyglycosylated polyphenols, only the sample of sucrose treated with 1 wt% rutin showed a difference compared to the control. Here the sucrose crystallized slightly faster than in the control: in four days. This reduction in the stability of amorphous sucrose was not observed in vapor sorption experiments. In those studies, sucrose treated with 1 wt% rutin showed no significant change in the time before crystallization and showed a 5% increase in the RH at which crystallization occurred when RH was increased stepwise. No clear cause for this difference was observed; however, samples stored in desiccators were taken as singlets and it may be possible this observation at day four was either an anomaly or partially crystalline.

Sucrose formulated with monoglycosylated polyphenols were more stable than the control for some treatments. A 1 wt% addition, α-arbutin and phenyl β-D-glucopyranoside produced no change, but at 5 wt% addition both sample types delayed sucrose crystallization by one week relative to the control. Results for samples treated with 5 wt% of these compounds are in line with vapor sorption data, which showed a relative increase in stability of sucrose of 2.7 and 2.0 times respectively when samples were held at 40% RH. For 1 wt% addition of these compounds, crystallization of sucrose was delayed by a factor of 1.8 and 2.4 times respectively, suggesting sucrose treated with 1 wt% phenyl β-D-glucopyranoside would delay crystallization of sucrose in the desiccator study to day 14, not day seven. This difference may be again be explained by the difference in storage RH between the two studies. Further, results for 1 wt%

addition of phenyl β-D-glucopyranoside in the vapor sorption study were somewhat unexpected: 1 wt% addition produced a greater effect than 5 wt% addition. If the measured enhancement of 2.4 times was an overestimate, crystallization of sucrose at day seven in the desiccator study would be expected. Addition of puerarin produced a substantial delay in sucrose crystallization, increasing the time until evidence of sucrose crystallinity was observed to 30 days for formulations of both 1 and 5 wt%.

In line with vapor sorption results, as a group, the bulk food ingredients provided the greatest enhancement in sucrose stability. While cocoa powder and matcha additions at 1 wt% resulted in no change in sucrose crystallization relative to the control, all other formulations delayed crystallization. A 5 wt% addition of green tea extract and green coffee produced the largest delay: samples remained amorphous at the end of the 30 day experiment, even at 33% RH. Formulation with 1 wt% GTE was able to extend amorphous sucrose stability to 30 days. Of all formulations including 1 wt% of additive, only GTE and puerarin demonstrated such an effect.

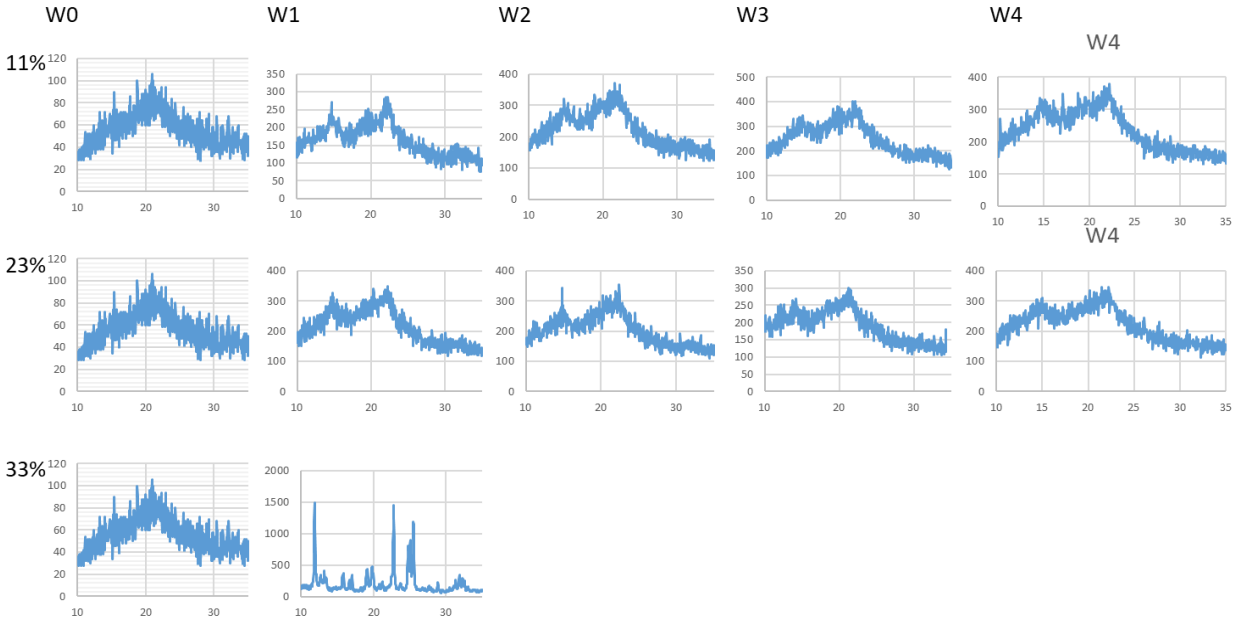


Figure 2.6 Set of diffractograms collected for control sucrose lyophiles stored in desiccators and analyzed weekly

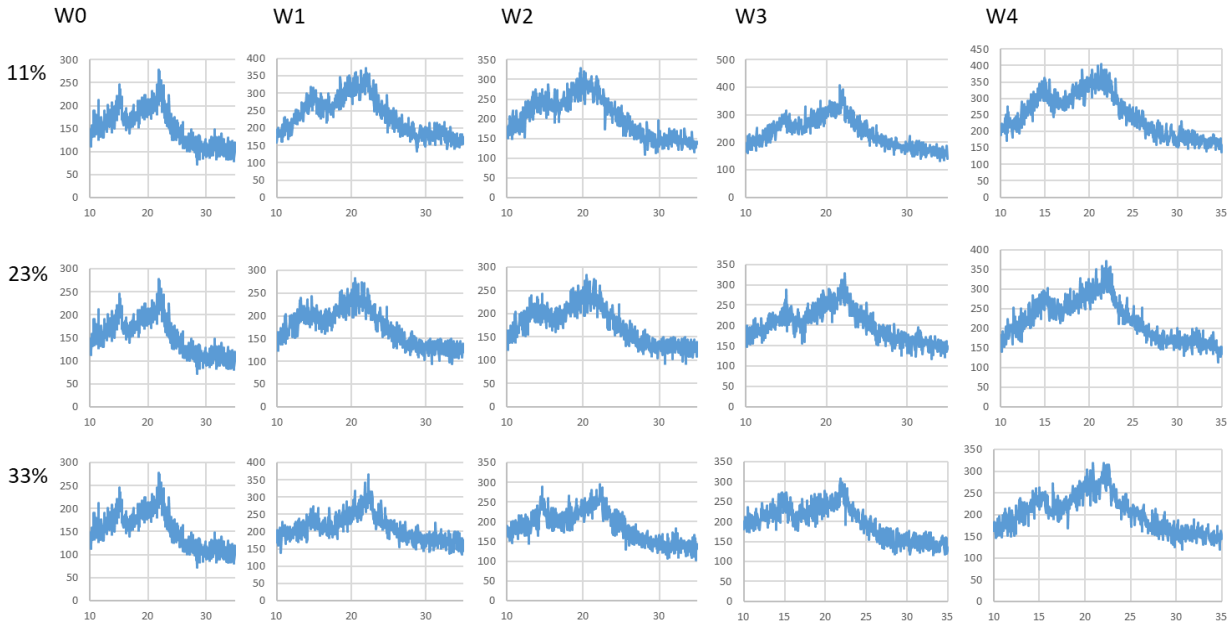


Figure 2.8 Set of diffractograms collected for sucrose treated with 5 wt% green coffee stored in desiccators and analyzed weekly

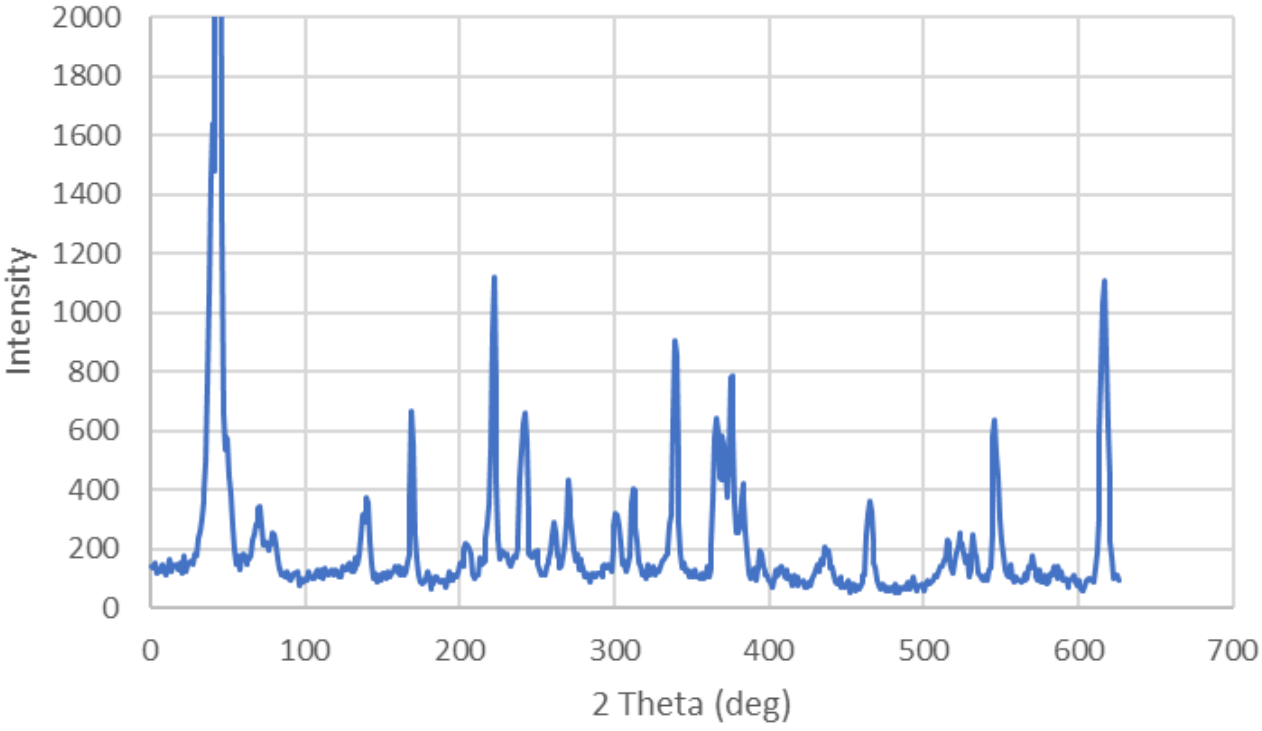


Figure 2.7 Diffractogram of pure crystalline sucrose

Examples of diffractograms collected for single formulations are presented in Figures 2.6 (control) and 7 (sucrose with 5 wt% green coffee). Figure 2.8 shows the diffractogram of pure crystalline sucrose. The clear onset of crystallization in control sucrose lyophiles was first observed after seven days of storage in 33% RH (Figure 2.6**)**. Further diffractograms of the sucrose control were collected for samples stored for one, two, and four days at 33% RH and all were observed to be amorphous. Figure 2.7 shows data collected for one of the formulations found to delay observations of crystallinity most substantially, addition of 5 wt% of green coffee. Here, amorphous patterns are observed for all samples tested. It is worth noting that diffractograms containing significant peaks do not indicate the sample has crystallized completely. Partially crystalline samples will show significant peaks; thus, these results indicate onset, not completion, of crystallization. It is also worth noting that all samples which were observed to be crystalline only showed evidence of peaks representative of sucrose. There was no significant evidence of peaks associated with the polyphenol additives in any sample analyzed.

Table 2.5 Length of time when evidence of crystallization is first observed for desiccator studies

Polyphenol Name	Polyphenol Type	1 wt%			5 wt%		
		11% RH	23% RH	33% RH	11% RH	23% RH	33% RH
Control	None	A	A	Day 7	A	A	Day 7
Apigenin	Nonglycosylated	A	A	Day 4	A	A	Day 14
Quercetin	Nonglycosylated	A	A	Day 7	A	A	Day 14
Daidzein	Nonglycosylated	A	A	Day 7	A	A	Day 7
Resveratrol	Nonglycosylated	A	A	Day 7	A	A	Day 7
α-Arbutin	Monoglycosylated	A	A	Day 7	A	A	Day 14
Puerarin	Monoglycosylated	A	A	Day 30	A	A	Day 30
Phenyl β-D-glucopyranoside	Monoglycosylated	A	A	Day 7	A	A	Day 14
Hesperidin	Polyglycosylated	A	A	Day 7	A	A	Day 7
Rutin	Polyglycosylated	A	A	Day 4	A	A	Day 7
Cocoa Powder	Bulk Food Ingredient	A	A	Day 7	A	A	Day 21
Green Tea Extract	Bulk Food Ingredient	A	A	Day 30	A	A	A
Matcha	Bulk Food Ingredient	A	A	Day 7	A	A	Day 30
Green Coffee	Bulk Food Ingredient	A	A	Day 14	A	A	A

2.4.4 Residual Moisture Content of Lyophilized Samples Stored Under P_2O_5

Residual moisture content of lyophilized sucrose has been reported to be in the range of 0.72 to 1.20% (26). In this study, residual moisture contents were found to be substantially higher despite being stored in desiccator chambers containing P_2O_5 which should have kept the headspaces at an RH close to 0%. `

Data were obtained for this portion of the study in the month of June. At this time the ambient RH was approximately 65% despite a dehumidifier being run continuously in the lab containing the device. While the time between each desiccator chamber being opened and the sample being added to the device was kept to a minimum, there was still 20 to 120 seconds of exposure to atmospheric RH for each sample. Further, due to the manner in which titrant is automatically added by the device, it was not possible to reduce or predict the variation between sample ambient exposure times. It is suspected that the variation in exposure to ambient RH conditions accounts for the majority of the variation in moisture content between and within

Table 2.6 Moisture content on a dry basis of lyophilized samples stored under P_2O_5 for one week

Polyphenol Name	**Polyphenol Type**	**Moisture Content (wt% dry basis)**	
		1 wt%	**5 wt%**
Control	None	1.93 +/- 0.20 (a)	
Apigenin	Nonglycosylated	1.55 +/- 1.00 (a)	2.07 +/- 0.18 (a)
Quercetin	Nonglycosylated	1.54 +/- 0.22 (a)	2.25 +/- 0.70 (a)
Daidzein	Nonglycosylated	1.62 +/- 0.20 (a)	2.05 +/- 0.35 (a)
Resveratrol	Nonglycosylated	2.12+/- 0.40 (a)	1.77 +/- 0.46 (a)
α-Arbutin	Monoglycosylated	1.86 +/- 0.26 (a)	2.03 +/- 0.36 (a)
Puerarin	Monoglycosylated	2.59 +/- 0.89 (a)	1.99 +/- 0.33 (a)
Phenyl β-D-glucopyranoside	Monoglycosylated	1.91 +/- 0.32 (a)	2.76 +/- 1.69 (a)
Hesperidin	Polyglycosylated	2.02 +/- 0.74 (a)	2.40 +/- 0.65 (a)
Rutin	Polyglycosylated	2.67 +/- 1.02 (a)	1.77 +/- 0.46 (a)
Cocoa Powder	Bulk Food Ingredient	2.77 +/- 0.20 (a)	2.34 +/- 0.74 (a)
Green Tea Extract	Bulk Food Ingredient	2.09 +/- 0.28 (a)	1.65 +/- 0.17 (a)
Matcha	Bulk Food Ingredient	3.36 +/- 0.59 (a)	2.59 +/- 0.54 (a)
Green Coffee	Bulk Food Ingredient	3.62 +/- 1.57 (a)	3.16 +/- 1.19 (a)

samples leading to the statistically insignificant results reported. Anecdotally, it was observed that some samples which were exposed to ambient RH conditions for longer periods of time were found to contain higher moisture contents than those exposed to lesser times; one sample left out for approximately 5 minutes before testing measured 4.85% compared to an average of 1.85% for other samples taken from the same batch of material which were exposed to ambient conditions for a shorter period of time.

Moisture loss in drying steps in vapor sorption profiling (Figure 2.3) ranged from 1.0 to 1.6 wt%. These samples were measured when ambient RH was approximately 40% and were exposed to ambient conditions for longer than samples tested through KF, approximately five minutes. Similarly, previously studied samples of sucrose and naringin and sucrose and glcyrrhizic acid were found to have moisture contents in the range of 1.79 to 2.07 wt% when samples were prepared in a glove box held at close to 0% RH (26).

2.4.5 Glass Transition Temperature of Lyophilized Samples

The T_g of dry sucrose has been reported to be in the range of 52 to 72.9 °C (21). In this study, observed T_gs were found to be significantly lower than this range. The presence of water will decrease the T_g of sucrose due to its comparatively low T_g of -137°C (34). This relationship is described by the Gordon-Taylor equation which is given in Equation 2.1 (25). Thus, this decrease in the observed T_g of the control relative to reported values likely indicates control samples were not dry despite piercing the pan prior to analysis. Since all samples were prepared in the same manner, it is likely all measured samples contained some quantity of water. This explains the high variability and lack of statistical significance. While pans were pierced to allow

$$T_{g,mix} \approx \frac{w_1 T_{g,1} + K w_2 T_{g,2}}{w_1 + K w_2} \qquad \text{Equation 2.1}$$

residual water to boil off to more accurately measure dry T_g, the maximum temperature the samples were exposed to was 100°C. Further, samples were immediately cooled once this temperature was reached. A higher temperature and/or a hold at this temperature could have boiled off more water; however, this risked inducing crystallization of the amorphous sucrose, which drove the selection of the specific protocol used. This water content is likely the result of moisture uptake from the atmosphere while pans were being loaded.

Table 2.7 Dry T_g of lyophilized samples stored under P_2O_5 for one week

Polyphenol Name	**Polyphenol Type**	**Glass Transition Temperature (onset, °C)**	
		1 wt%	**5 wt%**
Control	None	40.1 +/- 3.3 (a)	
Apigenin	Nonglycosylated	46 +/- 4.8 (a)	35.2 +/- 5.0 (a)
Quercetin	Nonglycosylated	45.5 +/- 2.5 (a)	38.9 +/- 10.3 (a)
Daidzein	Nonglycosylated	42.2 +/- 7.6 (a)	47.4 +/- 4.8 (a)
Resveratrol	Nonglycosylated	47.4 +/- 2.7 (a)	38.9 +/- 5.6 (a)
α-Arbutin	Monoglycosylated	45.4 +/- 0.3 (a)	44.6 +/- 5.0 (a)
Puerarin	Monoglycosylated	31.9 +/- 7.9 (a)	40.5 +/- 7.5 (a)
Phenyl β-D-glucopyranoside	Monoglycosylated	47.1 +/- 3.2 (a)	44.9 +/- 6.6 (a)
Hesperidin	Polyglycosylated	38.3 +/- 6.0 (a)	43.3 +/- 6.3 (a)
Rutin	Polyglycosylated	43.9 +/- 7.0 (a)	46.5 +/- 2.4 (a)
Cocoa Powder	Bulk Food Ingredient	40.2 +/- 5.7 (a)	37.0 +/- 5.5 (a)
Green Tea Extract	Bulk Food Ingredient	41.3 +/- 4.5 (a)	48.4 +/- 5.0 (a)
Matcha	Bulk Food Ingredient	39.4 +/- 7.0 (a)	45.6 +/- 2.9 (a)
Green Coffee	Bulk Food Ingredient	41.1 +/- 9.1 (a)	49.4 +/- 3.2 (a)

Figures 2.9, 10, and 11 each present a DSC scans for three samples. Figure 2.9 shows the samples run in triplicate for the pure sucrose control, Figure 2.10 shows the samples run in triplicate for sucrose treated with 5 wt% green tea extract, and Figure 2.11 shows the samples run

in triplicate for sucrose treated with 1 wt% quercetin. Due to the variability between samples of the same treatment, comparing the control to sucrose treated with either additive revealed no clear trends. For sucrose treated with 1 wt% quercetin, the general shape of the endothermic response was fairly consistent; however, for the sucrose control and sucrose treated with 5 wt% green tea extract, the shape of this response varied sample by sample.

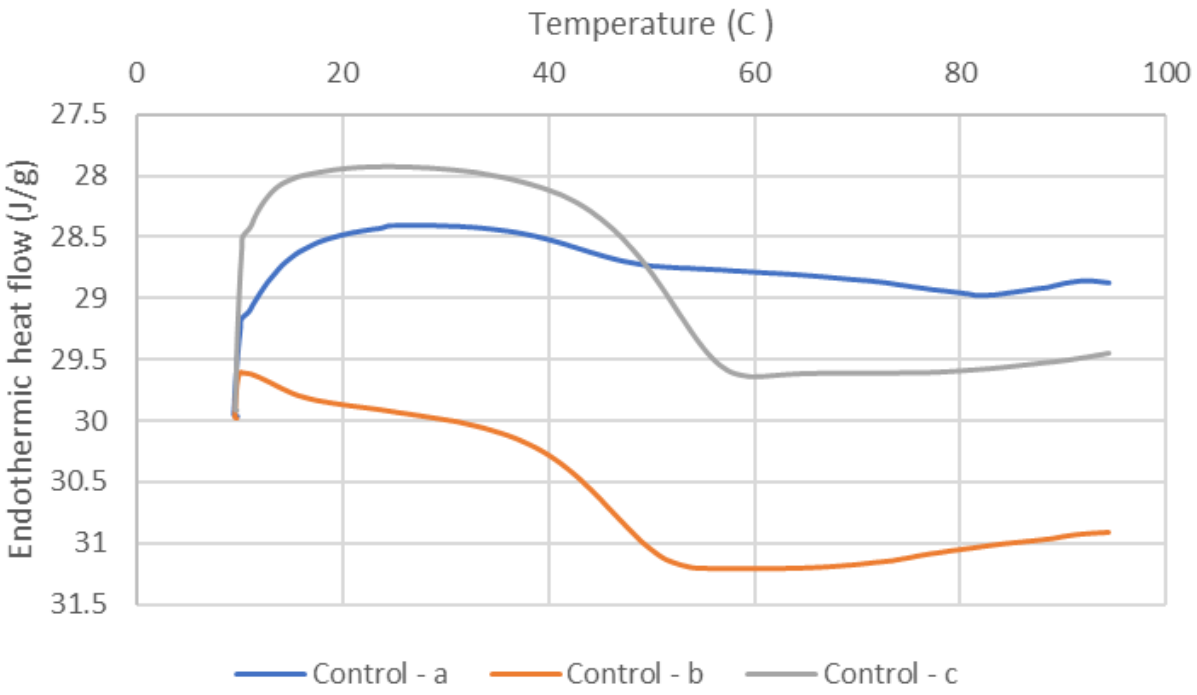


Figure 2.9 DSC scans for amorphous sucrose control lyophile stored under P_2O_5

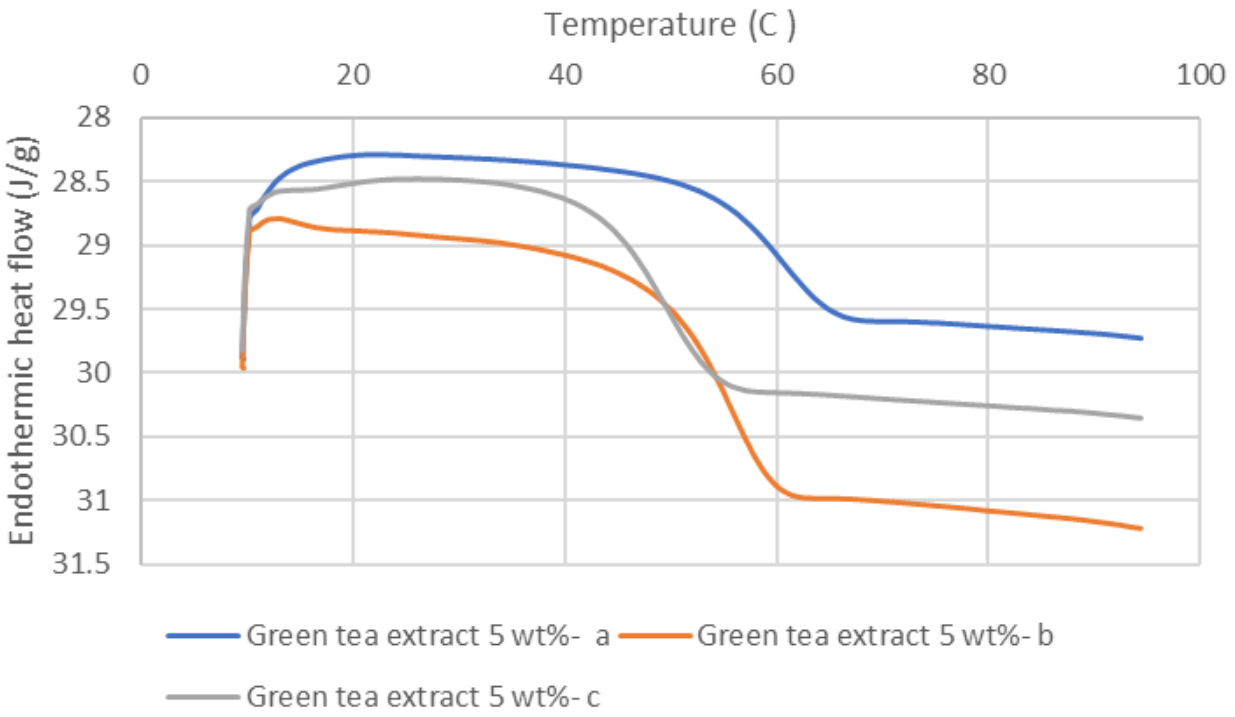


Figure 2.11 DSC scans for amorphous sucrose lyophile treated with 5 wt% green tea extract stored under P_2O_5

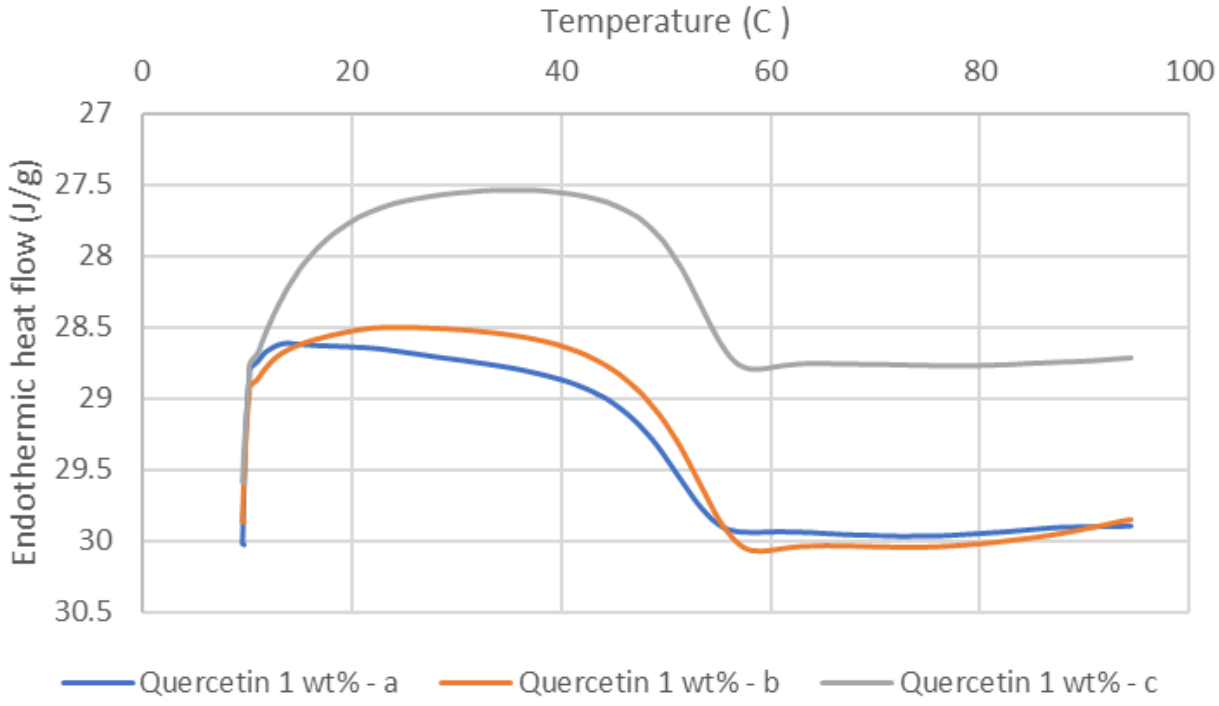


Figure 2.10 DSC scans for amorphous sucrose lyophile treated with 1 wt% quercetin stored under P_2O_5

2.4.6 Effect of Polyphenol Skeleton and The Number of Sugar Units on Crystallization Rate

One primary focus of this work was to better understand how the different polyphenol backbones affected crystallization of the sucrose. This was the motivation for selecting a few polyphenol classes for each number of sugar side groups. Ideally, at least one of each polyphenol class (flavone, flavonoid, flavanone, etc.) would be represented for each number of sugar side groups. Unfortunately, due to the low relative abundance of many of these forms in natural products and the difficulty and cost of purification or synthesis of these compounds, many were cost prohibitive for this study. While the present set of data does not contain as many compounds as would be necessary for more complete conclusions to be drawn, some conclusions and comparisons may be made.

Table 2.8 Structural features of naringin, glycyrrhizic acid, hesperidin and rutin

Compound	Terminal sugar unit	Non terminal sugar unit	Linkage
Naringin	α-L-rhamnopyranose	β-D-glucopyranose	2-1
Glycyrrhizic Acid	β-D-glucuronic acid	α-D-glucuronic acid	2-1
Hesperidin	α-L-rhamnopyranose	β-D-glucopyranose	6-1
Rutin	α-L-rhamnopyranose	β-D-glucopyranose	6-1

A provisional patent application by Thorat, et al. (19) showed that at 33% RH and 25°C, naringin was able to delay crystallization of sucrose to 21 days when added at 1 wt%. At 5 wt%, sucrose remained amorphous after 30 days. In this work, the pure sucrose control crystallized after seven days. Naringin contains a similar polyphenol backbone and identical sugar units to rutin and hesperidin with the primary differences being the linkage between sugars and functionality of the polyphenol. While naringin delayed sucrose crystallization, hesperidin and rutin did not. The aglycone of naringin is slightly less polar than both hesperidin and rutin which would be expected to enhance amorphous sucrose stability, however, this effect is likely minimal as the variations in functionality are minor when compared to the overall structure. It is proposed

the difference in linkage sites between the sugar units produced the observed differences. 6-1 linkages are known to be more flexible than 2-1 linkages (41). For all three of these compounds, the non terminal sugar unit is more structuraly similar to sucrose than the terminal unit. If the 2-1 linkage orients the terminal sugar in such a way that the non terminal sugar is more exposed, this may lead to a greater extent of interaction between the glycoside and sucrose, resulting in a higher stability of the amorphous state. This theory could be tested by repeating the experiments with a series of compounds that share an identical aglycone and sugar units and differ only in the linkage between sugar units.

Examining the monoglycosylated compounds studied, all produced delays in the crystallization of sucrose. While the sugar unit associated with puerarin is the least structurally similar to sucrose, addition of this compound produced the greatest delay in amorphous sucrose

Table 2.9 Structural features of puerarin, α-arbutin, and phenyl β-D-glucopyranoside

Compound	Sugar unit
Puerarin	1,5-anhydroglucitol
α-Arbutin	α-D-glucopyranose
Phenyl β-D-glucopyranoside	β-D-glucopyranose

crystallization of the three. Phenyl β-D-glucopyranoside and α-arbutin both contain phenol backbones while puerarin's aglycone is a polyphenol. It is proposed the greater effect observed for puerarin is a result of the larger size of the less-polar backbone.

Three direct comparisons between glycosylated polyphenols and aglycones can be made. Daidzein is the aglycone of puerarin, quercetin is the aglycone of rutin, and apigenin is the aglycone of naringin. While daidzein did not affect the rate of sucrose crystallization in this study, puerarin consistently delayed crystallization. Similarly, apigenin did not delay

crystallization while naringin did. Rutin, however, did not delay crystallization, nor did quercetin. It is proposed that, while sugar groups are necessary for polyphenols to delay amorphous sucrose crystallization, the specific unit and linkages between units are of importance. Specifically, it appears polyphenols with glucose units produced more of a delay than those containing rhamnose. For polyglycosylated polyphenols, it appears a 2-1 linkage allowed for the non-terminal sugar unit to interact with sucrose more significantly than a 6-1.

It is interesting to note that green tea extract, the additive that produced the most significant delays in sucrose crystallization of the materials tested, is essentially a blend of primarily nonglycosylated polyphenols. The package lists epigallocatechin gallate (ECG) as making up 51 wt% of the product. ECG does not contain any sugar side groups, though there are some known glycosylated catechins (38). The compound does contain a large number of hydroxyl groups which may allow for interaction with sucrose. Extract delayed crystallization more significantly than matcha which indicates the polyphenols are more responsible for this delay than the other components in green tea. Cocoa powder contains glycosylated forms of quercetin, specifically quercetin-3-O-α-D-arabinoside and quercetin-3-O-β-glucopuranoside, though the relative abundance of these forms is unclear (14). Rutin, quercetin-3-rutinoside, is structurally similar to these compounds. Green coffee's primary polyphenol constituent, chlorogenic acid, does not contain a sugar side group, but does contain a quinic acid group, which is somewhat structurally similar to a sugar (Figure 2.12) (17, 39). It may be worthwhile to

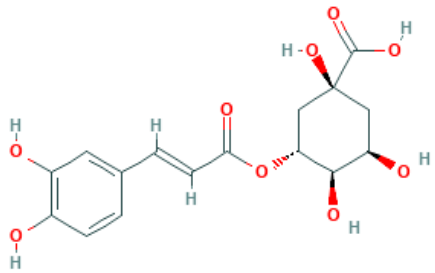


Figure 2.12 Structure of chlorogenic acid (39)

explore whether this and other quinic acid containing compounds produce an effect on the crystallization of sucrose when used as an isolated compound. It is difficult to make any claims about the effectiveness of any of these polyphenol constituents specifically as these products all contain a large number of compounds other than polyphenols. In future work, it would be worthwhile to study the effects of isolated polyphenols found in green coffee and cocoa powder on the crystallization of sucrose to determine whether these enhancements in the stability of the amorphous state are from the polyphenols or other constituents.

Comparing the results found in this work to previous research, the most effective classes of additives, bulk food ingredients and monoglycosylated polyphenols, produced delays in the crystallization of sucrose which were similar to the most effective additives reported in other studies (10, 18) when stored at 33% RH and 25°C. It is proposed that sugar units are necessary to produce delays in the crystallization of amorphous sucrose, provided the sugar units are structurally similar to sucrose and are not sterically hindered. Since polyphenols are generally viewed positively by the public and are associated with numerous health benefits, these compounds may be useful to increase shelf life in foods where amorphous sucrose is desired.

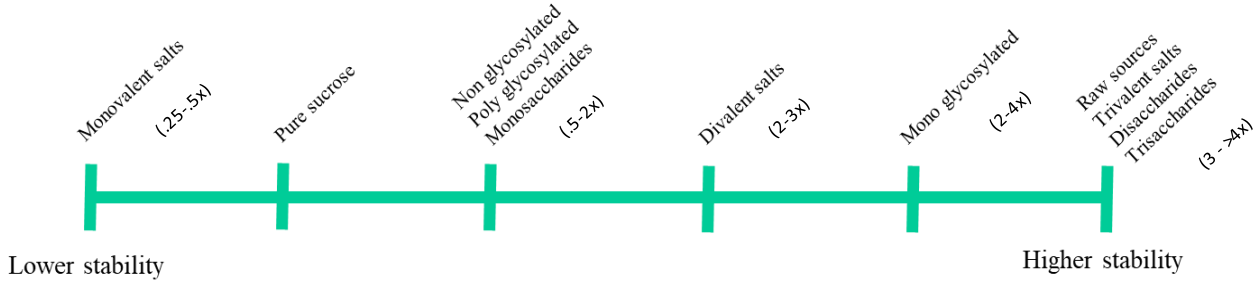


Figure 2.13 Comparison of the relative effects of additives on the stability of amorphous sucrose

2.5 Conclusions

In this chapter, the effects of polyphenols on the stability of amorphous sucrose were investigated. Four aglycones, three monoglycosylated, and two polyglycosylated polyphenols were studied at both 1 and 5 wt% of additive relative to sucrose. Four natural food sources rich in polyphenols were also included in this work. Of these materials, the natural food sources and monoglycosylated polyphenols provided the greatest increase in stability, increasing the lifetime of amorphous sucrose by a factor of 2.0 to greater than 6.4 when formulated at 5 wt%. For 1 wt% addition of these ingredients stability was increased by a factor of 1.7 to 2.4 times. In the context of previous work, general trends are provided in Figure 2.13. It is suspected that the structure of these compounds controls to what extent sucrose crystallization is delayed. Compounds where the aglycone portion of the polyphenol is a phenol perform worse than those where the aglycone is a polyphenol, likely due to their smaller size. Polyphenols with more than one attached sugar units delayed crystallization when the link between sugars was a 2-1 and did not when the linkage was 6-1. For these compounds, the flexibility of the 2-1 linkage likely allowed for a greater degree of association between the polyphenol and sucrose. These insights are valuable for guiding formulation decisions for powdered beverages. Though many of these materials listed may not make ideal ingredients in many food products, some ingredients such as matcha, coffee, and cocoa powder are already food ingredients common in beverages. Green tea extract may also be a potentially relevant additive as it would also convey potential health benefits and is viewed favorably by the public. Use of pure polyphenols may be less ideal food additives due to their bitter taste. Through this work, better understanding of interactions between polyphenols and amorphous sucrose was achieved which may lead to products with higher quality and shelf life.

2.6 References

(1) Kinugawa, Kohshi, et al. "Inhibitory Effects of Additives and Heat Treatment on the Crystallization of Freeze-Dried Sugar." *Journal of Food Engineering*, vol. 155, 2015, pp. 37–44., doi:10.1016/j.jfoodeng.2015.01.016.

(2) Barnstead™ E-Pure™ Ultrapure Water Purification Systems. Thermo Fisher Scientific, www.thermofisher.com/order/catalog/product/D4631.

(3) Arioglu-Tuncil, S., Bhardwaj, V., Taylor, L. S., & Mauer, L. J. (2017). Amorphization of thiamine chloride hydrochloride: A study of the crystallization inhibitor properties of different polymers in thiamine chloride hydrochloride amorphous solid dispersions. *Food Research International*, 99(March), 363–374. http://doi.org/10.1016/j.foodres.2017.05.021

(4) Murase, N., et al. "The Structural States of Freeze-Concentrated and Freeze-Dried Phosphates Studied by Scanning Electron Microscopy and Differential Scanning Calorimetry." *Cryobiology*, vol. 28, no. 4, 1991, pp. 364–375., doi:10.1016/0011-2240(91)90043-n.

(5) Tuberoso, C. I. G., & Orrù, C. D. (2008). Phenolic compounds in food. Progress in Food Chemistry, (4), 1–45. http://doi.org/10.1007/s00394-008-2002-2

(6) "Hesperidin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/hesperidin#section=Top.

(7) "Quercetin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/quercetin#section=Top.

(8) "Daidzein." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/daidzein.

(9) "Rutin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/rutin#section=Top.

(10) Thorat, A. A., Forny, L., Meunier, V., Taylor, L. S., & Mauer, L. J. 2017. Effects of Mono-, Di-, and Tri-saccharides on the Stability and Crystallization of Amorphous Sucrose. Manuscript submitted for publication.

(11) "Phenyl Beta-D-Glucopyranoside." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Phenylglucoside#section=Top.

(12) "4-Hydroxyphenyl-Alpha-D-Glucopyranoside." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/alpha-Arbutin#section=Top.

(13) "Puerarin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Puerarin.

(14) Rimbach, G., Melchin, M., Moehring, J., & Wagner, A. E. (2009). Polyphenols from cocoa and vascular health - A critical review. International Journal of Molecular Sciences, 10(10), 4290–4309. http://doi.org/10.3390/ijms10104290

(15) Smith, T. J. (2012). Green Tea Polyphenols in drug discovery - a success or failure? Expert Opinion on Drug Discovery, 6(6), 589–595. http://doi.org/10.1517/17460441.2011.570750.Green

(16) Graham, H. N. (1992). Green tea composition, consumption, and polyphenol chemistry. Preventive Medicine, 21(3), 334–350. http://doi.org/10.1016/0091-7435(92)90041-F

(17) Madhava Naidu, M., Sulochanamma, G., Sampathu, S. R., & Srinivas, P. (2008). Studies on extraction and antioxidant potential of green coffee. Food Chemistry, 107(1), 377–384. http://doi.org/10.1016/j.foodchem.2007.08.056

(18) Thorat, A. A., Forny, L., Meunier, V., Taylor, L. S., & Mauer, L. J. (2017). Effects of Chloride and Sulfate Salts on the Inhibition or Promotion of Sucrose Crystallization in Initially Amorphous Sucrose-Salt Blends. Journal of Agricultural and Food Chemistry, 65(51), 11259–11272. http://doi.org/10.1021/acs.jafc.7b04746

(19) Taylor, L.S., L.J. Mauer, and A. Thorat. 2017. Inhibition of sucrose crystallization using glycosides. Provisional Patent PRF 68033-01. Application No. 62580690.

(20) Shamblin, Sheri L., et al. "The Effects of Co-Lyophilized Polymeric Additives on the Glass Transition Temperature and Crystallization of Amorphous Sucrose." *Journal of Thermal Analysis*, vol. 47, no. 5, 1996, pp. 1567–1579., doi:10.1007/bf01992846.

(21) Roe, K. D., and T. P. Labuza. "Glass Transition and Crystallization of Amorphous Trehalose-Sucrose Mixtures." *International Journal of Food Properties*, vol. 8, no. 3, Jan. 2005, pp. 559–574., doi:10.1080/10942910500269824.

(22) Voelker, Adrienne L., et al. "Chemical Stability and Reaction Kinetics of Two Thiamine Salts (Thiamine Mononitrate and Thiamine Chloride Hydrochloride) in Solution." *Food Research International*, vol. 112, 2018, pp. 443–456., doi:10.1016/j.foodres.2018.06.056.

(23) Hartel, Richard W., et al. *Confectionery Science and Technology*. Springer International Publishing, 2018.

(24) Roos, Y H., et al. "Glass Transitions in Low Moisture and Frozen Foods: Effects on Shelf Life and Quality." *Food Technology*, vol. 50, no. 11, Nov. 1996, pp. 95–108.

(25) Hancock, B. C., & Zografi, G. (1994). The Relationship Between the Glass Transition Temperature and the Water Content of Amorphous Pharmaceutical Solids. *Pharmaceutical Research*. https://doi.org/10.1023/A:1018941810744

(26) Kamat, M. S., et al. Near-infrared spectroscopic determination of residual moisture in lyophilized sucrose through intact glass vials. *Pharmaceutical Research,* Vol. 6, no. 11, 1989.

(27) "Apigenin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Apigenin.

(28) "Resveratrol." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Resveratrol.

(29) Vang, Ole, et al. "What Is New for an Old Molecule? Systematic Review and Recommendations on the Use of Resveratrol." *PLoS ONE,* vol. 6, no. 6, 2011, doi:10.1371/journal.pone.0019881.

(30) Corriher, Shirley. *Learn How to Control Crystallization for Successful Sweets*. Fine Cooking, 1 Dec. 1999, www.finecooking.com/article/learn-how-to-control-crystallization-for-successful-sweets.

(31) Gabarra, P., and R. W. Hartel. "Corn Syrup Solids and Their Saccharide Fractions Affect Crystallization of Amorphous Sucrose." *Journal of Food Science*, vol. 63, no. 3, 1998, pp. 523–528., doi:10.1111/j.1365-2621.1998.tb15778.x.

(32) Bell, Leonard N., and Theodore P. Labuza. *Moisture Sorption: Practical Aspects of Isotherm Measurement and Use*. American Association of Cereal Chemists, 2000.

(33) Burnett, D.j., et al. "Determining the Critical Relative Humidity for Moisture-Induced Phase Transitions." *International Journal of Pharmaceutics*, vol. 287, no. 1-2, 2004, pp. 123–133., doi:10.1016/j.ijpharm.2004.09.009.

(34) Slade, Louise, et al. "Beyond Water Activity: Recent Advances Based on an Alternative Approach to the Assessment of Food Quality and Safety." *Critical Reviews in Food Science and Nutrition*, vol. 30, no. 2-3, 1991, pp. 115–360., doi:10.1080/10408399109527543.

(35) Yu, X., Kappes, S. M., Bello-Perez, L. A., & Schmidt, S. J. (2008). Investigating the Moisture Sorption Behavior of Amorphous Sucrose Using a Dynamic Humidity Generating Instrument. Journal of Food Science, 73(1), E25-E35. doi: 10.1111/j.1750-3841.2007.00596.x

(36) "Average Monthly Humidity in Indiana, United States of America." *World Weather and Climate Information*, weather-and-climate.com/average-monthly-Humidity-perc,indiana-pennsylvania-us,United-States-of-America.

(37) "Climate and Average Monthly Weather in: Phoenix, United States of America." *World Weather and Climate Information*, weather-and-climate.com/average-monthly-Rainfall-Temperature-Sunshine,phoenix,United-States-of-America.

(38) Plumb, Geoffrey W., et al. "Antioxidant Properties of Catechins and Proanthocyanidins: Effect of Polymerisation, Galloylation and Glycosylation." *Free Radical Research*, vol. 29, no. 4, 1998, pp. 351–358., doi:10.1080/10715769800300391.

(39) "Chlorogenic acid." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/chlorogenic_acid#section=Top.

(40) Smoliga, James M., et al. "Resveratrol and Health - A Comprehensive Review of Human Clinical Trials." *Molecular Nutrition & Food Research*, vol. 55, no. 8, 2011, pp. 1129–1141., doi:10.1002/mnfr.201100143.

(41) Javaroni, Fábio, et al. "The (α-1,6) Glycosidic Bond of Isomaltose: a Tricky System for Theoretical Conformational Studies." *Carbohydrate Research*, vol. 344, no. 10, 2009, pp. 1235–1247., doi:10.1016/j.carres.2009.04.022.

CHAPTER 3. ATTEMPTS TO PRODUCE NEW SALTS OF THIAMINE AND CHARACTERIZATION OF A KEY INTERMEDIATE, TCL·H_2O

3.1 Abstract

Thiamine is an essential nutrient that is naturally found in whole grains and other food products. Two salt forms of thiamine are available commercially: thiamine mononitrate (TMN) and thiamine chloride hydrochloride (TClHCl). The degradation rate of these thiamine forms is known to be a function of temperature, relative humidity, pH, formulation, and exposure to light. The anion associated with the thiamine is also known to affect stability. Thus, it is possible alternative salts of thiamine with different anions may have favorable properties in regard to shelf life and/or processing and storage attributes. The objective of this chapter is twofold: to describe a method developed to prepare alternate salt forms of thiamine, and to characterize an important intermediate in this reaction, thiamine chloride monohydrate (TCl·H_2O). TCl·H_2O has properties which differ from both TClHCl and TMN: its solubility of 459 mg/mL is intermediate between the two, and its stability in aqueous solutions is less than both. Activation energies observed for degradation ranged from 52 to 73 kJ/mol. Two approaches were taken to generate new forms: double replacement reactions and a method making use of silver nitrate. As of the publication of this work, no new salts have been successfully isolated through the double replacement reaction route. Five new salts have been produced through the use of the silver method; however, characterization of these new forms is limited while the synthesis is being developed and material quantities and purity are limited.

3.2 Introduction

Thiamine deficiency is an issue for both developing and developed nations. Inadequate food supply can lead to a thiamine deficiency, as can alcoholism (alcoholism reduces the body's ability to absorb thiamine). Severe side effects of thiamine deficiency include nerve damage, fatigue, and in extreme cases, death (4). Recommended daily allowance for thiamine intake in med is 1.2 mg/day and for women is 1.1 mg/day (12).

Thiamine is found most plentifully in the bran of whole grains, in pork, and in legumes (10). Processing of grains, either by removing the bran or through the application of heat, substantially reduces thiamine content. As a result, flours used for baked goods are often fortified with thiamine (5). An overview of the thiamine content of various foods is presented in Table 3.1.

Table 3.1 Thiamine content of natural foods, adapted from reference 10

Food	Thiamine content (μg/100g)
Wheat germ	2050
Dried brewer's yeast	1820
Soybeans	1300
Pork	600-950
Dried beans and peas	680
Dried milk whey	500
Nuts	300-560
Brown rice	300
Beef liver	300
Potatoes	170
Fish	50-90
Eggs	70
Vegetables	60-70
Whole milk	30-70
White rice	50

Thiamine is sold commercially in two salt forms; mononitrate and chloride hydrochloride. While the stability of TMN in some food systems, particularly solid foods, is higher than that of TClHCl (8), it has a low aqueous solubility and is still sensitive to a number of conditions regularly encountered in food systems (ultraviolet exposure, elevated temperature, etc.). Thus, new salts of thiamine with higher stability might increase the shelf life of food products which presently include TMN. Additionally, eliminating another source of nitrates added to foods may be viewed favorably by the public (2). TClHCl has a substantially higher aqueous solubility but is much more prone to degradation in solid food matrices (8). A number of anions relevant to food systems were selected for investigation. Sodium salts of these anions, such as sodium saccharine, sodium ascorbate, and trisodium citrate, were combined with thiamine chloride in water and precipitated with the addition of acetone and refrigeration. Some organic acids such as lactic and sulfuric acid were also tested in a similar manner. Following limited success with this method, a new method was developed which makes use of silver nitrate. At this time, five new forms have been isolated using this method.

3.2.1 Properties of Commercially Available Thiamine Salts

At the time of publication two forms of thiamine are commercially available as food ingredients. Both are salts, one, thiamine chloride hydrochloride and the other, thiamine

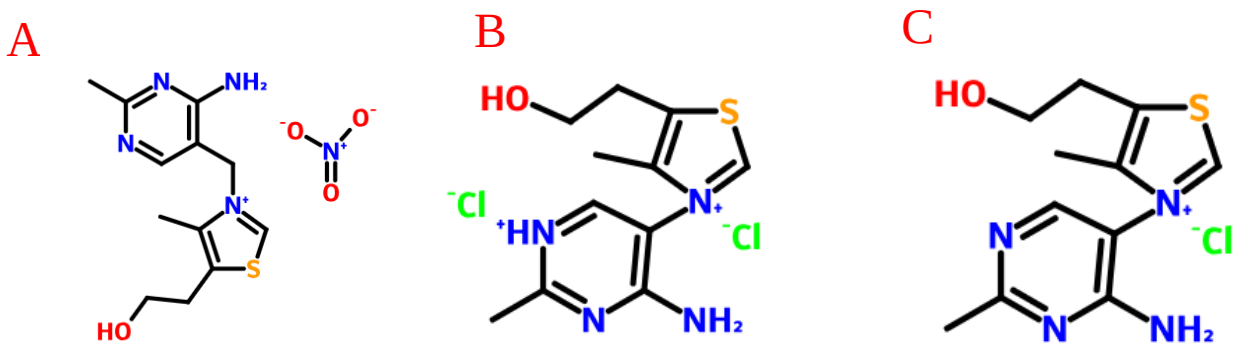


Figure 3.1 Structures of thiamine salt forms
A: Thiamine mononitrate (18)
B: Thiamine chloride hydrochloride (19)
C: Thiamine chloride (20)

mononitrate. The properties of each are quite distinct, leading to differing uses in food products. Generally, TClHCl is more often used in beverages while TMN is more often used in dry goods. These differences in use are driven by differences in their hygroscopicity, solubility, and stability. A summary of some properties of these salts is given in Table 3.2.

Table 3.2 Properties of crystalline TMN, TClHCl, and TCl·H_2O

	Thiamine Mononitrate	Thiamine Chloride Hydrochloride	Thiamine Chloride Monohydrate
Molecular Mass (g/mol)	327.36	337.26	318.82
Melting point (°C)	196-200 (10)	248-250 (10)	135-137
Aqueous Solubility @25°C (g/L)	27 (10)	570 (11)	459
pH in dilute aquous solution	6.5-7 (10)	3-3.5 (10)	7.8-8.1
Bulk density (kg/L)	0.5 (10)	0.4 (10)	No Data
Deliquesence point (%RH)	98.5 (11)	88 (10)	No Data

TClHCl is a di-salt, containing two points of charge, one on the pyrimidine ring and the other on the thiazole ring. The pK_a's of these two nitrogens are 4.8 and 9.2, respectively. Since most food products are neutral or slightly acidic, the pK_{a1} is the more relevant parameter. The use of TClHCl in beverage applications is motivated by its higher aqueous solubility, approximately 20 times that of TMN. The lower pH of TClHCl solutions also leads to a greater stability (11). It is worth noting that the solubility of TClHCl is often reported as greater than 1 g/mL. While 1 gram of TClHCl can be dissolved by 1 mL of water, the volume of TClHCl added is not negligible and thus the solubility of a saturated TClHCl solution is 570 mg of TClHCl per mL of solution (11).

Aqueous solubility of a crystalline material is related to the free energy of the crystal. One way to think about this is as follows: consider a single TClHCl molecule and a crystal of TClHCl spaced infinitely apart such that the extent of interaction between the two is zero. If the single molecule were physically brought to the crystal and allowed to add to the crystal, a certain

amount of energy would be released since the system is now more stable. If the same scenario were to occur with TMN, a greater quantity of energy would be released. When in solution, this greater stabilizing effect lends a greater driving force for TMN to remain as a crystal and thus reduces the likelihood of dissolution and thus lowers the solubility.

While TClHCl's higher aqueous solubility makes it a more ideal ingredient in beverages, it suffers from a lesser stability than TMN in model food systems. Labuza and Kamman (8) compared the stability of TMN and TClHCl when added to semolina flour and water. The dough was vacuum sealed and held at temperatures ranging from 70 to 95°C. Samples were taken over time and the quantity of undegraded thiamine was determined. From this, reaction kinetics were calculated. Results from this study are presented below in Table 3.3. These results indicate there are significant differences in the reaction kinetics when comparing TMN and TClHCl. At a given temperature and a_w, the activation energy for degradation is lower for TClHCl than for TMN, leading to a lower half life.

Table 3.3 Kinetic constants for thiamine loss in semolina dough, adapted from reference 8

Salt Form	a_w	Temperature (°C)	k (min-1)	r^2	Half Life (min)	Ea (kcal/mol)
TClHCl	0.58	75	3.72 +/- 0.01	0.986	1863	
		85	11.41 +/- 3.64	0.928	607	
		95	22.45 +/- 2.57	0.994	309	22.8
	0.86	75	5.35 +/- 2.57	0.888	1295	
		85	12.20 +/- 4.45	0.913	568	
		95	30.45 +/- 8.91	0.941	228	22.0
TMN	0.58	75	2.88 +/- 0.01	0.960	2406	
		85	7.91 +/- 0.01	0.993	876	
		95	22.69 +/- 2.57	0.990	305	26.1
	0.86	75	2.94 +/- 0.01	0.993	2357	
		85	8.31 +/- 0.01	0.997	834	
		95	23.89 +/- 0.01	0.999	290	26.5

3.2.2 Production of Commercially Available Thiamine Salts

While thiamine can be isolated from natural sources, at an industrial scale this is not cost efficient. Thus, production of thiamine generally follows one of two routes. One route constructs the thiazole ring onto a preformed pyrimidine intermediate. This method has been in use since 1937 (9). The other synthesizes the thiazole and pyrimidine rings individually and then binds the two together. This method was developed a year earlier in 1936 (6). An overview of synthetic routes to produce the thiazole used in the second method was compiled by David Burdick (10) and is presented in Figure 3.2. Each pathway has its own advantages and disadvantages related to cost, difficulty, and waste stream. Typically, the end product of each of these syntheses is TClHCl. To produce TMN, TClHCl is neutralized with base, nitric acid is added, and TMN is precipitated (10).

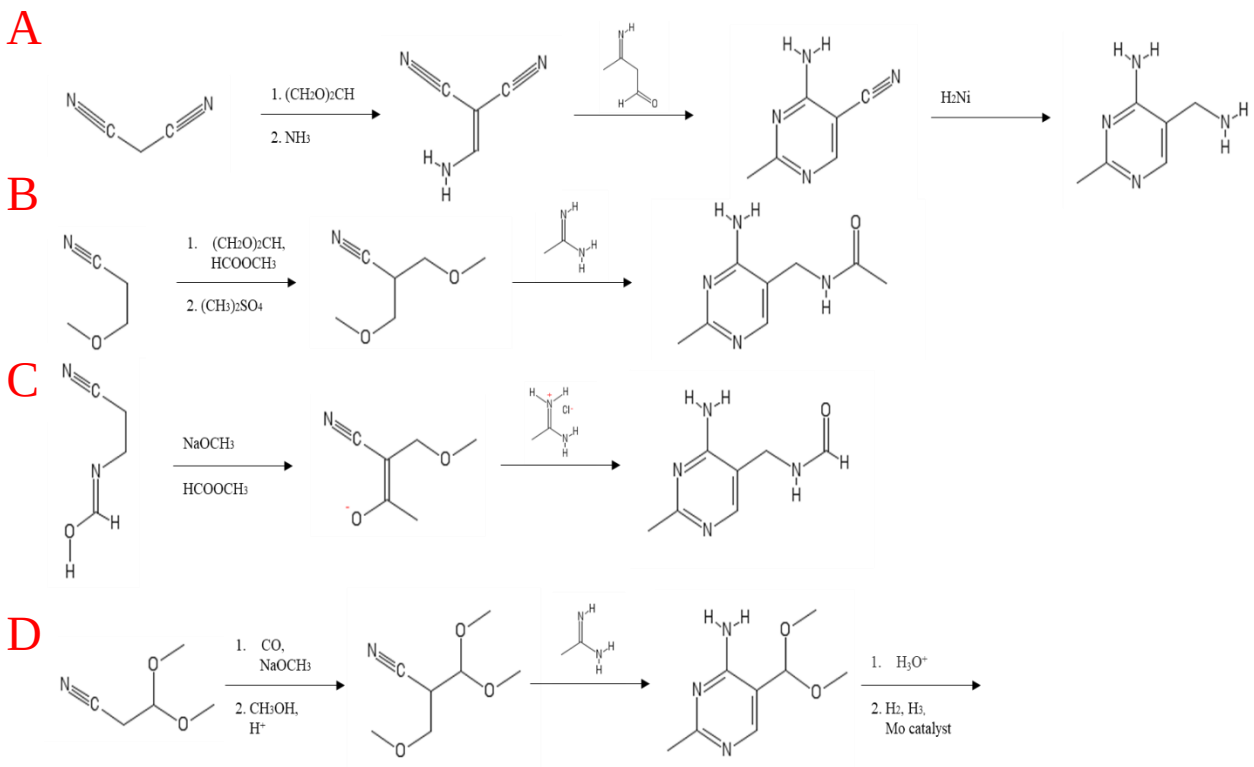


Figure 3.2 A selection of synthetic routes to produce thiamine, adapted from reference 10
A: Roche process
B: Shionogi process

3.2.3 X-Ray Crystallography

See Section 1.8.2 for information of X-ray crystallography as a general concept. In this chapter, scXRD was used in addition to pXRD. Powder XRD can be a more convenient measurement tool compared to scXRD as materials are often initially grown as small crystals early on in the development process. Powder XRD can also detect the presence of a new form even if it does not make up the entirety of the powder sample. Single crystal XRD requires crystals with a characteristic length of at least 50 µm, ideally 150 µm or more (3). As the growth of large crystals requires an understanding of the crystallization process or at least a bit of luck, it can be difficult to obtain crystals suitable for analysis by scXRD early on in the development process. The advantage of scXRD is that it is able to determine the exact lattice dimensions of the crystal, the placement of atoms in this crystal structure, and the presence of hydrates or solvates. In the context of this work, pXRD is used to survey reaction products and search for materials that produce a diffractogram which is different from its starting materials. Once such a material has been identified, larger crystals are grown and scXRD is used to determine the exact crystal structure and molecular formula.

3.3 Materials and Methods

3.3.1 Compounds

All materials purchased for use in this work were obtained from Fisher Scientific. In order to increase the likelihood of association between thiamine and the anion, acids with a pKa more than two units less than thiamine (<2.8) were selected (21). Similarly, sodium salts with a higher aqueous solubility than thiamine chloride (>459 mg/ml) were selected. Some materials which were close to fitting this criteria were also included as they were highly relevant to food systems. Acids tested include; sulfuric acid (Ricca chemical, 20% v/v in water, Arlington, TX

USA), L(+)-lactic acid (Acros Organics, 90% solution in water, NJ USA), phosphoric acid (Ricca Chemical, 25% v/v in water), iodic acid (Alfa Aesar, 99%, Tewksbury, MA USA), and citric acid monohydrate (Acros Organics, 99.5%, NJ USA). Sodium salts tested include; Sodium dihydrogen phosphate decahydrate (Alfa Aesar, 99%, Tewksbury, MA USA), sodium L-ascorbate (TCI, 98%, Portland, OR USA), trisodium citrate anhydrous (Alfa Aesar, 99%, Tewksbury, MA USA), taurine (Alfa Aesar, 99%, Tewksbury, MA USA), L(-)-tryptophan (Acros Organics, 99%, NJ USA), L-glutamic acid (Alfa Aesar, 99%, Tewksbury, MA USA), saccharin (Acros Organics, 99%, NJ USA), sodium DL-lactate (Alfa Aesar, 60% w/v in water, Tewksbury, MA USA), sodium bicarbonate (Acros Organics, 99.5%, NJ USA), and sodium benzoate (Acros Organics, 99%, NJ USA). A summary of the relevant properties of these materials can be found in Table 3.4

TCl·H_2O was prepared from TClHCl (Fisher Chemical, NJ USA) and TMN was purchased from BASF (NJ USA). Acetone was purchased from Fisher Chemical (NJ USA). Sodium hydroxide was manufactured by Sigma Aldrich (St. Louis, MO USA.

Table 3.4 Properties of materials used in attempts to form new thiamine salts

Salt	Aqueous Solubility		Reference
Trisodium citrate (anh.)	294	mg/mL	22
Sodium glutamate	739	mg/mL	23
Sodium saccharine	1000	mg/mL	24
Sodium benzoate	556	mg/mL	25
Sodium lactate	851	mg/mL	26
Sodium bicarbonate	100	mg/mL	27
Monosodium phosphate	600	mg/mL	28
Sodium ascorbate	620	mg/mL	29

Acid	pKa	Reference
Citric (monoh.)	3.13	30
Lactic	3.86	31
Phosphoric	2.12	32
Sulfuric	1.92	33
Iodic	0.78	34
Taurine	1.5	35
Tryptophan	2.83	36

All water used in this work was classified as ultrapure. House water supply was treated using a Barnstead E-Pure Ultrapure water purification system. According to the manufacturer,

resistivity of the water should have been greater than 18.2 MΩ-cm and total organic carbon concentrations should have been less than 10 ppb. A 0.2 μm filter should have eliminated possible bacterial and particulate contamination (2).

For use in HPLC experiments, HPLC grade Acetonitrile and trifluoroacetic acid were obtained from Sigma Aldrich.

3.3.2 Preparation of Thiamine Chloride

$TCl \cdot H_2O$ was the starting form of thiamine used in all salt metathesis reactions performed in this work. The monohydrate form was identified based on scXRD diffractograms. To prepare $TCl \cdot H_2O$, TClHCl was first dissolved to a concentration of 0.570 g/mL in UP water. An equimolar quantity of sodium hydroxide was added to neutralize the hydrochloric acid. As the solubility of $TCl \cdot H_2O$ is much less than TClHCl, precipitation occurs as soon as a sufficient quantity of NaOH was dissolved. After the NaOH had dissolved completely, five volumes of acetone were added. The slurry was then held at -2°C for 12 hours. The precipitate was filtered using a Whatman #1 filter paper and a Buchner funnel and was dried in a room temperature (approximately 20°C) vacuum oven overnight using the house vacuum line. Room temperature was selected as higher temperatures degraded and discolored samples in preliminary experiments.

HPLC assay indicated $TCl \cdot H_2O$ obtained from an initial precipitation has a purity of 88.5% by mass. Purity was obtained by comparing the peak area corresponding to the thiamine ion in the sample of unknown purity to a calibration curve consisting of varying molar concentrations of TClHCl. Since the thiamine salt will disassociate, only the molar concentration of thiamine effects peak area, not the anion. By multiplying by the molecular weight of $TCl \cdot H_2O$

and dividing by the mass of sample dissolved, purity was obtained. An example of a collected $TCl \cdot H_2O$ chromatogram is provided in Figure 3.3.

The remaining 11.5% is assumed to be sodium chloride as no evidence of degradation is present. The molar mass of $TCl \cdot H_2O$ is more than five times that of sodium chloride (318.8 vs

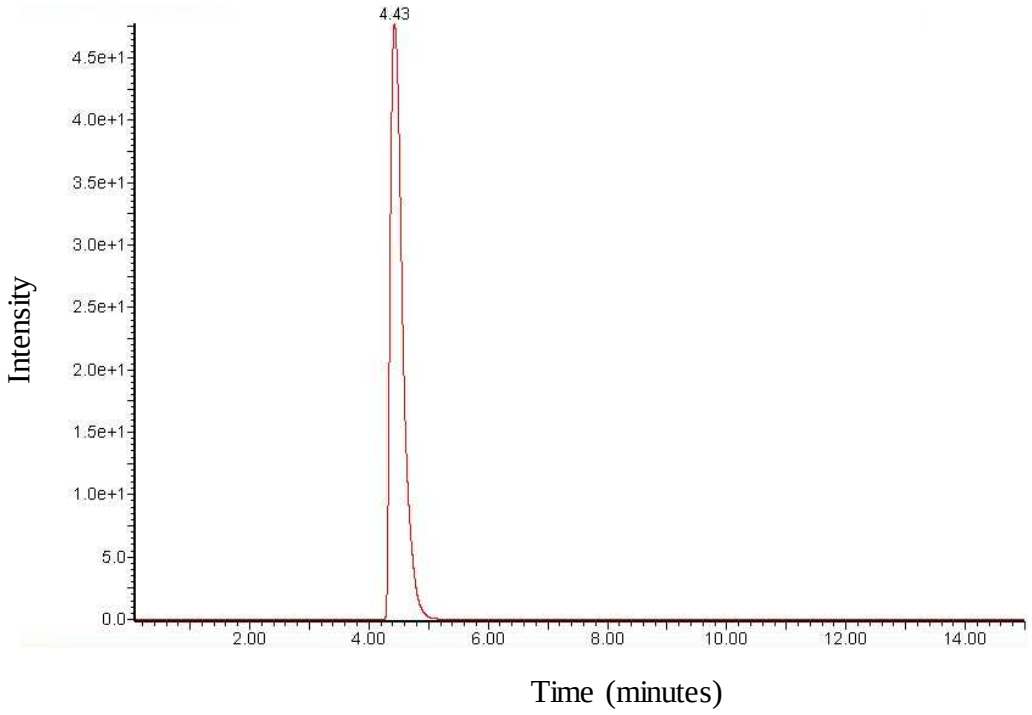


Figure 3.3 HPLC chromatogram of $TCl \cdot H_2O$

58.4 g/mol). Since each molecule contains one chloride ion, 11.5 wt% NaCl represents approximately 43% of the moles of chloride ions in a given quantity of material. As the reaction involving the silver-anion salt in the silver method is very sensitive to chloride concentration, excess chloride ions from NaCl will react with the silver cations, leaving the anion to precipitate as a sodium salt. Thus, it was necessary to remove as much of the NaCl as possible.

To remove NaCl, a series of recrystallizations were performed. HPLC assay indicates that following the first recrystallization, purity rose from 88.5 ± 2.1% to 98.2% ± 0.7%. A second recrystallization showed no improvement in purity and one recrystallization step was thus judged

as sufficient. Recrystallization was performed by dissolving $TCl \cdot H_2O$ to a concentration of 256 mg/mL in UP water. Five volumes of acetone were added in one volume increments with a period of 12 hours between each addition. After a period of 60 hours had elapsed the solution was stored at 7°C for 12 hours. The precipitate was filtered using a Whatman #1 filter paper and a Buchner funnel and was dried under house vacuum in a room temperature vacuum oven overnight.

3.3.3 Salt Forming Reactions

3.3.3.1 Salt Metathesis Reactions

In order to increase the chances of success a number of factors were considered when selecting compounds to test with this method. For sodium salts, high aqueous solubility (>256 mg/mL) was desired as this will help drive the reaction to produce the new thiamine salt. Similarly, acids were selected which have a pKa of at least two units below that of thiamine (less than 2.8, see Table 3.2). This also encourages the reaction to proceed in the desired direction. For all compounds, the relevancy of the anion to food systems was the first priority.

An example reaction procedure is given as follows. $TCl \cdot H_2O$ was dissolved at 256 mg/mL (.803 mmol/mL) in UP water. The desired molar equivalent of the anion was then added and dissolved. Molar ratios of $TCl \cdot H_2O$:anion of 1:1, 1:1.1, and 1:1.3 were tested. For trisodium citrate, which contains a trivalent anion, molar ratios of 3:1, 3:1.1, and 3:1.3 were used in addition to 1:1, 1:1.1, and 1:1.3. Ratios of greater than one part anion to one part thiamine were tested to determine if excess anion would help drive association with thiamine. Excess of $TCl \cdot H_2O$ was not tested as this would encourage precipitation of $TCl \cdot H_2O$, not new thiamine salts. Once the anion was dissolved, five volumes of acetone were added, and the mixture was refrigerated at 7°C for a minimum of 12 hours, up to 72 hours. If precipitant was observed, the

solution was then filtered using a Whatman #1 filter paper and Buchner funnel. The precipitant was then dried under in a RT vacuum for 12 hours after which it was stored in an airtight container away from light. All reactions were run as singlets.

For samples which used acids as a reactant, the reaction was tested both by addition of only acid and TCl·H_2O followed by precipitation and by addition of acid, TCl·H_2O, and NaOH followed by precipitation.

3.3.3.2 Silver Method

As stated in section 1.7.2, the double replacement reaction between silver nitrate and another salt to form a new salt and silver chloride is well known in introductory chemistry courses. Silver nitrate has an aqueous solubility of 256 g/100 mL water at 25°C while the solubility of silver chloride is only 0.19 mg/100 mL (1). As silver nitrate represents a practical upper limit on the solubility of silver salts and silver chloride represents a lower limit, it is likely other silver salts will have intermediate solubilities. In the context of this work, this is relevant as these new silver salts were used as an intermediate to react with thiamine chloride to form a new thiamine salt and silver chloride.

In this method, a 5M solution of silver nitrate was reacted with an aqueous solution of the sodium salt of the desired anion. For divalent anions a 2:1 molar ratio of silver nitrate:sodium salt was used, and for trivalent anions a 3:1 molar ratio was used. A white to off-white precipitant was immediately formed which was filtered using Whatman #1 filter paper and a Buchner funnel. This precipitate, a silver-anion salt, was then washed with UP water and dried under house vacuum in a 50°C vacuum oven for 1 hour. The silver-anion salt was not found to degrade under elevated temperatures, thus 50°C was used to accelerate the drying process and

minimize exposure to light. The silver-anion salt was dried and stored in an amber vial to protect the material from light and prevent discoloration.

TCl·H_2O was dissolved in UP water at a concentration of 256 mg/mL. To this, a quantity of silver-anion salt was added such that the molar ratio of thiamine:anion was 1:1. The suspension was mixed using a vortex mixer for 60 seconds. The suspension was then centrifuged at 7200xg for 3 minutes. The supernatant was then removed taking care not to collect any of the precipitant. Supernatant was then added dropwise to five volumes of acetone. The solution was stored at 7°C until precipitation is observed. Typically, crystals are observed after a period of 12-72 hours, though some samples took longer to precipitate. Precipitants were filtered, again using a Whatman #1 filter paper and Buchner funnel. The crystals were dried under house vacuum in a vacuum oven at ambient temperature overnight and were then ready for identification through scXRD.

3.3.4 X-Ray Crystallography Method Parameters

A Shimadzu LabX XRD-6000 (Kyoto, Kyoto Prefecture Japan) diffractometer was used to collect powder diffractograms used in this chapter. The instrument was equipped with a Cu-Kα radiation source. A range for 2θ of 5 to 40° was used with a scan rate of 2° per minute and a step size of 0.04°. Daily calibration was performed prior to analysis. A silicon standard (111) peak was confirmed to be measured between 28.423° and 28.463° 2θ. Samples were placed into a metal plate and were smoothed flat with a glass slide. Large crystals were lightly crushed to ensure an even surface.

A Bruker AXS D8 Quest CMOS (Billerica, MA USA) was used to collect single crystal diffractograms discussed in this chapter. Matthias Zeller of Purdue University operated the device to collect these diffractograms. The instrument was equipped with a Mo Kα radiation

source ($\lambda = 0.71073$ Å), a sealed fine focus X-ray tube, a single crystal curved graphite incident beam monochromator, and a Photon100 CMOS area detector (Bruker, Billerica, MA USA). Temperature was controlled using an Oxford Cryosystems Cryostream chiller (Long Hanborough, UK) with a set point of 150K. Diffraction data was processed using Bruker SAINT and APEX3 (Long Hanborough, UK) software and structure solution and refinement were performed by direct methods using SHELXS97 and SHELXL2017 (Göttingen, DE), respectively. Prior to analysis, single crystals of the compounds were coated with a trace quantity of Fomblin oil and were then transferred to the goniometer head of the diffractometer.

3.3.5 pH Measurement Method

Measurements of pH were taken using an Orion Model SA 720 (Waltham, MA USA) calibrated using buffer solutions of pH 7.00 (SB107 Fisher Chemical, Hampton, NH USA) and 8.00 (SB112 Fisher Chemical, Hampton, NH USA). Solutions of $TCl{\cdot}H_2O$ were prepared in duplicate at concentrations of 1, 5, 10, 27, 100, and 256 mg/mL using UP water. UP water is known to produce issues in pH measurements due to its low conductivity. Thus, KCl (Mallinckrodt, UK). was added at a concentration of 10 mg/mL to increase conductivity (13). This brought the measured pH of a 1 mg/mL solution from 8.1 to 6.9 which is consistent with expected results assuming a small amount of atmospheric CO_2 has dissolved.

3.3.6 Melting Point Analysis Method

Melting point was determined for $TCl{\cdot}H_2O$ using a Vernier Melt Station (Beaverton, OR USA). Crystals of $TCl{\cdot}H_2O$ were crushed into a powder with a metal spatula and were loaded into a glass capillary tube which was then placed into the device. Initially, a high heating rate was used to approximately determine the melting point. Once this was understood, the device was set at a heating rate based on an expected melting point of 140°C. Reported melting point is

given as a range: the first value is when melting is first observed and the second is when melting has reached completion. The experiment was run in triplicate and the values averaged. Results from this experiment are included in Table 3.2.

3.3.7 Fourier Transform Infrared Spectroscopy Method Parameters

All FTIR analysis used in this work was carried out using a TravelIR HCI instrument manufactured by SensIR Technologies (Danbury, CT USA). A new background was collected prior to each sample. Sixty-four scans were taken for each analysis and background with a resolution of 4 wavenumbers. The measured range was from 4000 to 650 wavenumbers. A TGS detector was used and data is presented as percent transmission. Samples were placed on the diamond window and were pressed down with a metal screw until sufficient contact had been made. A force gage is included on the device: a reading of six out of ten units was enough to achieve adequate contact. In between samples, the diamond window and metal screw were cleaned with water followed by acetone to remove any remaining sample.

3.3.8 High-Performance Liquid Chromatography Method Parameters

Samples analyzed with HPLC in this work made use of a Waters 2690 Separations Module (Milford, MA USA) using a Waters 2995 PDA detector (Milford, MA USA). The column used was 100mm x 3.9mm with a particle size of 3.5 µm and was manufactured by Waters Corporation (XTerraRP-C18, Milford, MA USA). The range of wavelengths scanned was 235-400 nm. The solvent system used was a gradient method where solvent A is 0.1% trifluoroacetic acid in UP water and solvent B is acetonitrile. The time-flow rate profile is as follows: 100/0 at 0 minutes, 97/3 at 4 minutes, 90/10 at 6 minutes, 100/0 at 10 minutes, and 100/0 at 15 minutes. Each change is flow rate was linear. This method was adapted from Voelker, et al. (11). For this system, typical retention times for thiamine ions were 4.5 minutes,

though variations in solvent preparation and the age of the column produced minor changes in the exact time.

For each day of testing a calibration curve was prepared to eliminate variations in the solvent preparation and PDA. A series of 6 dilutions of TClHCl of 10 mL each were prepared at the following concentrations (in mg/mL): 2.000, 1.000, 0.333, 0.111, 0.037, 0.012. This range covers the relevant range of concentrations observed in experiments and eliminates any variation due to preparation of the mobile phase and daily variations in the PDA. A correlation of <0.9999 is indicative of either poor sample preparation or a malfunctioning system.

Samples were prepared by dissolving approximately 15 mg of material in mobile phase such that the concentration was 1 mg/mL. Samples were prepared in triplicate. Since the thiamine salt will be fully dissociated, the anion will not affect the observed peak area: this area is only a function of the molar concentration of thiamine in solution. The relationship between peak area and molar concentration of thiamine was determined based on calibration data. This allows the calculation of the molar concentration of thiamine solutions of samples. By dividing the measured molar concentration by the expected molar concentration if the sample were pure, the percent purity of samples were obtained.

3.3.9 Sample Preparation for Degradation Studies

In order to evaluate the effects of both temperature and concentration on the stability of aqueous solutions of $TCl{\cdot}H_2O$, solutions ranging from 1 to 256 mg/mL of $TCl{\cdot}H_2O$ were prepared and were placed in amber scintillation vials. The standard cap for these vials was replaced by caps with a polyethylene liner (Wheaton, Millville, NJ USA). These caps, in conjunction with a single wrap of duct tape, were sufficient to eliminate evaporation due to extended exposure at high temperatures. In samples which didn't make use of these features,

evaporation of the solvent occurred, resulting in an artificial increase in concentration observed through HPLC analysis.

Incubators manufactured by Forma Scientific (Thermo Scientific, Waltham, MA USA) were used to store samples at 40, 60, and 70°C. An incubator room (Commercial Fixture Co. Inc., Indianapolis, IN USA) was used to maintain 25°C and a VWR (Radnor, PA USA) heating block was used to maintain 80°C.

Sample vials were opened, and a fraction of the solution was drawn out and diluted for measurement by HPLC. For samples of 1, 5, and 10 mg/mL, a total initial volume each of 15 mL was prepared. A 0.5 mL quantity of solution was diluted by 4.5 mL of mobile phase for 10 mg/mL samples, 0.5 mL of solution was diluted by 2 mL of mobile phase for 5 mg/mL samples, and 0.5 mL of solution was tested without dilution for 1 mg/mL samples. For concentrations of 27, 100, and 256 gm/mL, a total initial volume of 5 mL was prepared for each. A 0.1 mL quantity of solution was drawn for each sample and was diluted by 2.6, 9.9, and 25.5 mL of mobile phase for samples of 27, 100, and 256 mg/mL, respectively. Thus, each sample analyzed through HPLC would have a concentration of 1 mg/mL if no degradation were to occur. A calibration curve was prepared as described in Section 3.3.8. Each concentration and temperature combination were prepared in triplicate.

3.3.10 Degradation Reaction Kinetics of $TCl \cdot H_2O$ In Aqueous Solutions

Previous research has shown that thiamine degradation follows pseudo first order reaction kinetics (14). For first order reactions, concentration as a function of time is described by Equation 3.1 where x is the concentration at time, t, x_o is the initial concentration, and k is a rate constant, the unit of which is the inverse of the unit of time. The Arrhenius equation (Equation 3.2) describes the relationship between this rate constant, k, and temperature and is a

function of the activation energy. Here, A is the frequency factor of collision, taken to be equal to 1, E_a is the activation energy (with units of J/mol), R is the universal gas constant (8.314 J/molK), and T is the temperature in Kelvin.

$$\ln\left(\frac{x}{x_o}\right) = -kt \qquad \text{Equation 3.1}$$

$$k = Ae^{-\frac{E_a}{RT}} \qquad \text{Equation 3.2}$$

In order to calculate E_a, first a plot of $\ln\left(\frac{x}{x_o}\right)$ versus t was made. The opposite of the slope of this line was taken as k. Next, $-R\ln\left(\frac{k}{A}\right)$ was plotted versus 1/T. The slope of this line is E_a. This procedure was repeated for each concentration studied.

3.3.11 Solubility Measurement of $TCl{\cdot}H_2O$

Little information is available on the solubility of $TCl{\cdot}H_2O$; thus, measurements were taken to determine this value. Triplicate samples of excess $TCl{\cdot}H_2O$ were placed in a 10 mL beaker with a stir bar and a few mL of UP water. The beakers were placed in a 25°C room and were stirred for a period of 24 hours to ensure equilibration. After this period of time, excess solid was observed, indicating the solutions were not undersaturated. A small volume of this solution was diluted by a factor of 300x in UP water and was tested using the HPLC method described in section 3.3.X. The solubility measured by this method was 459.3 +/- 15.8 mg/mL.

3.3.12 Statistical Analysis

Statistical analysis was performed using single-variable ANOVA through the use of SAS 9.4 (SAS Institute, Cary, NC USA). Tukey's post hoc test was used to determine differences using a significance level of α=.05. For data concerning degradation of $TCl{\cdot}H_2O$ in aqueous solutions, capital letters are used to denote groupings within a single concentration over time and

lowercase letters are used to denote groupings over the range of concentrations tested for a given time point.

3.4 Results and Discussion

3.4.1 pH versus Concentration for TMN, TClHCl, and TCl·H_2O

The pH was measured of TCl·H_2O dissolved in UP water over a concentration range from 1 to 256 mg/mL. Solutions were prepared in triplicate and no variation was found between samples of the same concentration. This data is compared to pH measured by Voelker, et al. (11) and is presented in Table 3.11. TMN is not soluble above 27 mg/mL, which is why no higher concentrations are reported.

As thiamine stability in aqueous solutions is known to be strongly dependent on pH (1, 15), understanding the pH of the solutions studied was a key factor in understanding the observed stabilities. Since thiamine is more stable in acidic solutions, the relatively higher pH of TCl·H_2O solutions compared to TMN and TClHCl solutions suggests that the stability would be lower.

Table 3.5 pH of aqueous solutions of thiamine salts

Thiamine form	Aqueous concentration (mg/mL)						
	1	5	10	27	100	256	300
TMN (11)	6.42 ± 0.04	6.6 ± 0.3	6.8 ± 0.2	6.96 ± 0.03	No Data	No Data	No Data
TClHCl (11)	3.59 ± 0.03	3.30 ± .01	3.17 ± 0.00	2.99 ± 0.00	2.77 ± 0.00	No Data	2.53 ± 0.01
TCl·H2O	6.9 ± 0.0	7.5 ± 0.0	7.4 ± 0.0	7.3 ± 0.0	7 ± 0.0	6.8 ± 0.0	No Data

3.4.2 TCl·H_2O Stability Characterization and Comparison to TMN and TClHCl

Thiamine is known to be prone to degradation by a number of factors including heat, oxygen, sulfites, ultraviolet, and alkali conditions (14). As discussed in Section 3.2.1, the anion associate with the thiamine salt also has an effect on degradation rate. While the stability of

TMN and TClHCl have been studied individually (15-17) and, more recently, comparatively (11), no such characterization is known to have been performed for $TCl \cdot H_2O$.

Aqueous solutions of $TCl \cdot H_2O$ in the range of 1 to 256 mg/mL were prepared and stored at temperatures including 25, 40, 60, 70, and 80°C. Degradation was quantified through the use of HPLC and from this reaction kinetics were determined. Activation energy for degradation was calculated and compared to values reported for TMN and TClHCl in similar conditions. As expected, degradation rate increased with increasing temperature. At 80°C, solutions of 256 mg/mL contained only 25.1% of its initial thiamine content after 8 hours. At 25°C, solutions of the same concentration contained 80.2% of its initial thiamine content after 34 days.

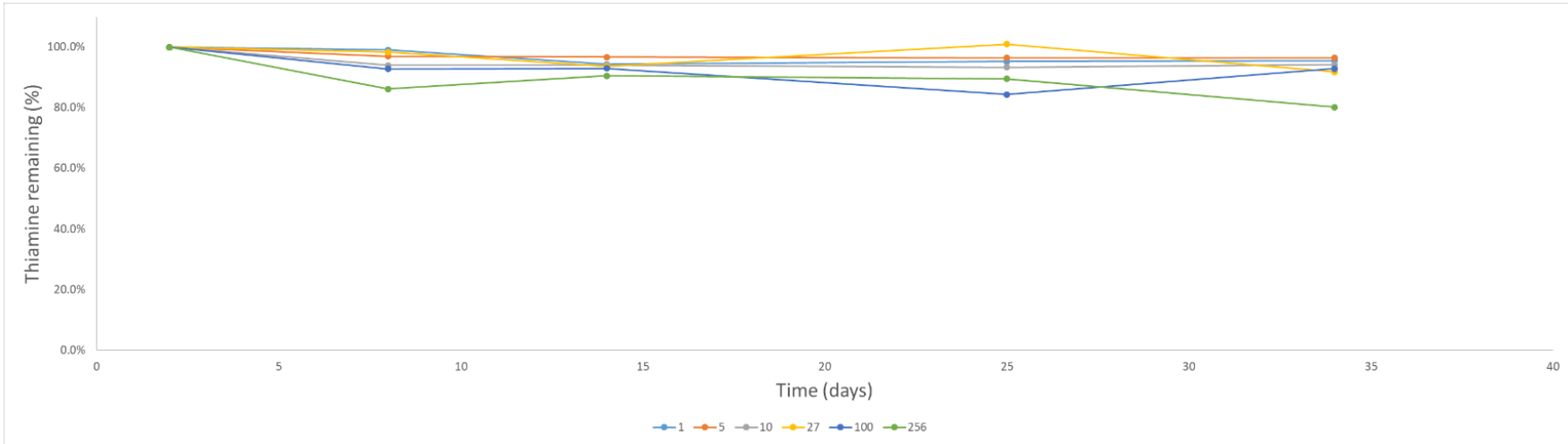


Figure 3.4 Degradation of $TCl{\cdot}H_2O$ at 25°C

Table 3.6 Degradation of $TCl{\cdot}H_2O$ at 25°C

Time (day)	Concentration (mg/ml)					
	1	5	10	27	100	256
0	No Data	No Data	No Data	No Data	No Data	No Data
2	100.0% +/- 0.5% (Aa)	100.0% +/- 1.9% (Aa)	100.0% +/- 1.1% (Aa)	100.0% +/- 2.8% (Aab)	100.0% +/- 0.6% (Aa)	100.0% +/- 1.7% (Aa)
8	99.1% +/- 1.2% (Aa)	96.9% +/- 3.7% (Aa)	96.9% +/- 3.7% (ABCa)	98.3% +/- 1.3% (Aab)	92.8% +/- 0.3% (Cb)	86.1% +/- 0.6% (Dc)
14	94.4% +/- 1.0% (Ab)	96.7% +/- 0.3% (ABCa)	96.7% +/- 0.3% (Aa)	93.6% +/- 2.5% (BCb)	93.0% +/- 0.1% (Bb)	90.5% +/- 0.7% (Cb)
25	95.2% +/- 1.0% (ABb)	96.5% +/- 2.3% (Aab)	96.5% +/- 2.3% (Aab)	101.0% +/- 3.9% (Aa)	84.3% +/- 6.2% (Cc)	89.5% +/- 1.7% (BCbc)
34	95.5% +/- 0.5% (BCb)	96.5% +/- 0.9% (Aa)	96.5% +/- 0.9% (Ba)	91.8% +/- 2.5%(Bca)	93.3% +/- 0.7% (BCb)	80.2% +/- 0.4% (Cc)

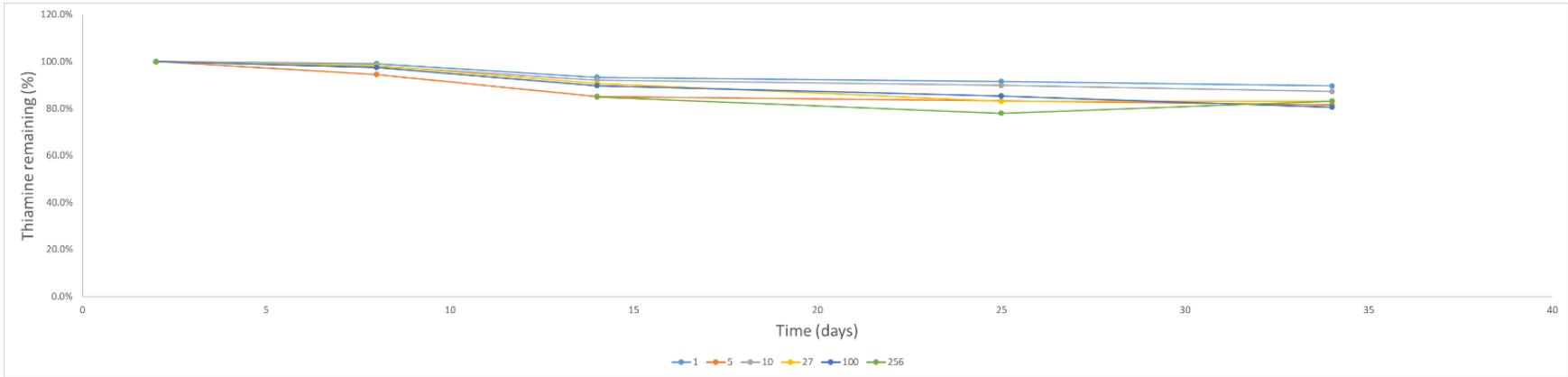


Figure 3.5 Degradation of $TCl{\cdot}H_2O$ at 40°C

Table 3.7 Degradation of $TCl{\cdot}H_2O$ at 40°C

Time (day)	Concentration (mg/ml)					
	1	5	10	27	100	256
0	No Data	No Data	No Data	No Data	No Data	No Data
2	100.0% +/- 2.0% (Aa)	100.0% +/- 7.2% (Aa)	100.0% +/- 5.6% (Aa)	100.0% +/- 5.2% (Aa)	100.0% +/- 1.1% (Aa)	100.0% +/- 0.1% (Aa)
8	99.2% +/- 1.4% (Aa)	94.5% +/- 4.0% (Aa)	98.1% +/- 2.3% (Aab)	98.2% +/- 1.3% (Aa)	97.6% +/- 1.8% (Aa)	No Data
14	93.3% +/- 1.6% (Ab)	85.2% +/- 2.7% (Aab)	92.1% +/- 2.2% (Aabc)	90.7% +/- 1.5% (Aa)	89.8% +/- 0.5% (Bb)	85.0% +/- 1.4% (Cb)
25	91.5% +/- 1.6% (Ab)	83.3% +/- 1.1% (Abc)	89.9% +/- 1.0% (Abc)	83.1% +/- 10.5% (Aab)	85.3% +/- 0.7% (Bc)	78.0% +/- 0.3% (Cc)
34	89.7% +/- 1.5% (Ac)	81.6% +/- 2.1% (Cc)	87.2% +/- 2.1% (ABc)	83.1% +/- 1.3%(Bac)	80.6% +/- 1.7% (Cd)	83.2% +/- 0.1% (BCd)

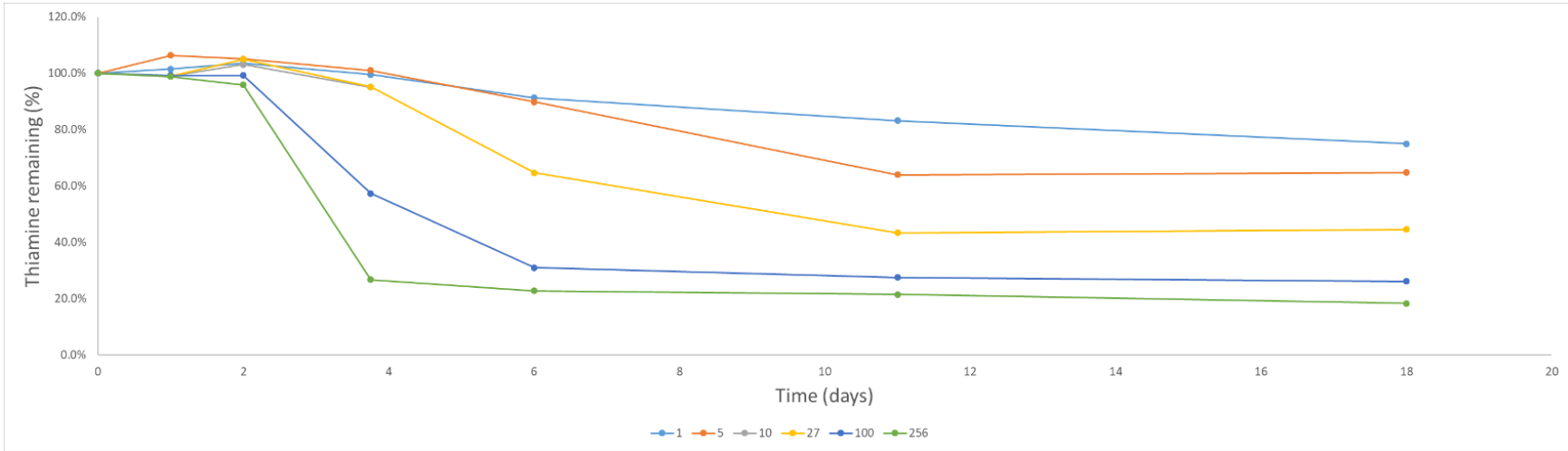


Figure 3.6 Degradation of $TCl \cdot H_2O$ at 60°C

Table 3.8 Degradation of $TCl \cdot H_2O$ at 60°C

Time (day)	Concentration (mg/ml)					
	1	5	10	27	100	256
0	100.0% +/- 0.4% (Aa)	100.0 +/- 1.0% (Ab)	100.0% +/- 0.2% (Aa)	100.0% +/- 1.0% (Aab)	100.0% +/- 0.4% (Aa)	100.0% +/- 0.8% (Aa)
1	101.5% +/- 0.3% (Aa)	106.4% +/- 0.6% (Aa)	98.9% +/- 2.6% (Aab)	99.1% +/- 0.6% (Ab)	99.3% +/- 1.0% (Aa)	98.8% +/- 0.7% (Aa)
2	103.5% +/- 1.2% (Aba)	105.1% +/- 2.4% (Aab)	103.1% +/- 2.4% (Aab)	105.0% +/- 2.1% (Aa)	99.3% +/- 2.5% (Bac)	95.8% +/- 0.8% (Cb)
3.75	99.5% +/- 0.3% (ABa)	101.0% +/- 1.6% (Aab)	95.0% +/- 2.6% (Bb)	95.1% +/- 2.3% (Bb)	57.3% +/- 2.1% (Cb)	26.7% +/- 0.4% (Dc)
6	91.3% +/- 0.2% (Ab)	89.8% +/- 0.1% (Ac)	84.0% +/- 0.9% (Bc)	64.7% +/- 1.9% (Cc)	30.9% +/- 0.6% (Dc)	22.8% +/- 0.2% (Ed)
11	83.2% +/- 3.0% (Ac)	63.9% +/- 3.1% (Bd)	65.7% +/- 0.5% (Bd)	43.4% +/- 0.4%(Cd)	27.5% +/- 0.2% (Dcd)	21.5% +/- 0.2% (Ed)
18	75.0% +/- 2.0% (Ad)	64.7% +/- 3.0% (Bd)	54.9% +/- 1.1% (Ce)	44.5% +/- 3.6%(Dd)	26.1% +/- 1.2% (Ed)	18.2% +/- 1.0% (Fe)

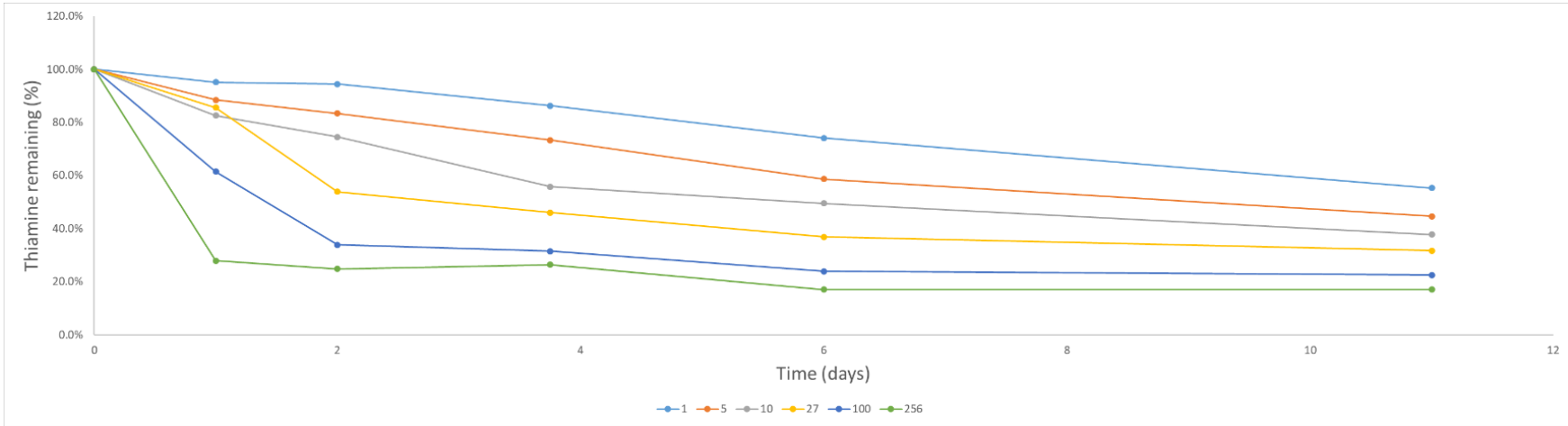


Figure 3.7 Degradation of $TCl \cdot H_2O$ at 70°C

Table 3.9 Degradation of $TCl \cdot H_2O$ at 70°C

Time (day)	Concentration (mg/ml)					
	1	5	10	27	100	256
0	100.0% +/- 0.2% (Aa)	100.0 +/- 2.0% (Aa)	100.0% +/- 1.1% (Aa)	100.0% +/- 1.7% (Aa)	100.0% +/- 2.5% (Aa)	100.0% +/- 1.7% (Aa)
1	95.2% +/- 0.5% (Ab)	88.4% +/- 3.8% (Ab)	82.6% +/- 2.6% (Ab)	85.6% +/- 4.4% (Aa)	61.5% +/- 9.8% (Bb)	27.9% +/- 0.5% (Cb)
2	94.5% +/- 1.2% (Ab)	83.4% +/- 2.4% (Bb)	74.5% +/- 1.9% (Cc)	53.9% +/- 1.7% (Db)	33.9% +/- 0.4% (Ec)	24.8% +/- 0.6% (Fb)
3.75	94.5% +/- 0.9% (Ab)	73.4% +/- 1.5% (Bc)	55.8% +/- 1.9% (Cd)	46.0% +/- 2.1% (Dc)	31.5% +/- 0.5% (Ec)	26.4% +/- 0.8% (Fb)
6	86.2% +/- 1.4% (Ac)	58.7% +/- 0.8% (Bd)	49.6% +/- 2.9% (Cd)	36.9% +/- 1.7% (Dd)	23.9% +/- 0.8% (Ed)	17.1% +/- 0.6% (Fc)
11	74.1% +/- 0.8% (Ad)	44.6% +/- 1.8% (Be)	39.2% +/- 3.3% (Ce)	32.9% +/- 2.5%(Dd)	22.6% +/- 0.4% (Ed)	17.1% +/- 1.4% (Ec)

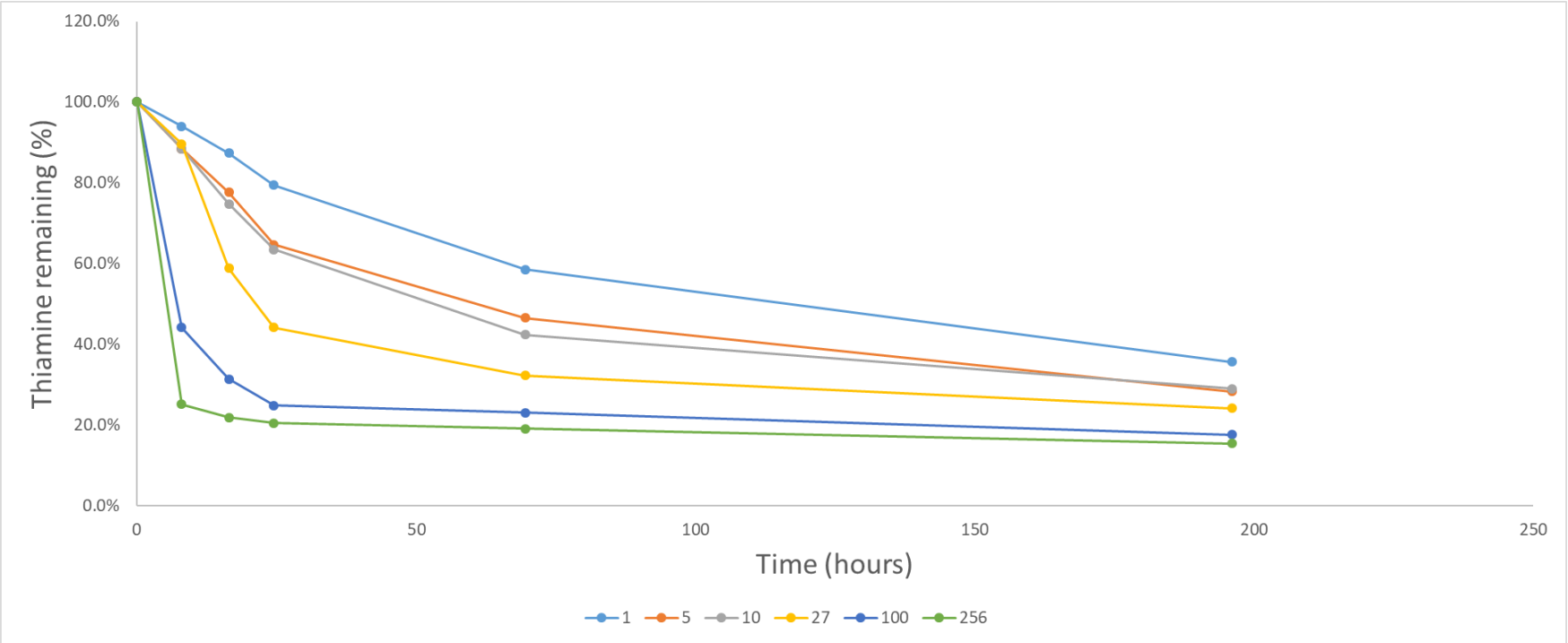


Figure 3.8 Degradation of $TCl{\cdot}H_2O$ at 80°C

Table 3.10 Degradation of $TCl{\cdot}H_2O$ at 80°C

Time (days)	Concentration (mg/ml)					
	1	5	10	27	100	256
0.00	100% +/- 3.4% (Aa)	100 +/- 2.5% (Aa)	100% +/- 2.5% (Aa)	100% +/- 2.6% (Aa)	100% +/- 0.2% (Aa)	100% +/- 0.6% (Aa)
0.33	94.0% +/- 1.0% (Aab)	88.5% +/- 3.6% (Ab)	88.4% +/- 3.2% (Ab)	89.6% +/- 3.0% (Ab)	44.1% +/- 11.3% (Bb)	25.1% +/- 1.7% (Cb)
0.69	87.4% +/- 0.5% (Abc)	77.6% +/- 1.3% (Bc)	74.8% +/- 3.2% (Bc)	58.8% +/- 3.5% (Cc)	31.3% +/- 4.0% (Dbc)	21.8% +/- 0.8% (Ec)
1.02	79.4% +/- 0.8% (Ac)	64.6% +/- 1.8% (Bd)	63.5% +/- 1.4% (Bd)	44.1% +/- 1.7% (Cd)	24.8% +/- 0.8% (Dc)	20.5% +/- 0.4% (Ecd)
2.90	58.5% +/- 5.4% (Ad)	46.5% +/- 1.2% (Be)	42.4% +/- 1.2% (Be)	32.3% +/- 0.4% (Ce)	23.0% +/- 0.2% (Dc)	19.1% +/- 0.4% (Dd)
8.17	35.6% +/- 3.6% (Ae)	28.3% +/- 3.6% (Abf)	29.0% +/- 0.3% (Abe)	24.2% +/- 1.7%(BCf)	17.5% +/- 0.8% (Cc)	15.4% +/- 0.3% (Ce)

At 25°C, seen in Figures 3.4 and Table 3.6, over a period of 34 days, degradation was minimal, reaching 19.8% loss at the highest concentration at the final time point tested. For solutions of 5, 10, and 27 mg/mL, degradation was not significant at the 34 day mark. Further, this data set was inconsistent between time points. Between days 8 and 14, the solutions of 256 mg/mL gained an average of 4.4% and between days 25 and 34, solutions of 100 mg/mL gained 9.0%. Due to the lack of significance and inconsistency between time points, it was not possible to calculate activation energy using this data.

At 40°C, seen in Figures 3.5 and Table 3.7, over a 34 day period, degradation was more substantial and was more consistent between time points over duration of the study. Unfortunately, due to issues with the HPLC, day 0 data are unavailable, thus the next time point, day 2, was used as the initial thiamine content. Over the 34 day period studied, degradation ranged from 11.3% for 1 mg/mL samples to 19.4% for 100 mg/mL samples. Degradation compared as a function of time was found to be statistically significant for all concentrations studied. Significance was not observed with increasing concentration. The combination of a greater extent of degradation and lesser fluctuation between time points for the same concentration allowed the calculation of activation energy using this data and data for 60, 70, and 80°C.

For solutions stored at 60°C, seen in Figures 3.6 and Table 3.8, the behavior between days zero and two are unexpected: a rise in concentration compared to day 0 was observed for samples of 1, 5, 10, and 27 mg/mL. However, following these time points, the data follows first order kinetics. Compared to 40°C, degradation occurs much more rapidly and to a greater extent. Here, samples were taken over a period of 18 days. Degradation as a function of concentration

was found to be statistically significant with higher concentrations degrading more rapidly. Data was also significant over time, excluding data from days 0 and 1.

For solutions stored at 70 (Figure 3.7 and Table 3.9) and 80°C (Figure 3.8 and Table 3.10), degradation follows the typical profile of first order reaction kinetics and did not suffer a delay in initial degradation or any instrument malfunctions. At 70°C, the total time over which samples were collected was 11 days. As was the case for data collected at 60°C, degradation was found to be significantly higher with increasing time and concentration. At 80°C, the time scale was further reduced to 8.17 days or 196 hours. Again, degradation was found to be significantly higher with increasing time and concentration, though degradation rate for 5 and 10 mg/mL samples were not statistically significant at any time points analyzed.

Using this data, activation energy for degradation was calculated for each concentration. These results, shown in Table 3.11, are then compared to activation energies published by Voelker, et al. (11) in order to compare the effect of pH and the anion associated with thiamine

Table 3.11 Activation energy versus concentration for TMN, TClHCl, and TCl·H2O

Vitamin salt form	Concentration (mg/mL)	Activation energy (kJ/mol)	pH
TMN (11)	1	94	6.42 ± 0.04
	5	88	6.6 ± 0.3
	10	88	6.8 ± 0.2
	27	103	6.96 ± 0.03
TClHCl (11)	1	133	3.59 ± 0.03
	5	100	3.30 ± .01
	10	124	3.17 ± 0.00
	27	120	2.99 ± 0.00
	100	131	2.77 ± 0.00
	300	135	2.53 ± 0.01
$TCl \cdot H_2O$	1	68	6.9 ± 0.0
	5	73	7.5 ± 0.0
	10	62	7.4 ± 0.0
	27	52	7.3 ± 0.0
	100	52	7.0 ± 0.0
	256	52	6.8 ± 0.0

on degradation. Comparatively, $TCl \cdot H_2O$ showed a lower activation energy than TMN which showed a lower activation energy than TClHCl. This indicates $TCl \cdot H_2O$ is the least stable of the three salts in aqueous solutions, followed by TMN, and then TClHCl. Degradation rate of thiamine in solution is known to be strongly dependent on pH (11, 15-17), with stability being greatest in acidic solutions. Comparing the measured pH of each concentration for each anion, solutions of $TCl \cdot H_2O$ have the highest pH, followed by TMN, and TClHCl. This is in line with observed degradation rates: higher pH correlates with higher degradation rates.in aqueous solutions. In order to elucidate the effect the specific anion has on degradation rate of thiamine in aqueous solutions, a study would need to be performed where pH is controlled and only the nature of anion is varied. This could be accomplished through the use of buffer solutions; however, care must be taken to ensure interactions between the buffer and thiamine do not affect degradation apart by changing pH.

3.4.3 3.4.3 Unsuccessful Attempts to Form New Thiamine Salts

The overwhelming majority of reactions attempted in this chapter failed to produce new forms of thiamine. Mechanisms of failure came in many forms. Most of these failures merely precipitated $TCl \cdot H_2O$. A handful failed to precipitate even after storage at 7°C for a period of two weeks. In these samples, two aqueous phases of differing densities and viscosities were observed which were metastable over the two week storage time. Another observed outcome were precipitant pastes which were difficult to dry and typically discolored rapidly enough that samples had changed color noticeably over the time required to remove residual moisture under house vacuum in a RT vacuum oven.

Of all materials tested using the double replacement reaction method, all fail to produce new forms. Materials which produce $TCl{\cdot}H_2O$ include: sulfuric acid, L(+)-lactic acid, phosphoric acid, citric acid monohydrate, sodium dihydrogen phosphate decahydrate, sodium L-ascorbate, taurine, L(-)-tryptophan, L-glutamic acid, saccharin, sodium L-lactate, sodium bicarbonate, and sodium benzoate. Examples of characterization of reactions which produced $TCl{\cdot}H_2O$ can be seen in Figure 3.9. Here, FTIR spectra are presented comparing the starting material, $TCl{\cdot}H_2O$, to the collected precipitant from a solution of $TCl{\cdot}H_2O$ and monosodium glutamate. No new peaks are observed. Had the glutamate ion successfully replaced the chlorine, new peaks corresponding to this group would have appeared. Diffractograms collected through pXRD similarly showed no differences in crystal structure.

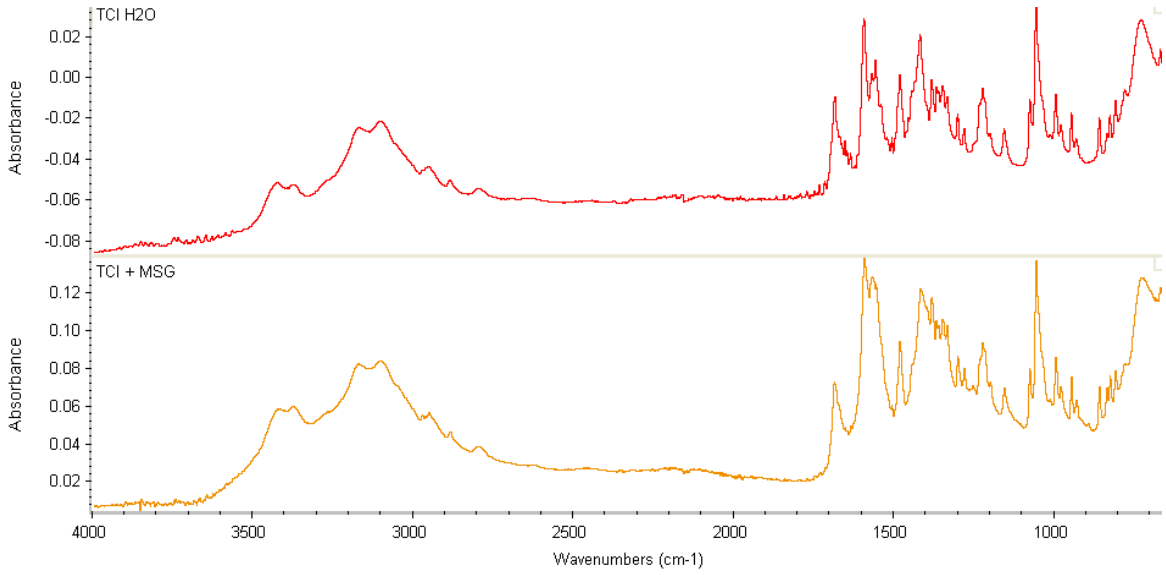


Figure 3.9 FTIR spectra of $TCl{\cdot}H_2O$ and the precipitant from a solution of $TCl{\cdot}H_2O$ and monosodium glutamate

When $TCl{\cdot}H_2O$ was combined with iodic acid and NaOH, a paste was produced which was unique when measured by XRD and FTIR but upon HPLC analysis showed a purity of approximately 40%. Figure 3.10 shows the corresponding FTIR spectra: a loss of the peak

centered around 3200 cm^{-1} as well as in the range of 1700-1000 cm^{-1} was observed. Figure 3.11 shows the corresponding diffractograms: differences were observed at 13, 15, 19, and 34°. Figure 3.12 shows the HPLC chromatogram: here the peak observed at 1.12 minutes corresponds to the iodate ion and the peak at 4.79 corresponds to the thiamine ion. Based on a TClHCl calibration curve, purity of the sample is calculated to be 42% ± 2%. An image of this precipitant may be found in Figure 3.13, which shows a material that is orange in color. Color was found to darken from a light yellow to the pictured color quite rapidly. Color further darkened over a period of days to a rust color. The total yield for this reaction was quite low, 57%. Factoring in purity, an overall yield of 24% was obtained. The low yield coupled with a low purity was the motivation to stop pursuing development of this precipitant, despite the unique FTIR and pXRD results obtained.

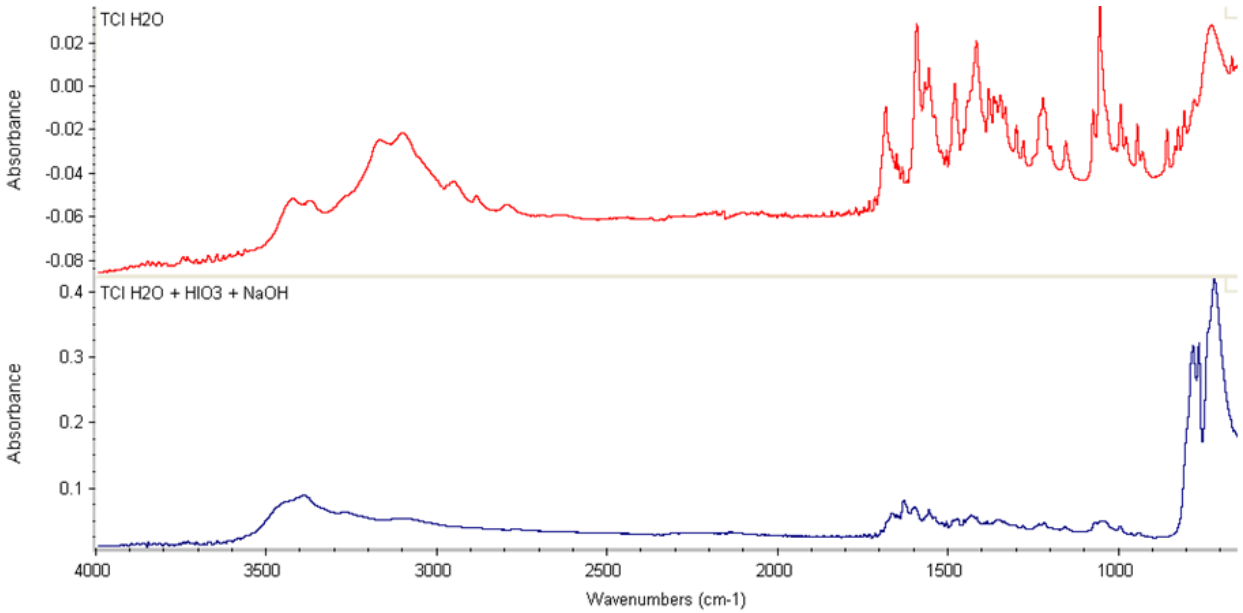


Figure 3.10 FTIR spectra of $TCl \cdot H_2O$ and the precipitant from a solution of $TCl \cdot H_2O$, HIO_3, and NaOH

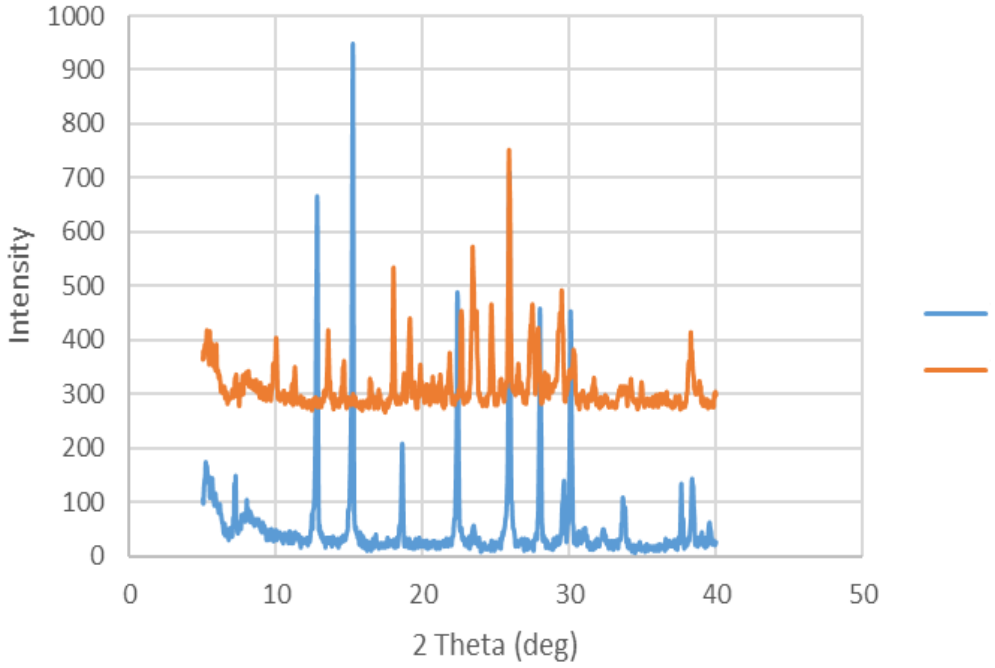


Figure 3.11 pXRD diffractogram of $TCl{\cdot}H_2O$ and the precipitant from a solution of $TCl{\cdot}H_2O$, HIO_3, and NaOH

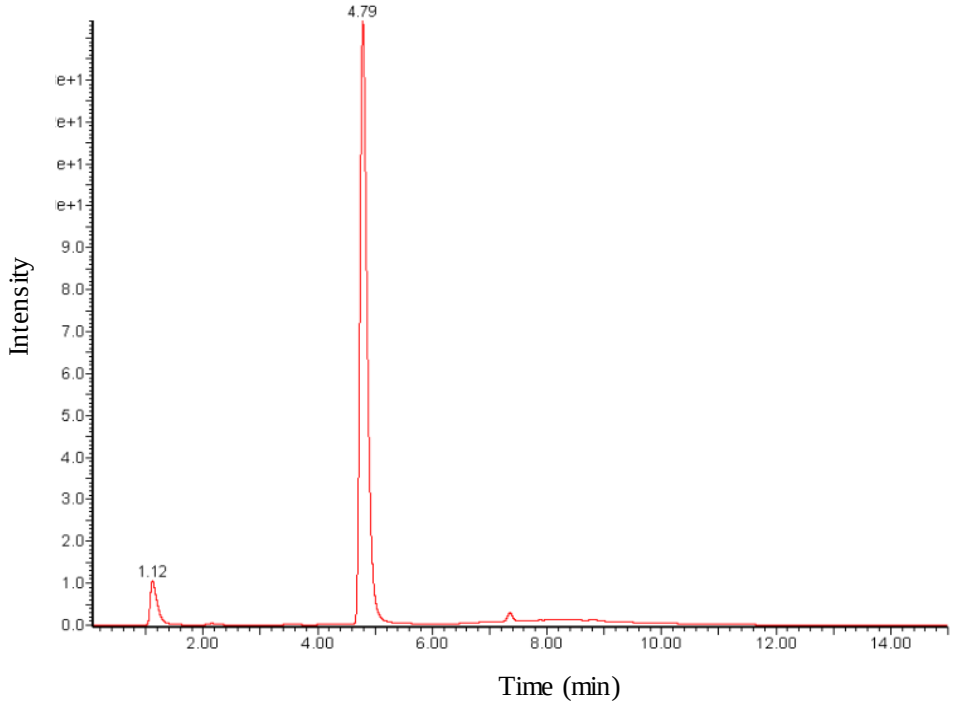


Figure 3.12 HPLC chromatogram of $TCl{\cdot}H_2O$ and the precipitant from a solution of $TCl{\cdot}H_2O$, HIO_3, and NaOH

Figure 3.13 Image of precipitant from a solution of $TCl{\cdot}H_2O$, HIO_3, and NaOH

Reaction with trisodium citrate also produced a material which showed a unique FTIR spectrum (Figure 3.14) and pXRD diffractogram (Figure 3.15**)** in initial experiments. In Figure 3.14, changes in peak intensity are observed around 3400 and 1700 cm^{-1}. In Figure 3.15, new peaks are observed at 13 and 32° which do not appear to be coming from trisodium citrate. When this sample was analyzed by scXRD, the specific crystal analyzed was determined to be $TCl{\cdot}H_2O$. It is possible a portion of the chlorine had been replaced with citrate, as evidenced by the slight differences in FTIR spectra and pXRD diffractograms; however, successive recrystallization attempts did not produce phase pure material as measured by scXRD. Given further development time a method may possibly be developed which could separate a potential thiamine citrate salt from $TCl{\cdot}H_2O$ but at this time, no such method has been developed.

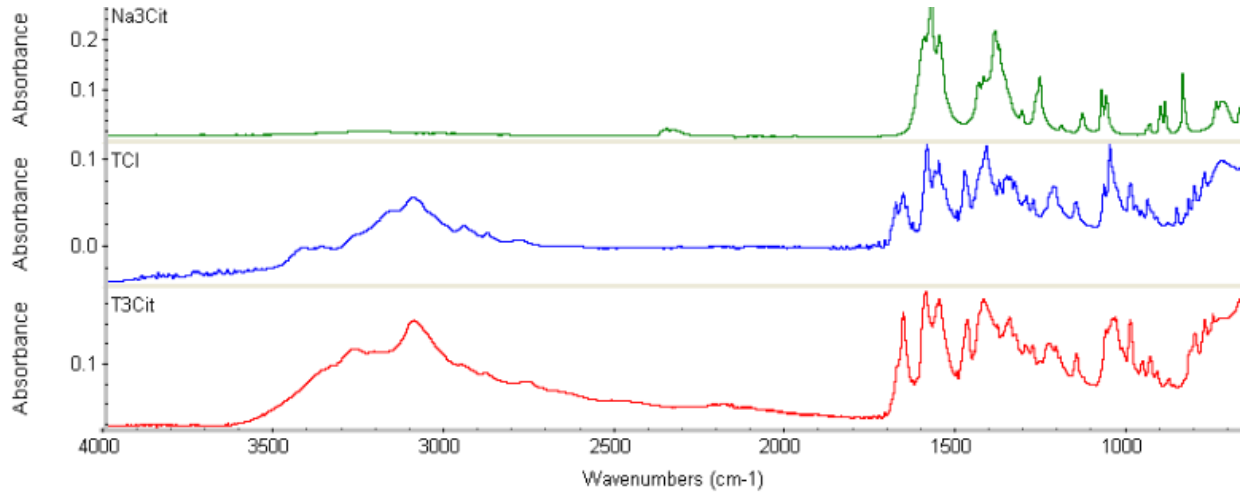


Figure 3.14 FTIR spectra for TCl·H_2O, Na_3Citrate, and the precipitant formed from the reaction between these two materials

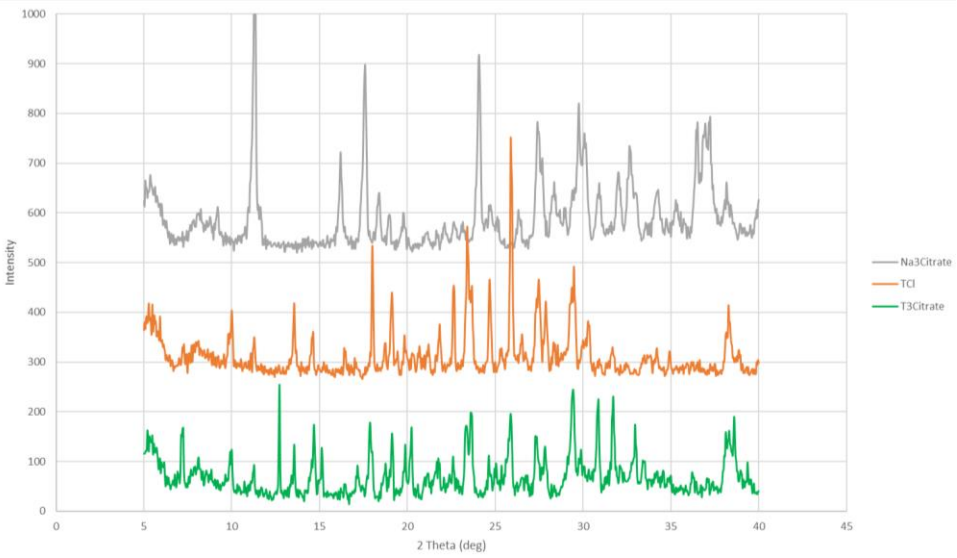


Figure 3.15 pXRD diffractograms for TCl·H_2O, Na_3Citrate, and the precipitant formed from the reaction between these two materials

3.4.4 3.4.4 Successful Attempts to Form New Thiamine Salts

Having met with very limited success using the double replacement reaction route, a new method was explored. The motivation behind developing this method was to eliminate the chloride ion completely from the system, thus making the formation of TCl·H_2O impossible. To

date, through the use of this method, five new thiamine salts have been prepared. Presently, further development of the process to synthesize these salts and characterization of their properties is ongoing. No further information about these materials can be given at this time.

3.5 Conclusion

In this chapter various salts of thiamine (vitamin B1) are explored and compared. Two methods were explored in an attempt to create new salts of thiamine. One of these methods was successful in producing new salt forms. Degradation of the starting material for these methods, $TCl{\cdot}H_2O$, was determined in aqueous solutions of concentrations ranging from 1 to 256 mg/mL. It was found that the activation energy of degradation was lower than has been reported for both TMN and TClHCl. Properties of $TCl{\cdot}H_2O$ such as melting point and solubility were also determined and are presented. With further development and characterization these new salt forms may find their place as food ingredients due to differences in hygroscopicity and/or stability compared to TMN and TClHCl. Characterization of $TCl{\cdot}H_2O$ assists in this development by increasing the understanding of its solid state properties and susceptibility to degradation in aqueous solutions which is how these salt forming reactions are carried out.

3.6 References

(1) Dwivedi, Basant K., and Roy G. Arnold. "Chemistry of Thiamine Degradation." *Journal of Food Science* 37.6 (1972): 886-88.

(2) Mitrovich, Velo. Curing Conundrum. Meat Packing Journal, meatpacking.info/2017/01/25/curing-conundrum/.

(3) Clark, Christine M, and Barbara L Dutrow. “Single-Crystal X-Ray Diffraction.” Geochemical Instrumentation and Analysis, Science Education Resource Center, serc.carleton.edu/research_education/geochemsheets/techniques/SXD.html.

(4) Lonsdale, Derrick. "A Review of the Biochemistry, Metabolism and Clinical Benefits of Thiamin(e) and Its Derivatives." *Evidence-Based Complementary and Alternative Medicine*, vol. 3, no. 1, 2006, pp. 49–59., doi:10.1093/ecam/nek009.

(5) Fattal-Valevski, Aviva. "Thiamine (Vitamin B1)." *Journal of Evidence-Based Complementary & Alternative Medicine*, vol. 16, no. 1, 2011, pp. 12–20., doi:DOI: 10.1177/1533210110392941.

(6) R. R. Williams, J. K. Kline, *Journal of the American Chemical Society*, 1936, p. 58

(7) Contant, P., et al. "ChemInform Abstract: A New Convergent Synthesis of Thiamine Hydrochloride." *ChemInform*, vol. 21, no. 45, June 1990, doi:10.1002/chin.199045320.

(8) Labuza, T. P., and J. F. Kamman. "Comparison of Stability of Thiamin Salts at High Temperature and Water Activity." *Journal of Food Science*, vol. 47, no. 2, 1982, pp. 664–665., doi:10.1111/j.1365-2621.1982.tb10146.x.

(9) Todd, A. R., and F. Bergel. "Aneurin. Part VII. A Synthesis of Aneurin." *Journal of the Chemical Society (Resumed)*, 1937, p. 364., doi:10.1039/jr9370000364.

(10) Burdick, David. "Thiamine (B1)." *Kirk-Othmer Encyclopedia of Chemical Technology*, 4 Dec. 2000, doi:https://doi.org/10.1002/0471238961.2008090102211804.a01.

(11) Voelker, Adrienne L., et al. "Chemical Stability and Reaction Kinetics of Two Thiamine Salts (Thiamine Mononitrate and Thiamine Chloride Hydrochloride) in Solution." *Food Research International*, vol. 112, 2018, pp. 443–456., doi:10.1016/j.foodres.2018.06.056.

(12) *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. National Academies Press, 1998. 0-309-59725-0

(13) Youmans, Hubert. "Measurement of PH of Distilled Water." *Journal of Chemical Education*, vol. 49, no. 6, 1972, p. 429., doi:10.1021/ed049p429.

(14) S. Damodaran, K.L. Parkin, O.R. Fennema (Eds.), Fennema's food chemistry (4th ed.), CRC Press/Taylor & Francis, Boca Raton (2008), pp. 439-521

(15) Farrer, K. T. H. "The Thermal Destruction of Vitamin B1." *British Journal of Nutrition*, vol. 2, no. 03, 1948, p. 242., doi:10.1079/bjn19480042.

(17) Williams, Robert R., and A. E. Ruehle. "Studies of Crystalline Vitamin B1. XI. Presence of Quaternary Nitrogen1." *Journal of the American Chemical Society*, vol. 57, no. 10, 1935, pp. 1856–1860., doi:10.1021/ja01313a029.

(18) "Thiamine Mononitrate." *Thiamine Mononitrate*, Royal Society of Chemistry, www.chemspider.com/Chemical-Structure.10308.html?rid=b003ac45-7e1b-47b3-a158-b883760ceee7.

(19) "Thiamine hydrochloride." *Thiamine hydrochloride*, Royal Society of Chemistry, www.chemspider.com/Chemical-Structure.10308.html?rid=b003ac45-7e1b-47b3-a158-b883760ceee7.

(20) "Thiamine chloride." *Thiamine chloride*, Royal Society of Chemistry, www.chemspider.com/Chemical-Structure.10308.html?rid=b003ac45-7e1b-47b3-a158-b883760ceee7.

(21) Turfus, S C., et al. "Chapter 25 - Pharmacokinetics." *Pharmacognosy*, Academic Press, 2017, pp. 495–512. https://doi.org/10.1016/B978-0-12-802104-0.00025-1

(22) "Sodium Citrate." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Sodium_citrate#section=Solubility.

(23) "Monosodium Glutamate." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/ Monosodium_Glutamate #section=Solubility.

(24) No DCR. Saccharin and its salts. 2th Ed 1987

(25) "Sodium Benzoate." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/ sodium_benzoate#section=Solubility.

(26) "Sodium Lactate Product Ingredient for Lactic Acid." *DrugBank*, www.drugbank.ca/salts/DBSALT001765.

(27) "Sodium Bicarbonate." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/ sodium_bicarbonate#section=Solubility.

(28) "Sodium Phosphate Monobasic." *Chemical Book*, www.chemicalbook.com/ChemicalProductProperty_EN_CB6219354.htm.

(29) "Sodium ascorbate." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/ sodium_ascorbate#section=Solubility.

{30) "Citric Acid Monohydrate." *Sigma-Aldrich*, www.sigmaaldrich.com/catalog/product/sial/c1909?lang=en®ion=US.

(31) "Lactic Acid." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/lactic_acid#section=pKa.

(32) Hall, James F. *Experimental Chemistry*. Houghton Mifflin, 2003. Appendix 5

(33) "Sulfuric Acid." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/sulfuric_acid#section=pKa.

(34) "Principles of General Chemistry." *Sociological Perspectives on Education*, 2012books.lardbucket.org/books/principles-of-general-chemistry-v1.0/index.html. Appendix C: Dissociation Constants and pKa Values for Acids at 25°C

(35) "Taurine." *DrugBank*, www.drugbank.ca/drugs/DB01956.

(36) Hunt, Ian R. "Ch27 PKa and PI Values." *Chapter 27: Amino Acids, Peptides and Proteins*, University of Calgary, www.chem.ucalgary.ca/courses/350/Carey5th/Ch27/ch27-1-4-2.html.

CHAPTER 4. SUMMARY AND FUTURE DIRECTIONS

4.1 Summary

This work discussed two separate explorations into solid food ingredients. The first piece discussed advancement in the understanding of the crystallization of amorphous sucrose; specifically, the affects a range of polyphenols had on the crystallization rate. Aglycones, monoglycosylated polyphenols, polyglycosylated polyphenols, and food ingredients known to be rich in polyphenols were lyophilized with sucrose at both 1 and 5 wt% relative to sucrose. Amorphous sucrose and sucrose-polyphenol blends were stored at 11%, 23%, and 33% RH and 25°C and XRD was used to track the transition from amorphous to crystalline solid state. Vapor sorption profiles were generated to further understand this transition. Samples were held at a constant 40% RH and 25°C and the time for sucrose to crystallize was measured. Samples were also held in an environment wherein RH was increased stepwise from 0 to 80% RH in 5% increments. KF was used to determine moisture content and DSC was used to determine T_g.

Of the materials tested, the bulk food ingredients produced the most substantial changes in amorphous state stability; at 5 wt% these ingredients increased the time to crystallize by a factor of two to greater than six. Monoglycosylated polyphenols produced the next most substantial change, increasing the time to crystallize by a factor of two to three when added at 5 wt%. Polyglycosylated and nonglycosylated did not produce significant effects in the majority of experiments performed. The lack of effect observed by addition of polyglycosylated polyphenols was unexpected bases on previous research into the effects of naringin and glycyrrhizic acid on the crystallization of amorphous sucrose. It is believed the rigid 6-1 linkage between sugar units in hesperidin and rutin did not allow for association between sucrose and the non-terminal sugar, resulting in the lack of observed effects.

In the context of previous research, monoglycosylated polyphenols and raw plant ingredients performed as well as any materials considered, namely trivalent salts, disaccharides, and trisaccharides. These results may help guide formulation decisions in applications where amorphous sucrose is the desired solid state. Properties such as softer textures and more rapid dissolution make amorphous solids ideal in certain applications such as powdered beverages or baked goods. Previously tested materials which have been shown to substantially increase amorphous sucrose stability may not represent ideal food ingredients due to strong flavors at the concentrations studied. While monoglycosylated polyphenols may contribute bitter flavors, these raw food ingredients are already present in many foods and represent a more appealing choice for formulating products than previously studied materials. Another benefit of formulating products with these ingredients is the potential for positively impacting the health of consumers.

The second portion of this work explored different salts of thiamine. A method was developed which was used to successfully create previously unreported salts of thiamine. Five new salts have been produced, albeit in limited quantities and purity. Development of their synthesis and characterization of these forms is ongoing. The method used to produce these salts made use of silver nitrate, a water soluble salt of silver. Silver nitrate was reacted with a sodium-anion salt, replacing the sodium with silver and forming a silver-anion salt. This salt was then reacted with $TCl \cdot H_2O$, producing a thiamine-anion salt. Attempts were made initially to produce these salt forms without a silver intermediate through a simple double replacement reaction between either the sodium-anion salt or the acids of these anions with $TCl \cdot H_2O$; however, this was not met with success. At this time, limited quantities and purity of materials have been obtained and characterization of these new forms is presently limited.

In addition to work preparing new salt forms, characterization of the key intermediate, TCl·H_2O, was performed. While this material has been previously reported, limited information on its properties was available. Degradation kinetics in aqueous solutions of concentrations ranging from 1 to 256 mg/mL were determined. Solutions were stored at 25, 40, 60, 70, and 80°C and HPLC was used to track the portion of intact thiamine remaining over time. TCl·H_2O was found to have a stability which was less than that of TMN and TClHCl at all concentrations studied. As the pH of aqueous solutions of TCl·H_2O were found to be greater than those of TMN and TClHCl at a given concentration, this result was expected and is in line with previously reported data. Melting point of TCl·H_2O was also determined to be 135-137°C which is substantially lower than that of TMN and TClHCl. Finally, the solubility of TCl·H_2O was measured to be 459.3 mg/mL at 25°C.

The potential usefulness of TCl·H_2O as a food ingredient is limited as judged by the data collected in this work. Its lower aqueous solubility and lesser stability in solution compared to TClHCl makes it a less ideal ingredient in beverage applications. Stability in solid food systems was not explored, thus its usefulness in these applications cannot be compared to TMN. The new thiamine salts which have been produced through the silver method may have properties beneficial to food systems; however, further development is needed before proper characterization may be performed and recommendations made.

4.2 Future Directions

More can be done to understand the mechanism behind the increase in stability of amorphous sucrose observed when sucrose is lyophilized in the presence of some polyphenols. As there were differences observed in the effects of polyglycosylated polyphenols which are believed to be due to the different linkages between sugar units, it would be helpful to directly

compare identical glycosylated polyphenols which only differ in the location of this linkage. It also would help to be able to directly compare one aglycone associated with different monosaccharides such as glucopyranoside, fructopyranoside, and galactopyranoside. The primary hurdle in these studies would be the cost of the polyphenols. Many cost on the order of $100/mg which renders these kinds of experiments cost prohibitive.

Another benefit to understanding may come from investigating which fraction of the raw food ingredients were producing the increase in stability observed. While green tea extract is labeled as consisting entirely of polyphenols, green coffee, matcha, and cocoa powder all contain many other components such as simple sugars, lipids, etc. One or more of these ingredients could be selected and all materials which represent a sizeable fraction of the total mass can be lyophilized with sucrose individually. Thus, the effect from lipids could be distinguished from the effects of simple sugars and so on. This study would be significantly less expensive as these ingredients are produced on a much larger scale than purified polyphenols.

It would also be worthwhile to test the lifetime of amorphous sucrose treated with polyphenols when held at RHs above 33%, temperatures about 25°C, and for periods greater than 30 days. Many foods have a water activity of greater than .33 and any amorphous sucrose in these products will thus be exposed to a higher RH than was studied in this work. Storage temperature can often exceed 25°C, at least temporarily, especially once the product has been purchased by the consumer. Similarly, for most food products, the desired shelf life is much greater than 30 days. Understanding the behavior at RHs above 33%, temperatures above 25°C, and over periods of time greater than 30 days is thus necessary for understanding the behavior of real food products.

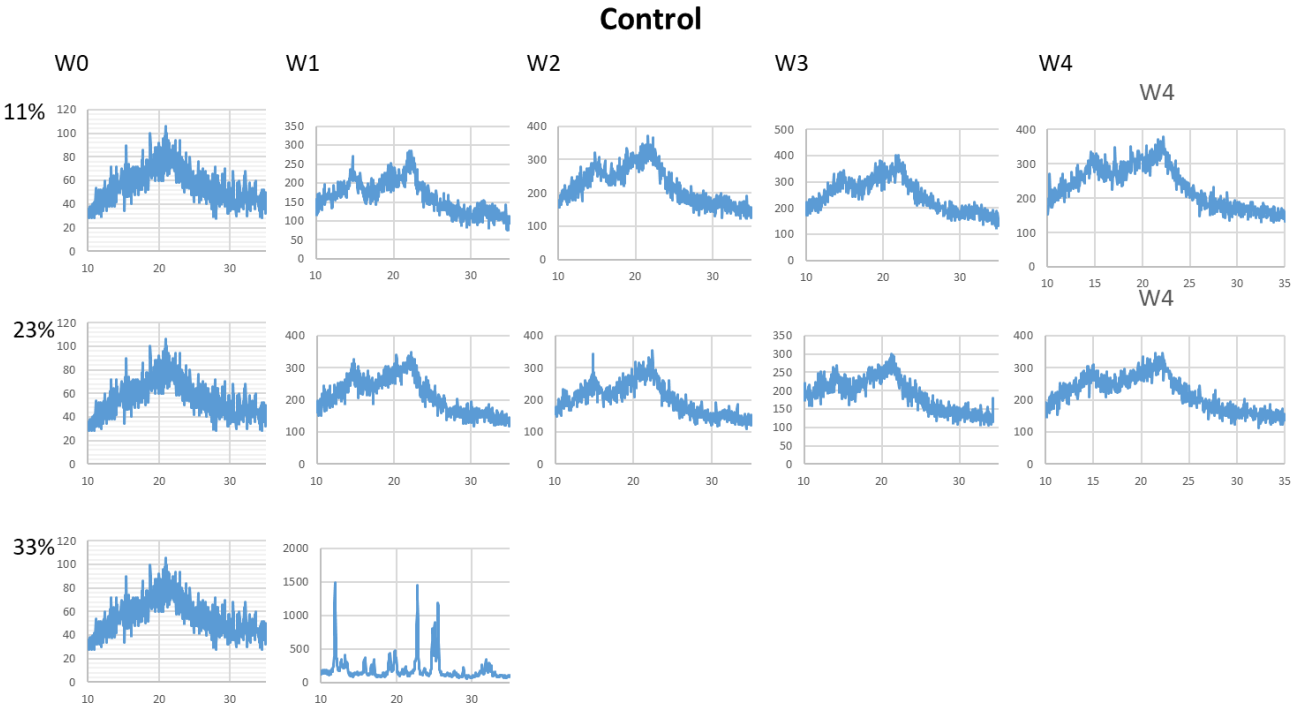
Once such experiments have been performed, more effective suggestions for formulating real food products can be made. One example of such a guidance may be lyophilizing or spray drying sucrose with ground roasted coffee to be used as a sweetener for coffee. The amorphous sucrose will dissolve more rapidly, and the addition of coffee would not negatively impact the flavor of the final product. Another potential formulation improvement may come through the addition of polyphenols. In a product like hot cocoa mix, additional cocoa polyphenols may be added to further increase the stability of amorphous sucrose, thus increasing product quality and shelf life. This use will be limited by the quantity of polyphenols which can be added without adding a bitter taste.

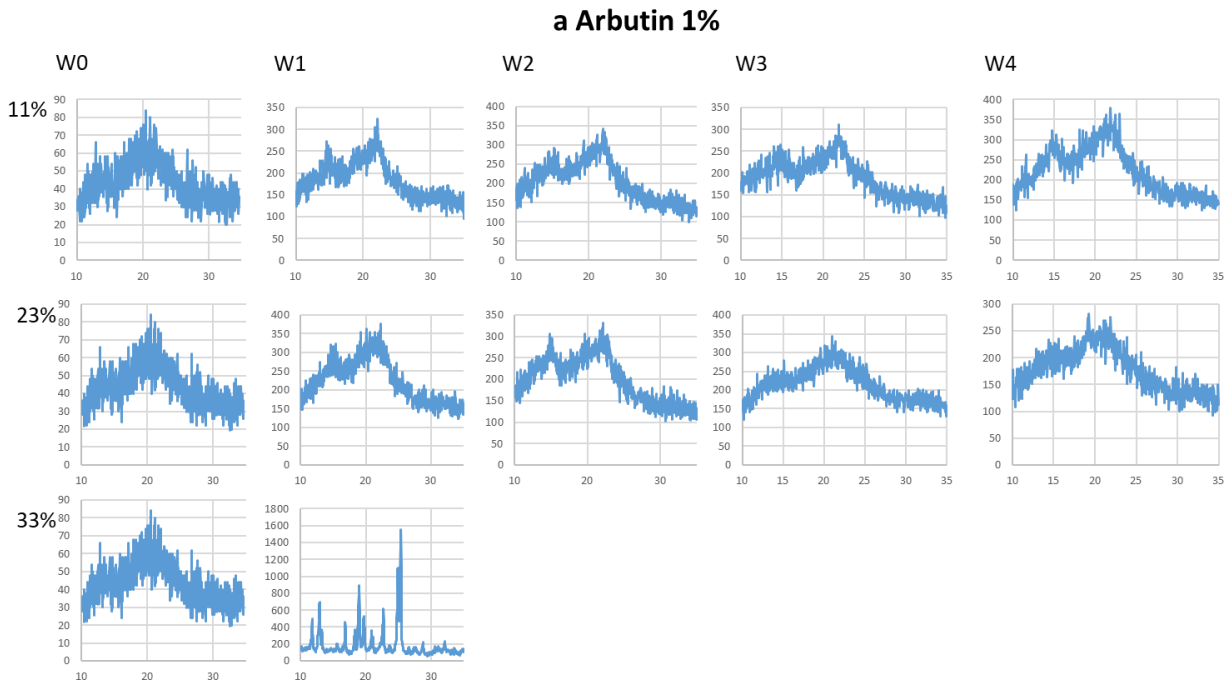
Regarding new salt forms of thiamine, two steps must be achieved before these materials may be of any use in food products. First, pure forms of these salts must be produced on a larger scale. To accomplish this, work needs to be done to further develop the reactions involved in their production. Two critical points were identified as needing the most development. First, the intermediate silver-anion salt must be more extensively characterized. The anions which have been used successfully are all multivalent. It is presently not understood whether the silver-anion salt obtained contains any material with only a portion of the sodium exchanged for silver. Also, discoloration was observed over a short time frame in these materials; for some, mere minutes of exposure to light and ambient conditions is enough to turn the originally crème colored powder a light grey. Over the time scale required to dry these solids some degree of discoloration is presently unavoidable. By understanding these qualities, these salts may be used more effectively in their reaction with thiamine. The second critical point involves the purification of these new salt forms. Simultaneous precipitation of thiamine-anion salt with $TCl{\cdot}H_2O$ has been observed. A reliable method to separate these two salts has not been fully developed at this time. In addition,

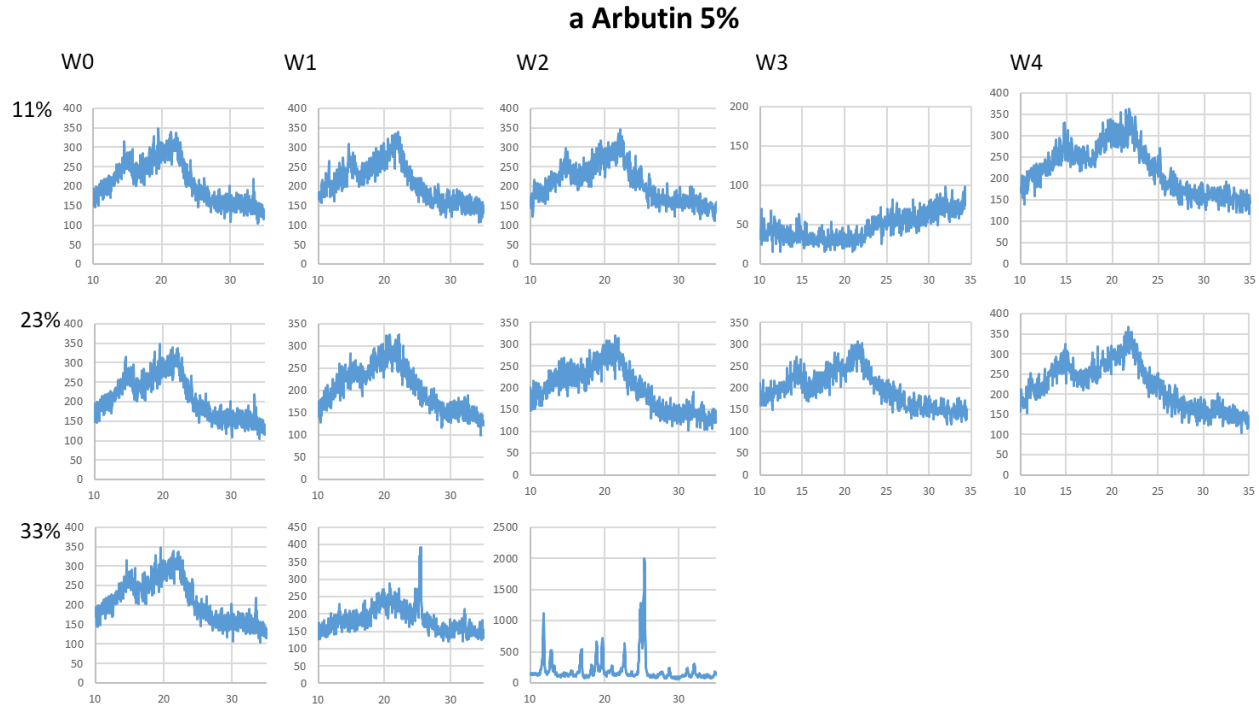
if a portion of the silver-anion salt contains sodium, precipitation of NaCl can be expected. Thus, the final product may contain a blend of thiamine-anion salt, $TCl \cdot H_2O$, and NaCl. Without a means of isolating pure thiamine-anion salt, no meaningful characterization of their properties can be obtained. Once these critical points have been addressed, the process can be scaled up. With quantities of material on the gram scale, a number of critical characterizations can be made. These include: moisture sorption isotherms, melting point, FTIR spectra, pH at various concentrations, deliquescence point, solid and aqueous degradation kinetics, and solubility. With these characterizations obtained, the potential applications of these new forms may be determined allowing their use in food products.

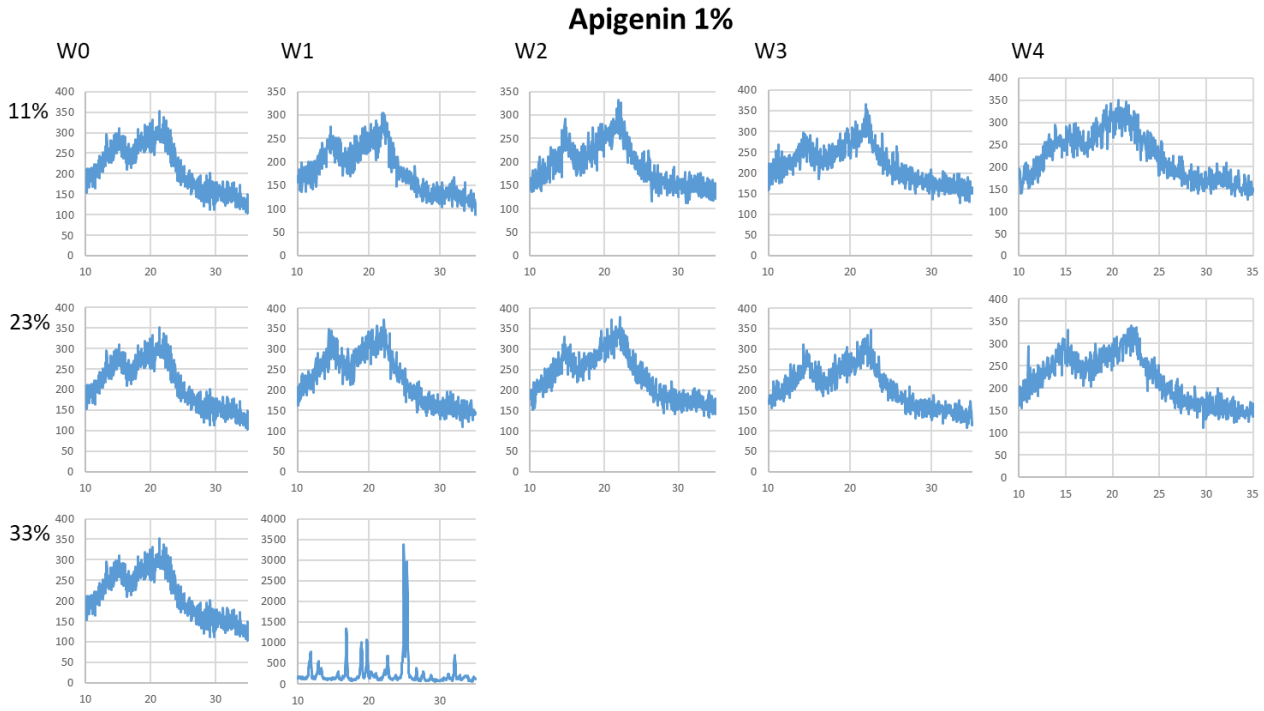
APPENDIX

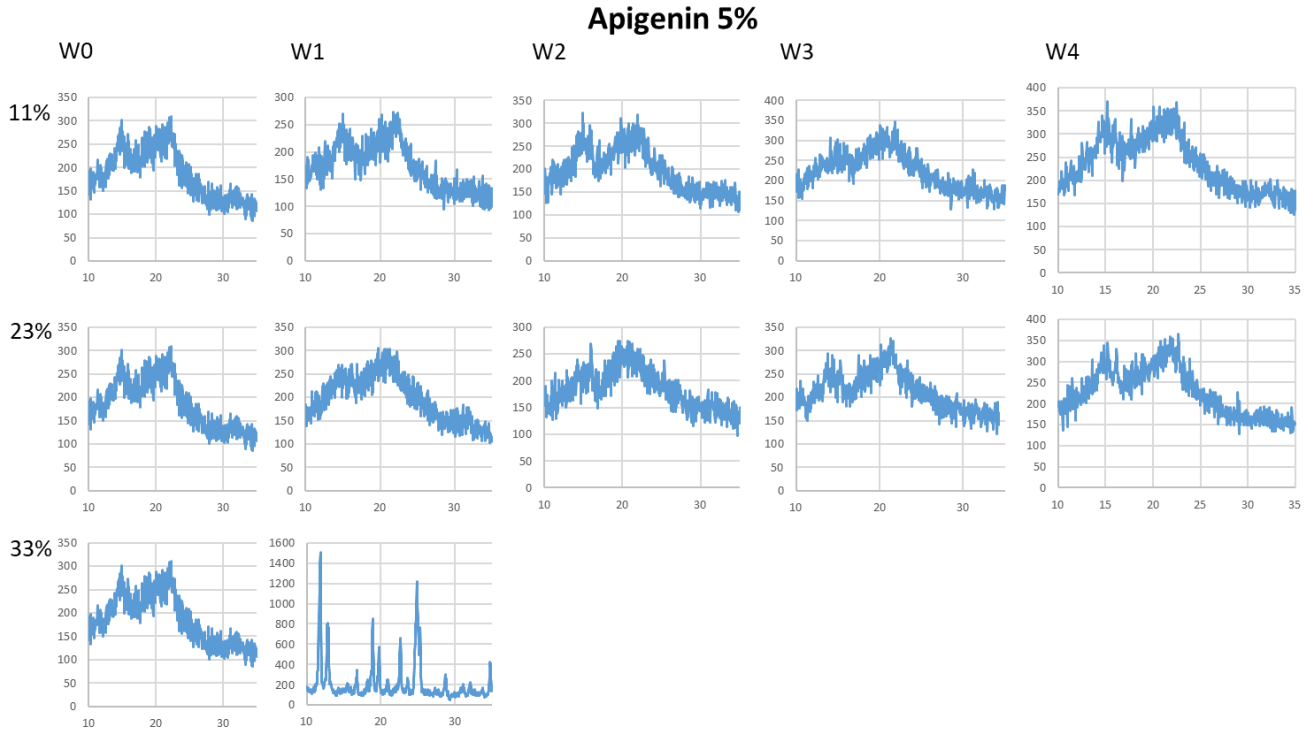
DIFFRACTOGRAMS COLLECTED IN DESICCATOR STUDIES



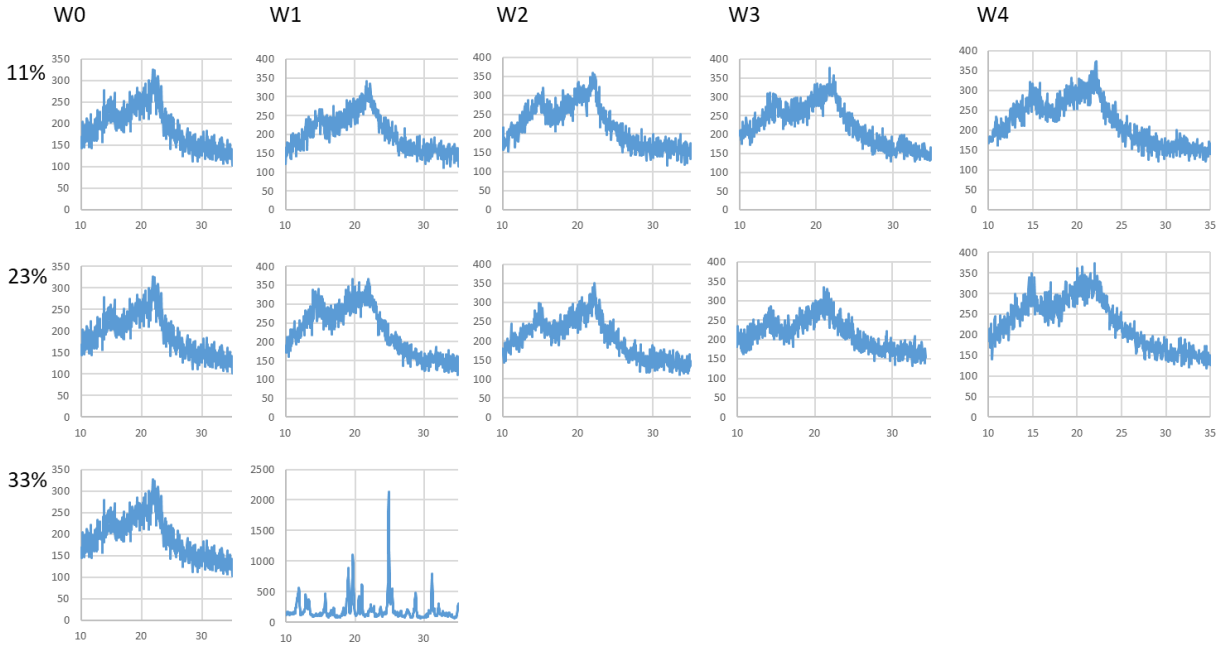

a Arbutin 1%
W0
W1
W2
W3
W4
11%
23%
33%


a Arbutin 5%
W0
W1
W2
W3
W4
11%
23%
33%

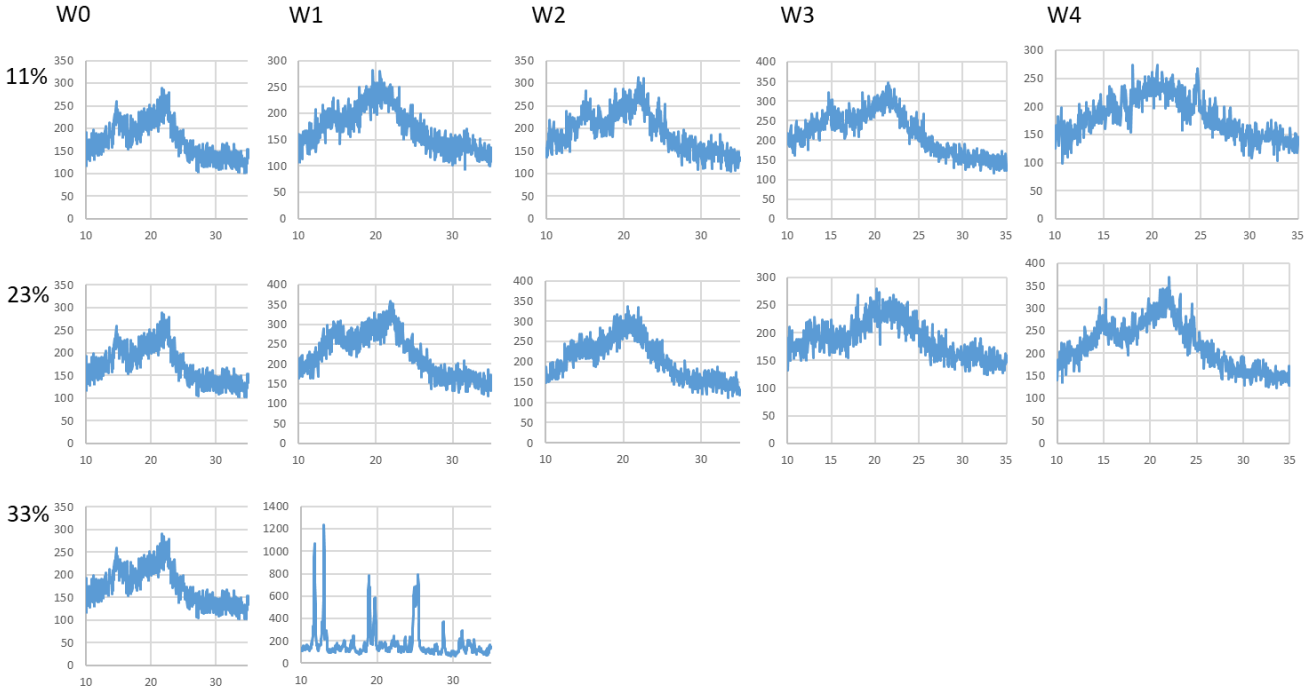

Apigenin 1%
W0
W1
W2
W3
W4
11%
23%
33%


Apigenin 5%
W0
W1
W2
W3
W4
11%
23%
33%

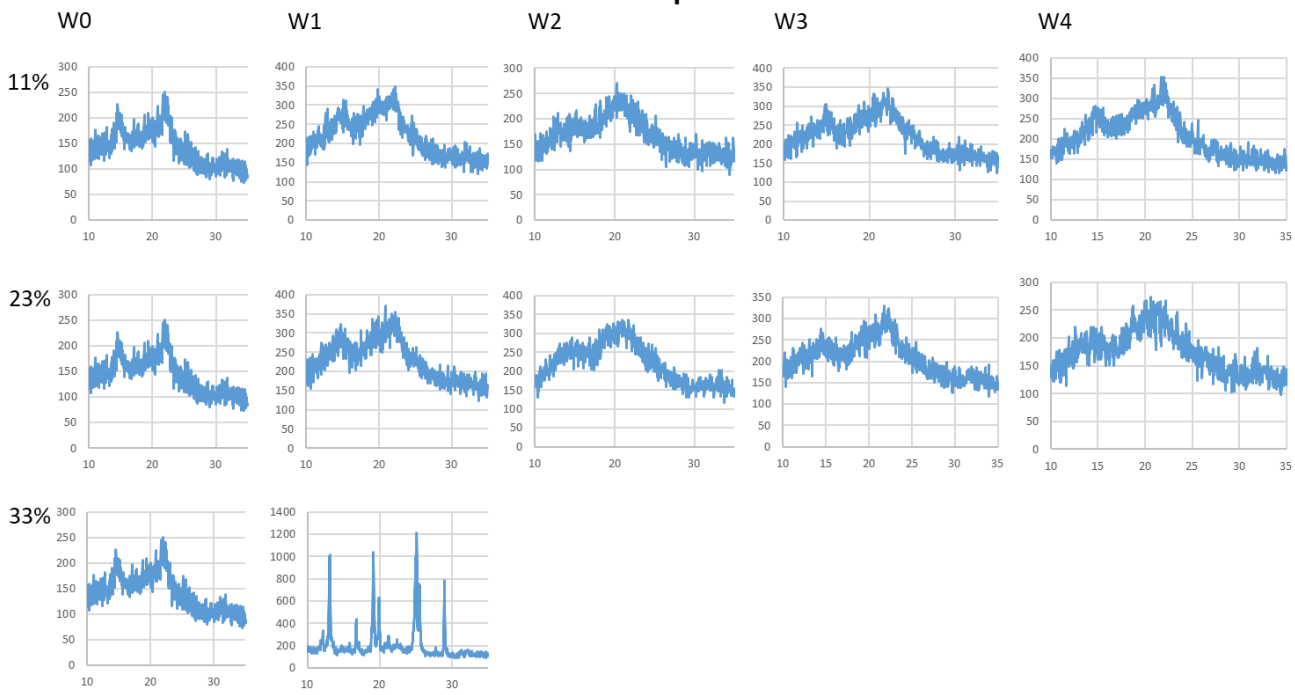
Daidzein 1%



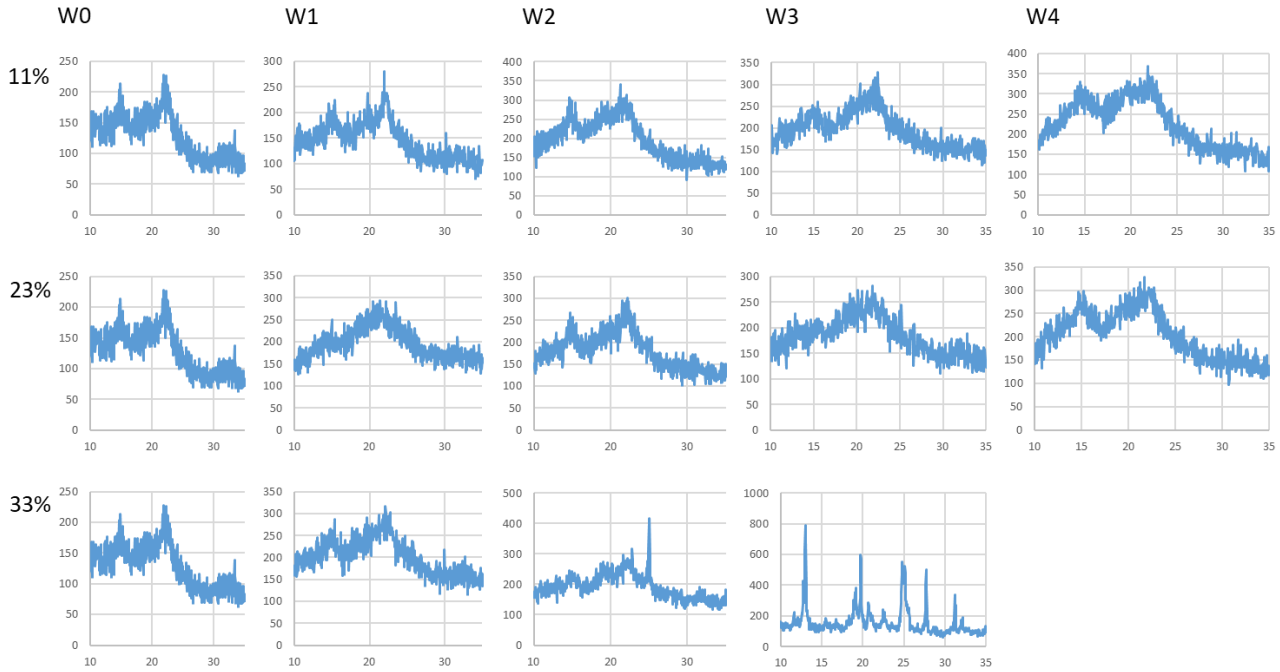
Daidzein 5%



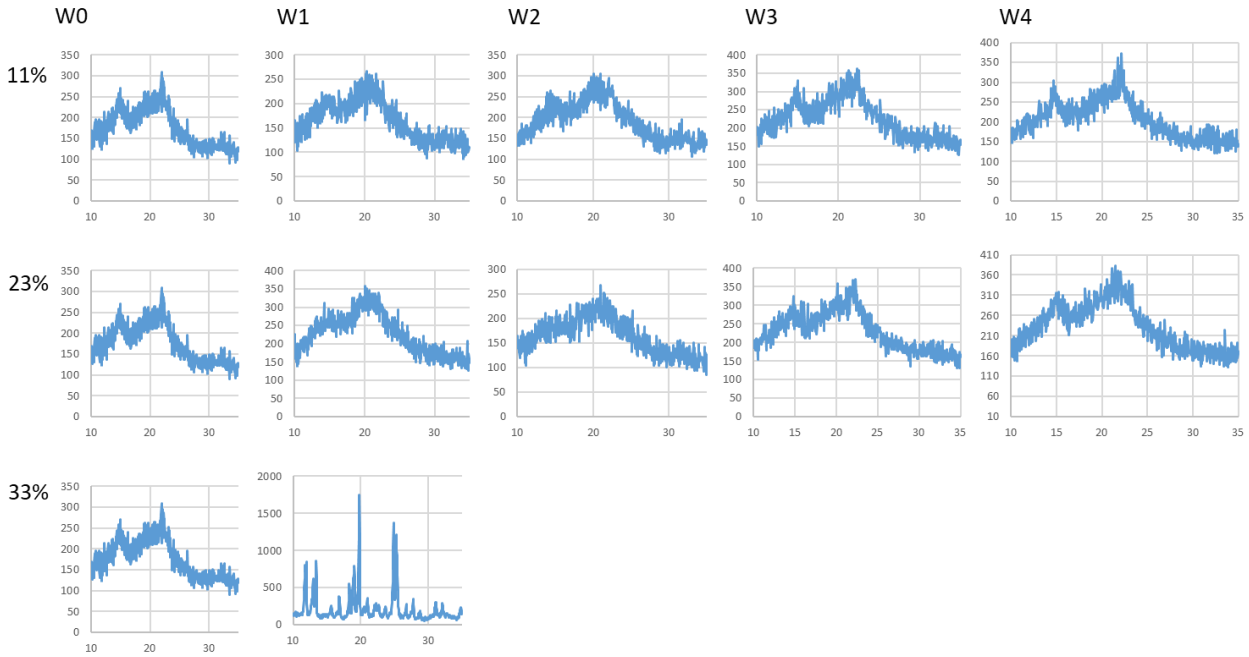
Cocoa powder 1%



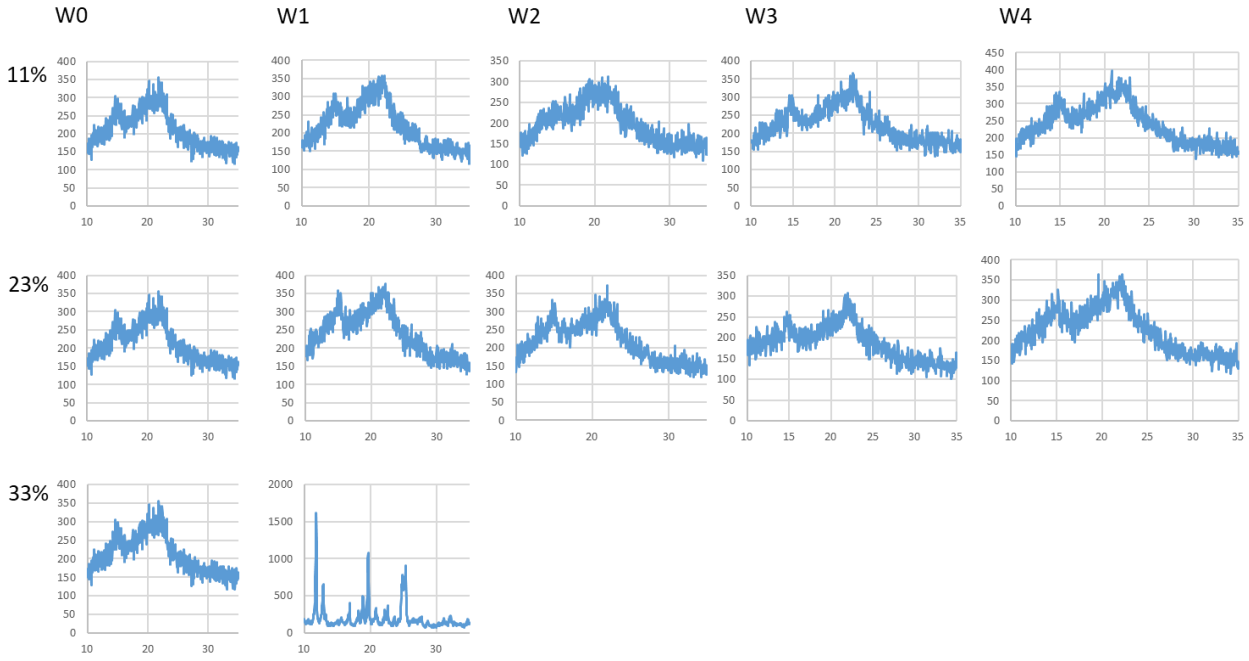
Cocoa powder 5%

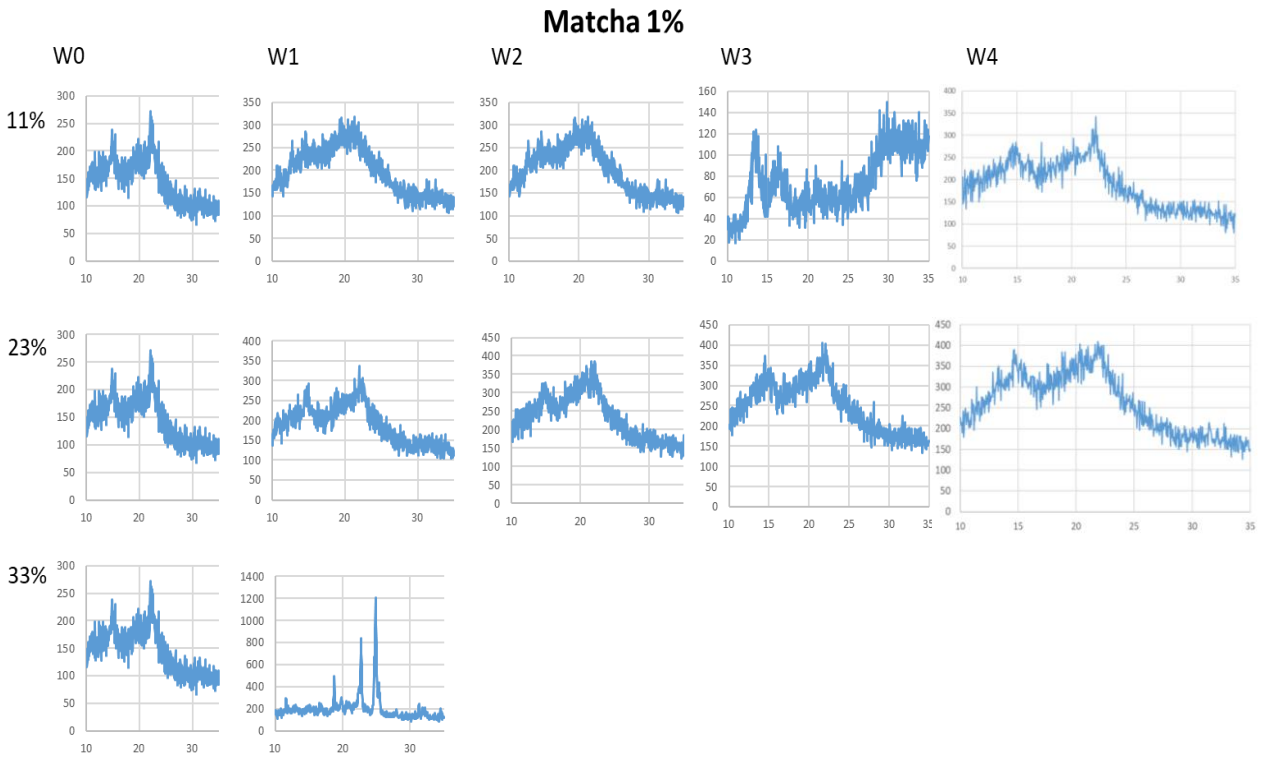


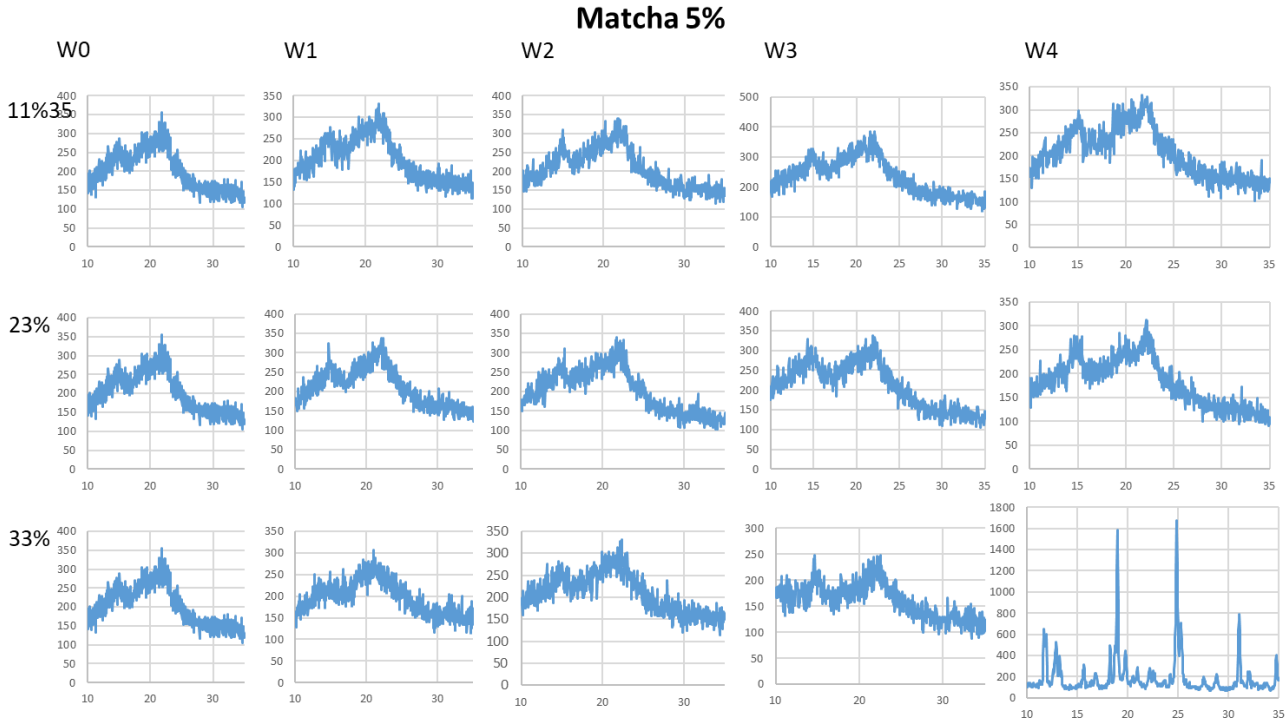
Rutin 1%

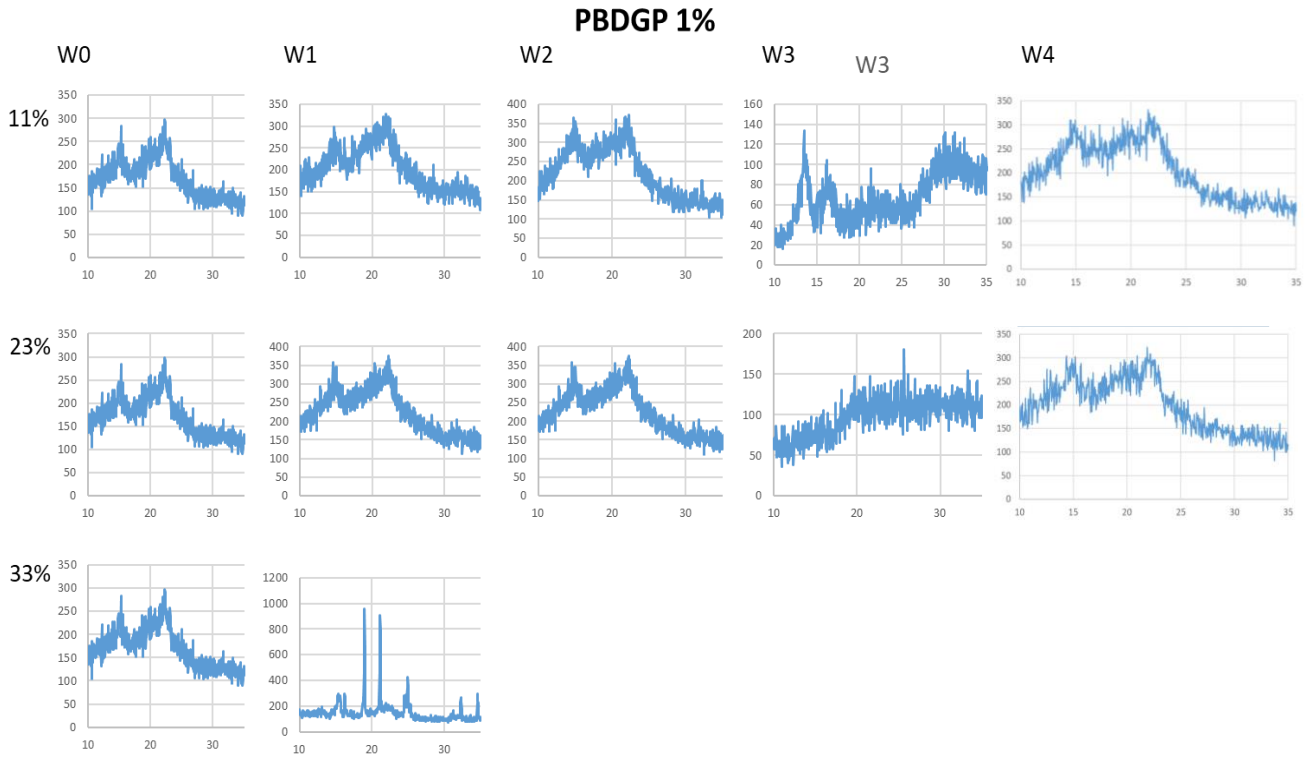


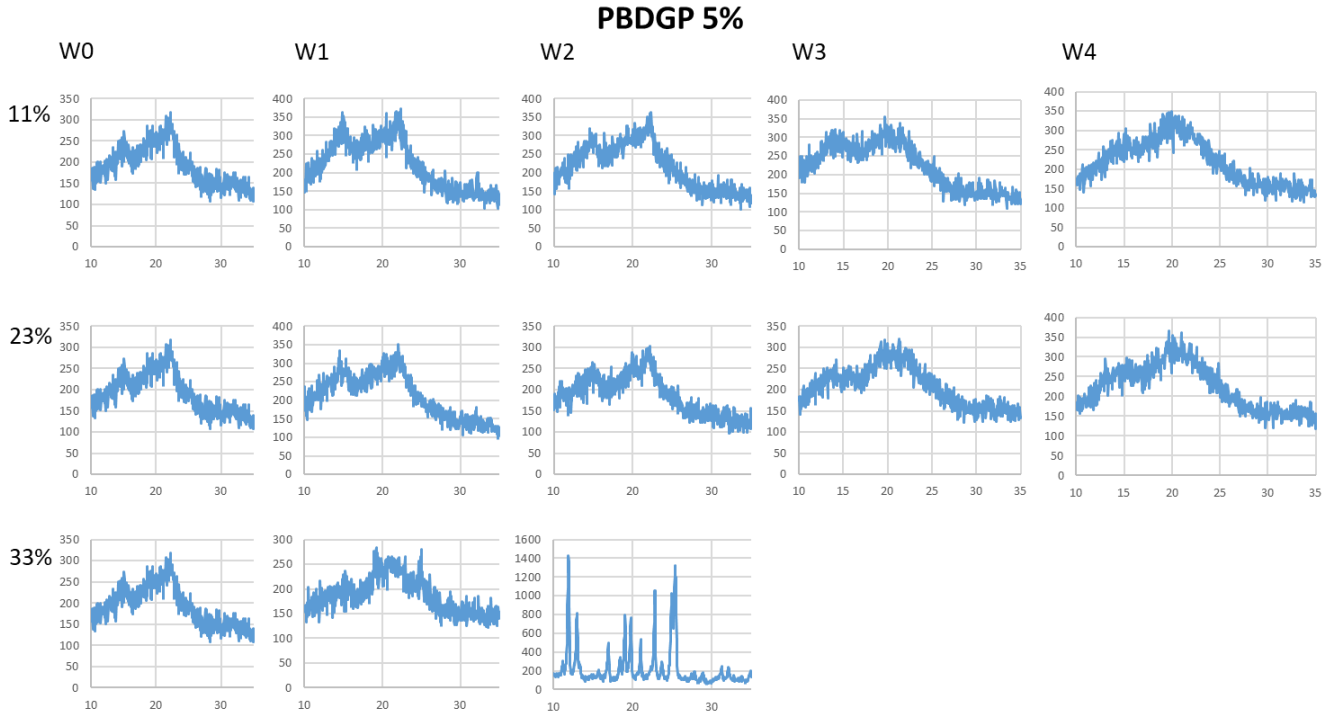
Rutin 5%



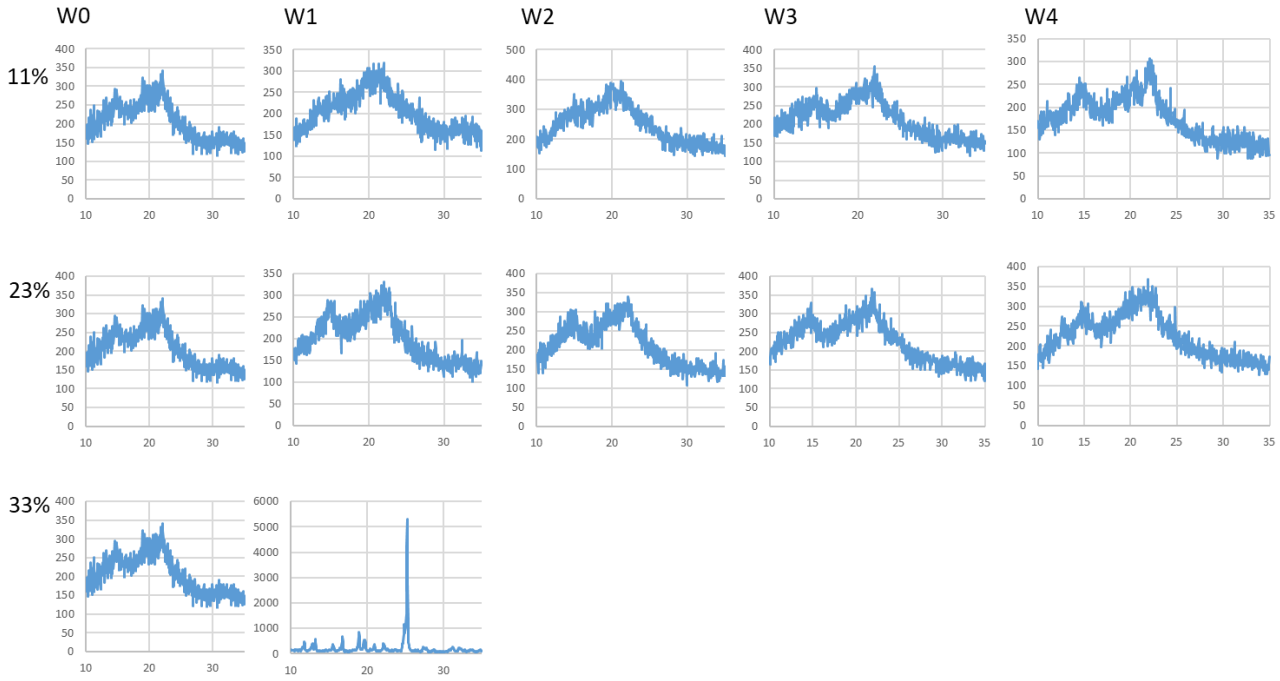

Matcha 1%
W0
W1
W2
W3
W4
11%
23%
33%


Matcha 5%
W0
W1
W2
W3
W4
11%
23%
33%

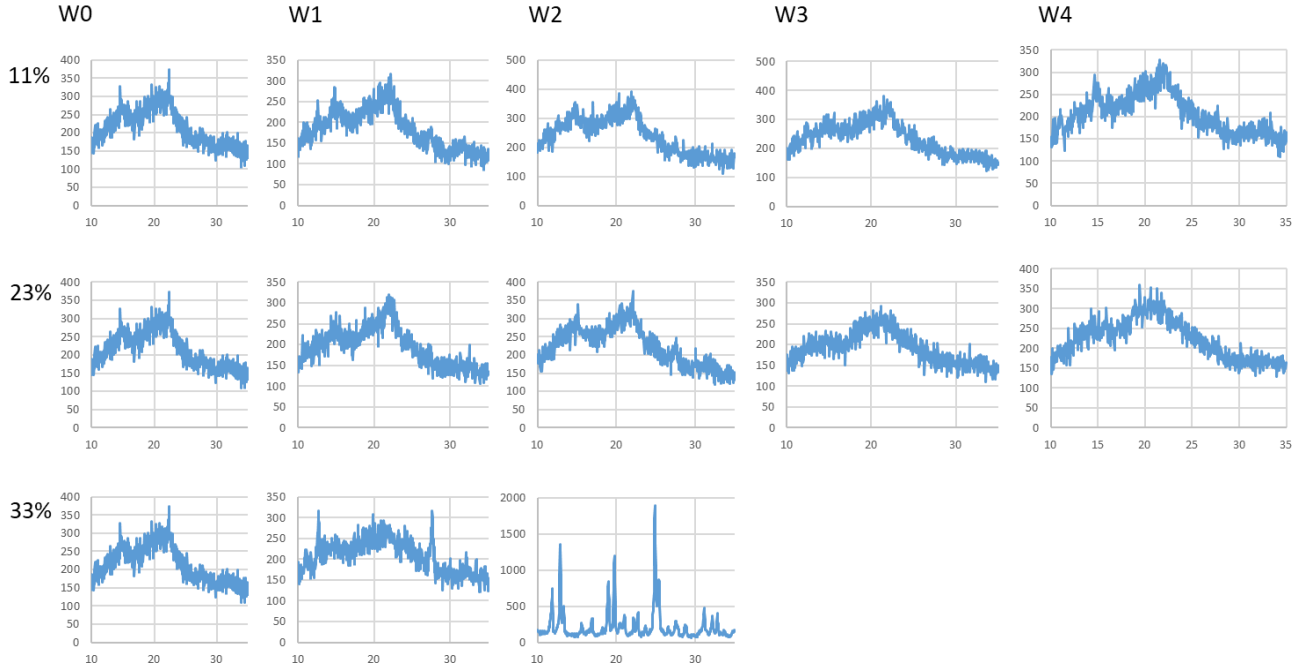

PBDGP 1%
W0
W1
W2
W3
W3
W4
11%
23%
33%

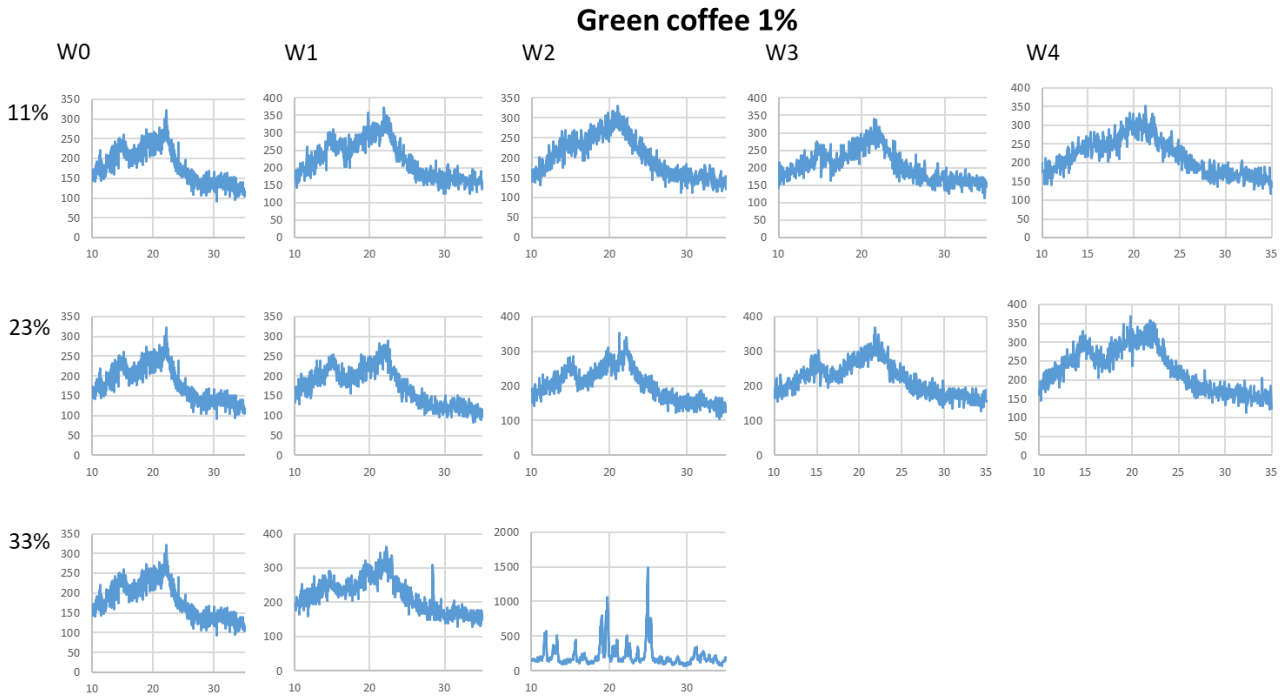

PBDGP 5%
W0
W1
W2
W3
W4
11%
23%
33%

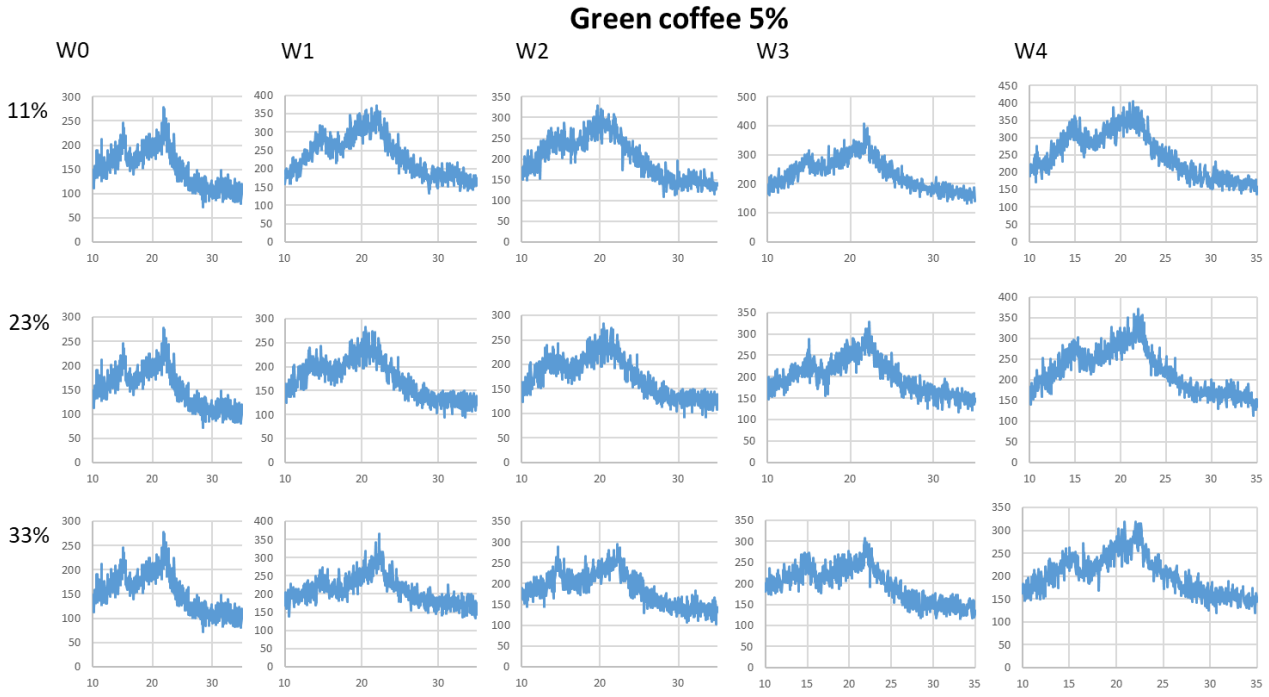
Quercetin 1%

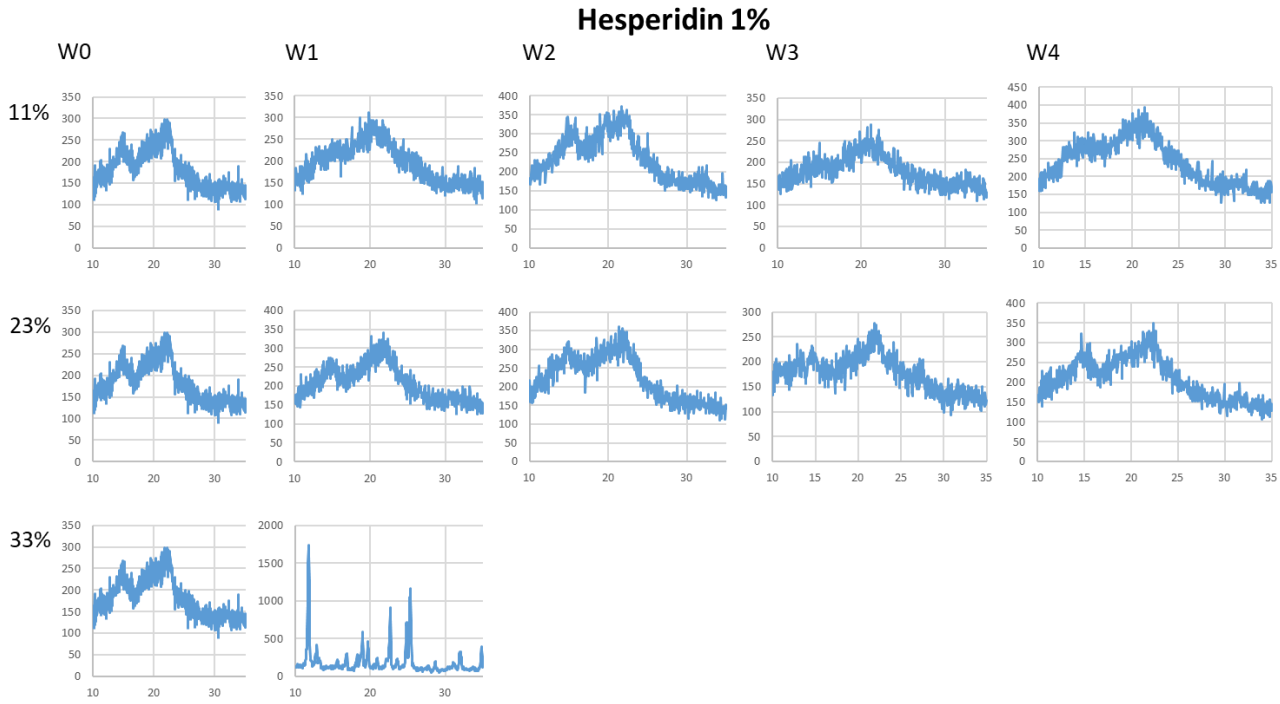


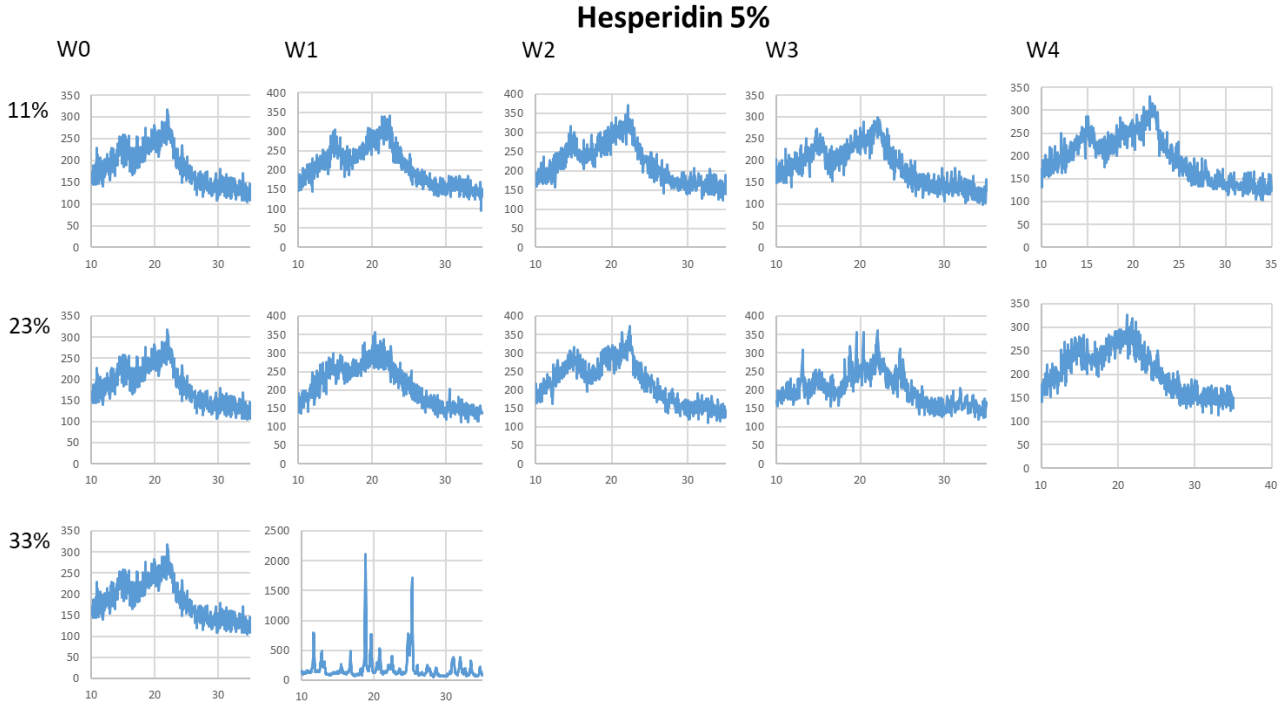
Quercetin 5%

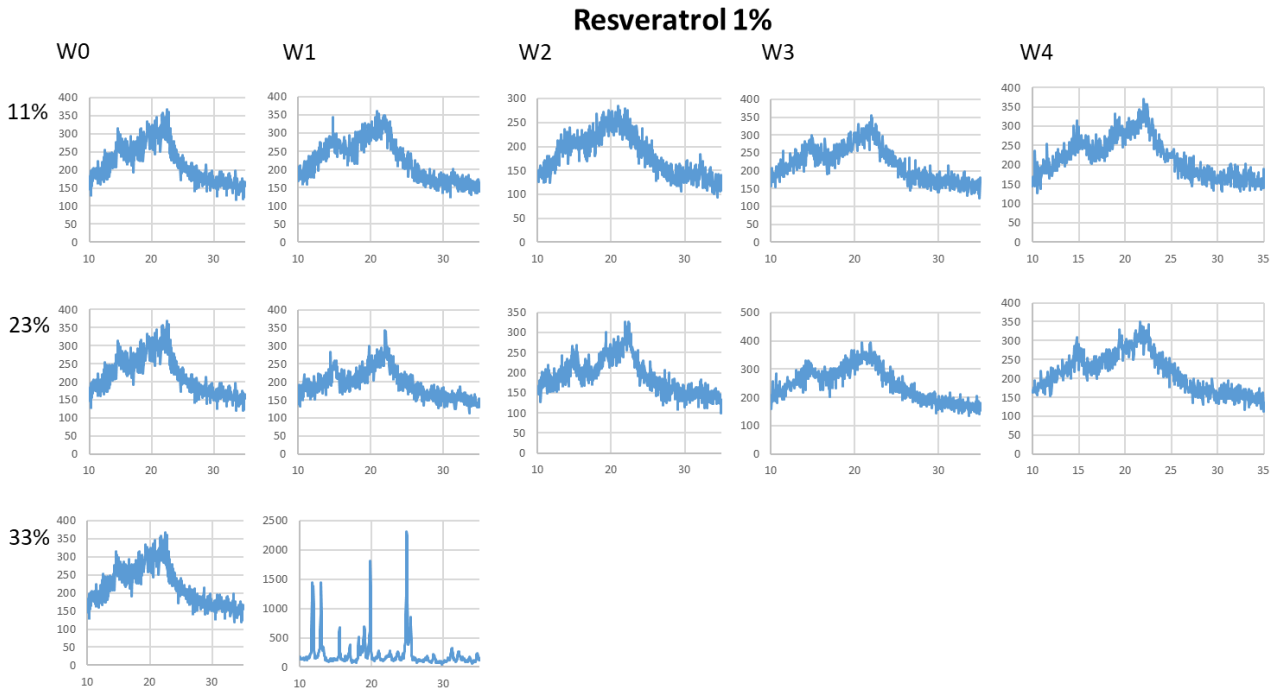


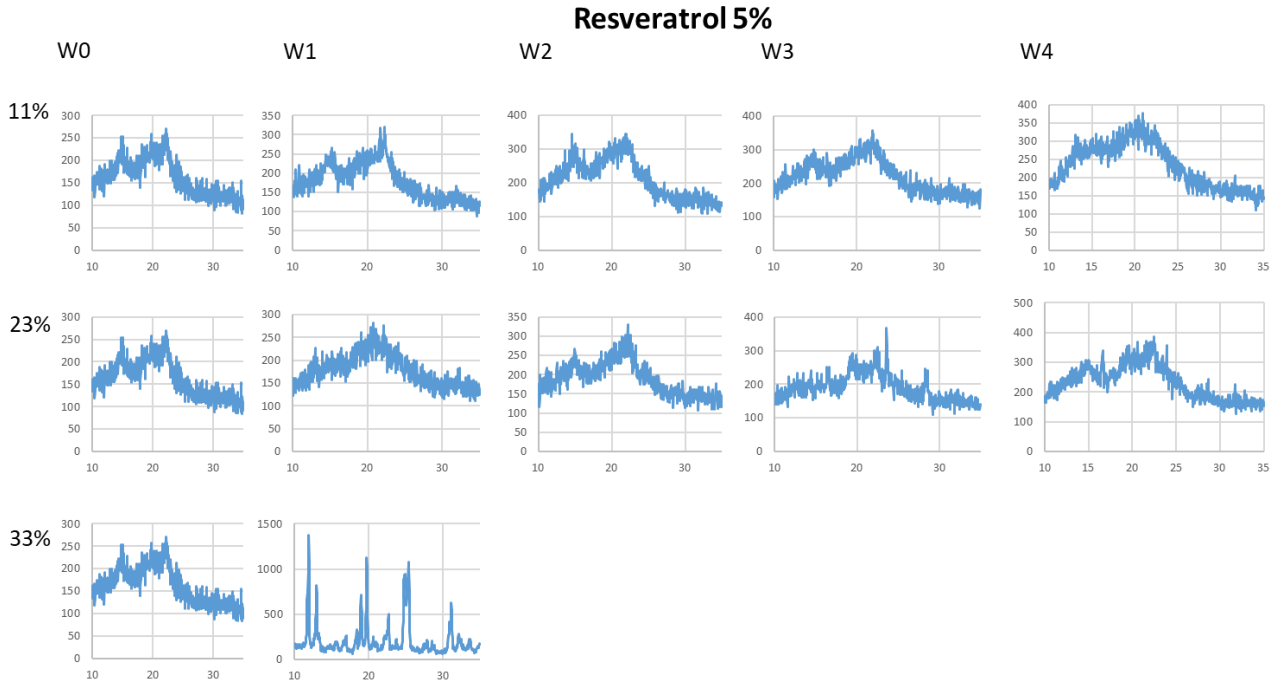

Green coffee 1%
W0
W1
W2
W3
W4
11%
23%
33%


Green coffee 5%
W0
W1
W2
W3
W4
11%
23%
33%

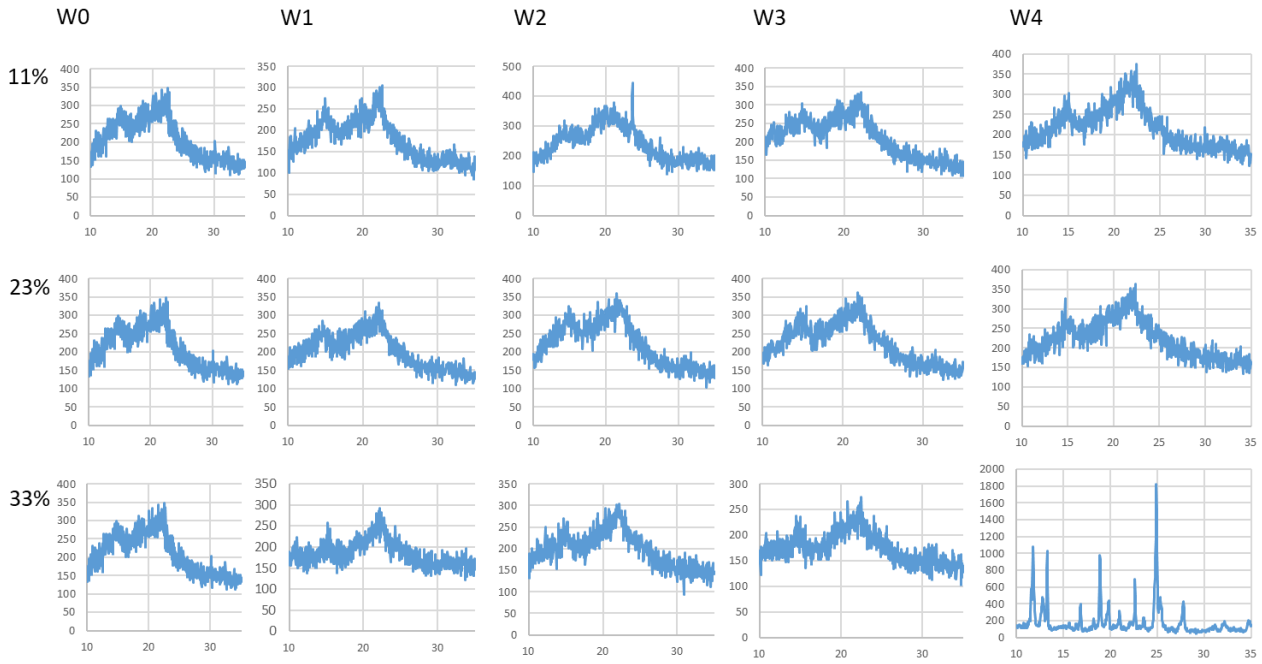

Hesperidin 1%
W0
W1
W2
W3
W4
11%
23%
33%


Hesperidin 5%
W0
W1
W2
W3
W4
11%
23%
33%

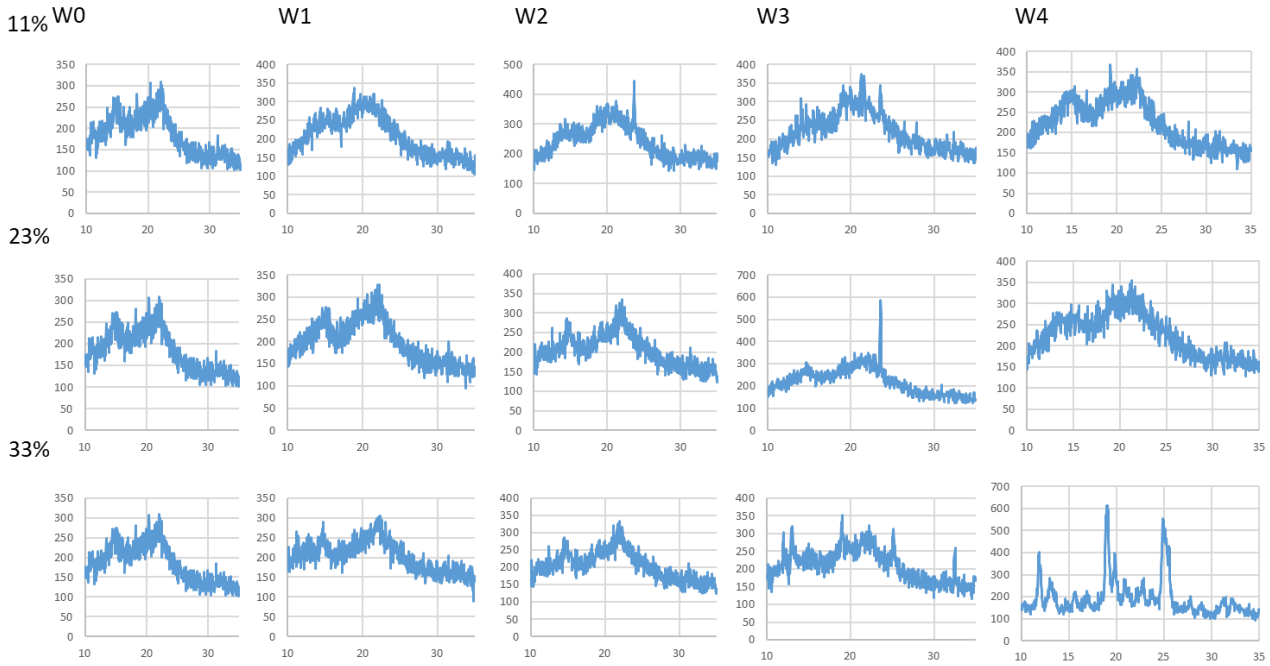

Resveratrol 1%
W0
W1
W2
W3
W4
11%
23%
33%

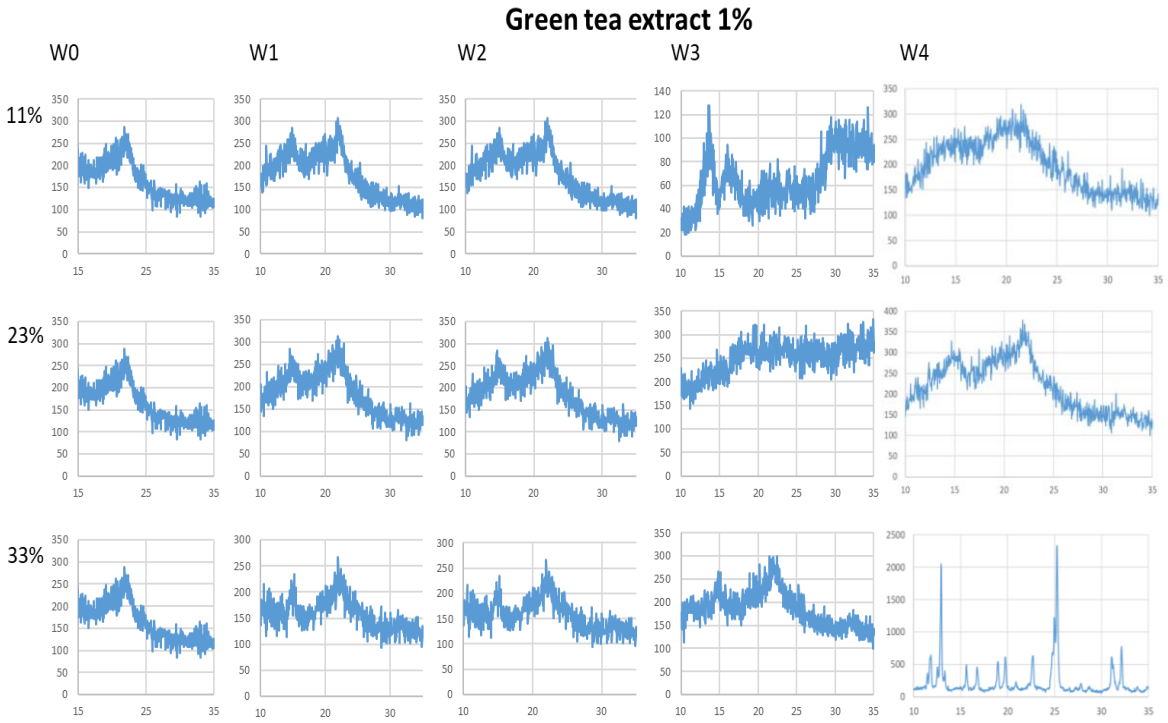

Resveratrol 5%
W0
W1
W2
W3
W4
11%
23%
33%

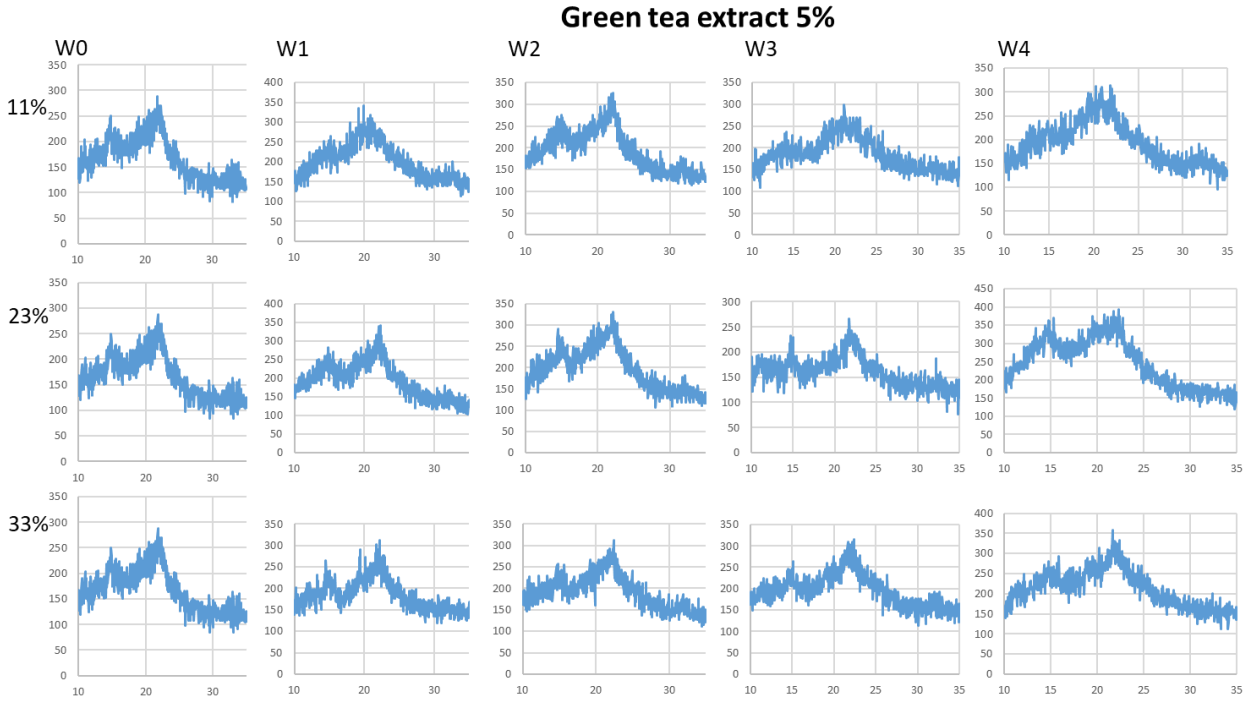
Puerarin 1%



Puerarin 5%




Green tea extract 1%
W0
W1
W2
W3
W4
11%
23%
33%


Green tea extract 5%
W0
W1
W2
W3
W4
11%
23%
33%

REFERENCES

(1) Seidell, Atherton; Linke, William F. (1919). Solubilities of Inorganic and Organic Compounds (2nd ed.). New York City*:* D. Van Nostrand Company. pp. 617–619

(2) Cramer, R. E., & Carrie, M. J. J. (1989). Structure of thiamin hydrochloride hemisulfate monohydrate. Acta Crystallographica Section C Crystal Structure Communications, 45(12), 1899–1902. http://doi.org/10.1107/S0108270189003422

(3) Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev 2000;52:673–751.

(4) Beckman CH. Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defense responses in plants? *Physiological Molecular Plant Pathology* 2000;57:101–110.

(5) Tuberoso, C. I. G., & Orrù, C. D. (2008). Phenolic compounds in food. *Progress in Food Chemistry*, (4), 1–45. http://doi.org/10.1007/s00394-008-2002-2

(6) Tomás-Navarro, M., Vallejo, F., & Tomás-Barberán, F. A. (2013). Bioavailability and Metabolism of Citrus Fruit Beverage Flavanones in Humans. *Polyphenols in Human Health and Disease*, 1, 537–551. http://doi.org/10.1016/B978-0-12-398456-2.00040-2

(7) "Flavylium." (R,R)-Stilbene Dibromide | C14H12Br2 | ChemSpider, Royal Society of Chemistry, www.chemspider.com/Chemical-Structure.128674.html.

(8) "Flavonol." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/3-Hydroxyflavone#section=Top.

(9) "Furan; 2-Phenylchromen-4-One." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/89705386#section=Top.

(10) "P-Coumaric acid." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/p-Coumaric%20acid#section=Top.

(11) "Flavanone, (-)-." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/_2S_-Flavanone#section=Top.

(12) "Gonane." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/gonane#section=Top.

(13) “Steviol.” PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Steviol#section=Top.

(14) “Sapogenin A.” PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/23265676#section=Top.

(15) Zografi, George. “States of Water Associated with Solids.” *Drug Development and Industrial Pharmacy*, vol. 14, no. 14, 1988, pp. 1905–1926, doi:10.3109/03639048809151997. (16) Brovchenko, Ivan, and Alla Oleinikova. “Multiple Phases of Liquid Water.” *ChemPhysChem*, vol. 9, no. 18, 2008, pp. 2660–2675., doi:10.1002/cphc.200800639. (17) Wei Yao, Xin Yu, Joo Won Lee, Xiaoda Yuan & Shelly J. Schmidt (2011) Measuring the Deliquescence Point of Crystalline Sucrose as a Function of Temperature Using a New Automatic Isotherm Generator, *International Journal of Food Properties*, 14:4, 882-893, DOI: 10.1080/10942910903474393

(18) Ritchie, Gary E. “General Chapters: Water-solid interactions in pharmaceutical systems.” US Pharmacopeia, www.pharmacopeia.cn/v29240/usp29nf24s0_c1241.html.

(19) Aguilera, Josém., et al. “Caking Phenomena in Amorphous Food Powders.” *Trends in Food Science & Technology*, vol. 6, no. 5, 1995, pp. 149–155., doi:10.1016/s0924-2244(00)89023-8.

(20) Zafar, U., et al. “A Review of Bulk Powder Caking.” *Powder Technology*, vol. 313, 2017, pp. 389–401., doi:10.1016/j.powtec.2017.02.024.

(21) El-Yafi, Abdul Khaliq El-Zhry, and Hind El-Zein. “Technical Crystallization for Application in Pharmaceutical Material Engineering: Review Article.” *Asian Journal of Pharmaceutical Sciences*, vol. 10, no. 4, 2015, pp. 283–291., doi:10.1016/j.ajps.2015.03.003.

(22) Libretexts. Crystalline and Amorphous Solids. Libretexts, 1 Feb. 2015, chem.libretexts.org/Textbook_Maps/General_Chemistry/Map:_Chemistry_(Averill_and_Eldredge)/12:_Solids/12.1:_Crystalline_and_Amorphous_Solids.

(23) Hartmann, Markus, and Stefan Palzer. “Caking of Amorphous Powders — Material Aspects, Modelling and Applications.” *Powder Technology*, vol. 206, no. 1-2, 2011, pp. 112–121., doi:10.1016/j.powtec.2010.04.014.

(24) Khan, Naghma, and Hasan Mukhtar. “Tea Polyphenols for Health Promotion.” *Life Sciences*, vol. 81, no. 7, 2007, pp. 519–533., doi:10.1016/j.lfs.2007.06.011.

(25) Scalbert, Augustin, et al. “Polyphenols: Antioxidants and Beyond.” *The American Journal of Clinical Nutrition*, vol. 81, no. 1, Jan. 2005, doi:10.1093/ajcn/81.1.215s.

(26) Sies, Helmut. “Polyphenols and Health: Update and Perspectives.” *Archives of Biochemistry and Biophysics*, vol. 501, no. 1, 2010, pp. 2–5., doi:10.1016/j.abb.2010.04.006.

(27) Pojer, Elisa, et al. “The Case for Anthocyanin Consumption to Promote Human Health: A Review.” *Comprehensive Reviews in Food Science and Food Safety*, vol. 12, no. 5, 2013, pp. 483–508., doi:10.1111/1541-4337.12024.

(28) Herrmann, K. “Flavonols and Flavones in Food Plants: A Review.” *International Journal of Food Science & Technology*, vol. 11, no. 5, 1976, pp. 433–448., doi:10.1111/j.1365-2621.1976.tb00743.x.

(29) Taofiq, Oludemi, et al. “Hydroxycinnamic Acids and Their Derivatives: Cosmeceutical Significance, Challenges and Future Perspectives, a Review.” *Molecules*, vol. 22, no. 2, 2017, p. 281., doi:10.3390/molecules22020281.

(30) Jr, John P. Munafo, and Thomas J. Gianfagna. “Chemistry and Biological Activity of Steroidal Glycosides from the Lilium Genus.” *Natural Product Reports*, vol. 32, no. 3, 2015, pp. 454–477., doi:10.1039/c4np00063c.

(31) Ceunen, Stijn, and Jan M. C. Geuns. “Steviol Glycosides: Chemical Diversity, Metabolism, and Function.” *Journal of Natural Products*, vol. 76, no. 6, 2013, pp. 1201–1228., doi:10.1021/np400203b.

(32) Oakenfull, David. “Saponins in Food—A Review.” *Food Chemistry*, vol. 7, no. 1, 1981, pp. 19–40., doi:10.1016/0308-8146(81)90019-4.

(33) McNaught, Alan D., and Andrew Wilkinson. *IUPAC Compendium of Chemical Terminology*. Royal Society of Chemistry, 2000.

(34) Makower, Benjamin, and W. B. Dye. “Sugar Crystallization, Equilibrium Moisture Content and Crystallization of Amorphous Sucrose and Glucose.” *Journal of Agricultural and Food Chemistry*, vol. 4, no. 1, 1956, pp. 72–77., doi:10.1021/jf60059a010.

(35) Todd, A. R., and F. Bergel. “Aneurin. Part VII. A Synthesis of Aneurin.” *Journal of the Chemical Society (Resumed)*, 1937, p. 364., doi:10.1039/jr9370000364.

(36) R. R. Williams, J. K. Kline, *Journal of the American Chemical Society*, 1936, p. 58

(37) Contant, P., et al. “ChemInform Abstract: A New Convergent Synthesis of Thiamine Hydrochloride.” *ChemInform*, vol. 21, no. 45, June 1990, doi:10.1002/chin.199045320.

(38) Nave, R. “Bragg's Law.” *Centripetal Force*, Georgia State University, hyperphysics.phy-astr.gsu.edu/hbase/quantum/bragg.html.

(39) Labuza, T. P., and J. F. Kamman. “Comparison of Stability of Thiamin Salts at High Temperature and Water Activity.” *Journal of Food Science*, vol. 47, no. 2, 1982, pp. 664–665., doi:10.1111/j.1365-2621.1982.tb10146.x.

(40) Ritchie, Gary E. “General Chapters: WATER-SOLID INTERACTIONS IN PHARMACEUTICAL SYSTEMS.” US Pharmacopeia, www.pharmacopeia.cn/v29240/usp29nf24s0_c1241.html.

(41) Different Types of SEM Imaging – BSE and Secondary Electron Imaging. Azom Materials, www.azom.com/article.aspx?ArticleID=14309.

(42) Doyle, Walter M. Principles and applications of Fourier transform infrared (FTIR) process analysis. Technical note AN-906 Rev. C, Hellma Axiom, Tustin, Ca. http://www.hellma-axiom.com/pdf/technical_notes/AN-906.pdf

(43) Belanger, Jacqueline M. R., et al. “High Performance Liquid Chromatography (HPLC): Principles and Applications .” *Instrumental Methods in Food Analysis* , 1997.

(44) Voelker, Adrienne L., et al. “Chemical Stability and Reaction Kinetics of Two Thiamine Salts (Thiamine Mononitrate and Thiamine Chloride Hydrochloride) in Solution.” *Food Research International*, vol. 112, 2018, pp. 443–456., doi:10.1016/j.foodres.2018.06.056.

(45) Kinugawa, Kohshi, et al. “Inhibitory Effects of Additives and Heat Treatment on the Crystallization of Freeze-Dried Sugar.” *Journal of Food Engineering*, vol. 155, 2015, pp. 37–44., doi:10.1016/j.jfoodeng.2015.01.016.

(46) Barnstead™ E-Pure™ Ultrapure Water Purification Systems. Thermo Fisher Scientific, www.thermofisher.com/order/catalog/product/D4631.

(47) Arioglu-Tuncil, S., Bhardwaj, V., Taylor, L. S., & Mauer, L. J. (2017). Amorphization of thiamine chloride hydrochloride: A study of the crystallization inhibitor properties of different polymers in thiamine chloride hydrochloride amorphous solid dispersions. Food Research International, 99(March), 363–374. http://doi.org/10.1016/j.foodres.2017.05.021

(48) Murase, N., et al. “The Structural States of Freeze-Concentrated and Freeze-Dried Phosphates Studied by Scanning Electron Microscopy and Differential Scanning Calorimetry.” *Cryobiology*, vol. 28, no. 4, 1991, pp. 364–375., doi:10.1016/0011-2240(91)90043-n.

(48) Tuberoso, C. I. G., & Orrù, C. D. (2008). Phenolic compounds in food. Progress in Food Chemistry, (4), 1–45. http://doi.org/10.1007/s00394-008-2002-2

(49) "Hesperidin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/hesperidin#section=Top.

(50) "Quercetin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/quercetin#section=Top.

(51) "Daidzein." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/daidzein.

(52) "Rutin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/rutin#section=Top.

(53) "Lactic Acid." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/lactic_acid#section=pKa.

(54) "Phenyl Beta-D-Glucopyranoside." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Phenylglucoside#section=Top.

(55) "4-Hydroxyphenyl-Alpha-D-Glucopyranoside." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/alpha-Arbutin#section=Top.

(56) "Puerarin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Puerarin.

(57) Rimbach, G., Melchin, M., Moehring, J., & Wagner, A. E. (2009). Polyphenols from cocoa and vascular health - A critical review. International Journal of Molecular Sciences, 10(10), 4290–4309. http://doi.org/10.3390/ijms10104290

(58) Smith, T. J. (2012). Green Tea Polyphenols in drug discovery - a success or failure? Expert Opinion on Drug Discovery, 6(6), 589–595. http://doi.org/10.1517/17460441.2011.570750.Green

(59) Graham, H. N. (1992). Green tea composition, consumption, and polyphenol chemistry. Preventive Medicine, 21(3), 334–350. http://doi.org/10.1016/0091-7435(92)90041-F

(60) Madhava Naidu, M., Sulochanamma, G., Sampathu, S. R., & Srinivas, P. (2008). Studies on extraction and antioxidant potential of green coffee. Food Chemistry, 107(1), 377–384. http://doi.org/10.1016/j.foodchem.2007.08.056

(61) "Principles of General Chemistry." *Sociological Perspectives on Education*, 2012books.lardbucket.org/books/principles-of-general-chemistry-v1.0/index.html. Appendix C: Dissociation Constants and pKa Values for Acids at 25°C

(62) "Taurine." *DrugBank*, www.drugbank.ca/drugs/DB01956.

(63) Shamblin, Sheri L., et al. "The Effects of Co-Lyophilized Polymeric Additives on the Glass Transition Temperature and Crystallization of Amorphous Sucrose." *Journal of Thermal Analysis*, vol. 47, no. 5, 1996, pp. 1567–1579., doi:10.1007/bf01992846.

(64) Roe, K. D., and T. P. Labuza. "Glass Transition and Crystallization of Amorphous Trehalose-Sucrose Mixtures." *International Journal of Food Properties*, vol. 8, no. 3, Jan. 2005, pp. 559–574., doi:10.1080/10942910500269824.

(65) "Sulfuric Acid." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/sulfuric_acid#section=pKa.

(66) Hartel, Richard W., et al. *Confectionery Science and Technology*. Springer International Publishing, 2018.

(67) Roos, Y H., et al. "Glass Transitions in Low Moisture and Frozen Foods: Effects on Shelf Life and Quality." *Food Technology*, vol. 50, no. 11, Nov. 1996, pp. 95–108.

(68) Hancock, B. C., & Zografi, G. (1994). The Relationship Between the Glass Transition Temperature and the Water Content of Amorphous Pharmaceutical Solids. *Pharmaceutical Research*. https://doi.org/10.1023/A:1018941810744

(68) Kamat, M. S., et al. Near-infrared spectroscopic determination of residual moisture in lyophilized sucrose through intact glass vials. *Pharmaceutical Research*, Vol. 6, no. 11, 1989.

(69) "Apigenin." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Apigenin.

(70) "Resveratrol." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Resveratrol.

(71) Vang, Ole, et al. "What Is New for an Old Molecule? Systematic Review and Recommendations on the Use of Resveratrol." *PLoS ONE*, vol. 6, no. 6, 2011, doi:10.1371/journal.pone.0019881.

(72) Corriher, Shirley. *Learn How to Control Crystallization for Successful Sweets*. Fine Cooking, 1 Dec. 1999, www.finecooking.com/article/learn-how-to-control-crystallization-for-successful-sweets.

(73) Gabarra, P., and R. W. Hartel. "Corn Syrup Solids and Their Saccharide Fractions Affect Crystallization of Amorphous Sucrose." *Journal of Food Science*, vol. 63, no. 3, 1998, pp. 523–528., doi:10.1111/j.1365-2621.1998.tb15778.x.

(74) Bell, Leonard N., and Theodore P. Labuza. *Moisture Sorption: Practical Aspects of Isotherm Measurement and Use*. American Association of Cereal Chemists, 2000.

(75) Burnett, D.j., et al. "Determining the Critical Relative Humidity for Moisture-Induced Phase Transitions." *International Journal of Pharmaceutics*, vol. 287, no. 1-2, 2004, pp. 123–133., doi:10.1016/j.ijpharm.2004.09.009.

(76) Slade, Louise, et al. "Beyond Water Activity: Recent Advances Based on an Alternative Approach to the Assessment of Food Quality and Safety." *Critical Reviews in Food Science and Nutrition*, vol. 30, no. 2-3, 1991, pp. 115–360., doi:10.1080/10408399109527543.

(77) Yu, X., Kappes, S. M., Bello-Perez, L. A., & Schmidt, S. J. (2008). Investigating the Moisture Sorption Behavior of Amorphous Sucrose Using a Dynamic Humidity Generating Instrument. Journal of Food Science, 73(1), E25-E35. doi: 10.1111/j.1750-3841.2007.00596.x

(78) "Average Monthly Humidity in Indiana, United States of America." *World Weather and Climate Information*, weather-and-climate.com/average-monthly-Humidity-perc,indiana-pennsylvania-us,United-States-of-America.

(79) "Climate and Average Monthly Weather in: Phoenix, United States of America." *World Weather and Climate Information*, weather-and-climate.com/average-monthly-Rainfall-Temperature-Sunshine,phoenix,United-States-of-America.

(80) Plumb, Geoffrey W., et al. "Antioxidant Properties of Catechins and Proanthocyanidins: Effect of Polymerisation, Galloylation and Glycosylation." *Free Radical Research*, vol. 29, no. 4, 1998, pp. 351–358., doi:10.1080/10715769800300391.

(81) "Chlorogenic acid." PubChem Compound Database, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/chlorogenic_acid#section=Top.

(82) Smoliga, James M., et al. “Resveratrol and Health - A Comprehensive Review of Human Clinical Trials.” *Molecular Nutrition & Food Research*, vol. 55, no. 8, 2011, pp. 1129–1141., doi:10.1002/mnfr.201100143.

(83) Dwivedi, Basant K., and Roy G. Arnold. "Chemistry of Thiamine Degradation." *Journal of Food Science* 37.6 (1972): 886-88.

(84) Mitrovich, Velo. Curing Conundrum. Meat Packing Journal, meatpacking.info/2017/01/25/curing-conundrum/.

(85) Clark, Christine M, and Barbara L Dutrow. “Single-Crystal X-Ray Diffraction.” Geochemical Instrumentation and Analysis, Science Education Resource Center, serc.carleton.edu/research_education/geochemsheets/techniques/SXD.html.

(86) Lonsdale, Derrick. “A Review of the Biochemistry, Metabolism and Clinical Benefits of Thiamin(e) and Its Derivatives.” *Evidence-Based Complementary and Alternative Medicine*, vol. 3, no. 1, 2006, pp. 49–59., doi:10.1093/ecam/nek009.

(87) Fattal-Valevski, Aviva. “Thiamine (Vitamin B1).” *Journal of Evidence-Based Complementary & Alternative Medicine*, vol. 16, no. 1, 2011, pp. 12–20., doi:DOI: 10.1177/1533210110392941.

(88) R. R. Williams, J. K. Kline, *Journal of the American Chemical Society*, 1936, p. 58

(89) Contant, P., et al. “ChemInform Abstract: A New Convergent Synthesis of Thiamine Hydrochloride.” *ChemInform*, vol. 21, no. 45, June 1990, doi:10.1002/chin.199045320.

(8) Labuza, T. P., and J. F. Kamman. “Comparison of Stability of Thiamin Salts at High Temperature and Water Activity.” *Journal of Food Science*, vol. 47, no. 2, 1982, pp. 664–665., doi:10.1111/j.1365-2621.1982.tb10146.x.

(90) Todd, A. R., and F. Bergel. “Aneurin. Part VII. A Synthesis of Aneurin.” *Journal of the Chemical Society (Resumed)*, 1937, p. 364., doi:10.1039/jr9370000364.

(91) Burdick, David. “Thiamine (B1).” *Kirk-Othmer Encyclopedia of Chemical Technology*, 4 Dec. 2000, doi:https://doi.org/10.1002/0471238961.2008090102211804.a01.

(92) Hall, James F. *Experimental Chemistry*. Houghton Mifflin, 2003. Appendix 5

(93) *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. National Academies Press, 1998. 0-309-59725-0

(94) Youmans, Hubert. "Measurement of PH of Distilled Water." *Journal of Chemical Education*, vol. 49, no. 6, 1972, p. 429., doi:10.1021/ed049p429.

(95) S. Damodaran, K.L. Parkin, O.R. Fennema (Eds.), Fennema's food chemistry (4th ed.), CRC Press/Taylor & Francis, Boca Raton (2008), pp. 439-521

(96) Farrer, K. T. H. "The Thermal Destruction of Vitamin B1." *British Journal of Nutrition*, vol. 2, no. 03, 1948, p. 242., doi:10.1079/bjn19480042.

(97) Hunt, Ian R. "Ch27 PKa and PI Values." *Chapter 27: Amino Acids, Peptides and Proteins*, University of Calgary, www.chem.ucalgary.ca/courses/350/Carey5th/Ch27/ch27-1-4-2.html.

(98) Williams, Robert R., and A. E. Ruehle. "Studies of Crystalline Vitamin B1. XI. Presence of Quaternary Nitrogen1." *Journal of the American Chemical Society*, vol. 57, no. 10, 1935, pp. 1856–1860., doi:10.1021/ja01313a029.

(99) "Thiamine Mononitrate." *Thiamine Mononitrate*, Royal Society of Chemistry, www.chemspider.com/Chemical-Structure.10308.html?rid=b003ac45-7e1b-47b3-a158-b883760ceee7.

(100) "Thiamine hydrochloride." *Thiamine hydrochloride*, Royal Society of Chemistry, www.chemspider.com/Chemical-Structure.10308.html?rid=b003ac45-7e1b-47b3-a158-b883760ceee7.

(101) "Thiamine chloride." *Thiamine chloride*, Royal Society of Chemistry, www.chemspider.com/Chemical-Structure.10308.html?rid=b003ac45-7e1b-47b3-a158-b883760ceee7.

(102) Turfus, S C., et al. "Chapter 25 - Pharmacokinetics." *Pharmacognosy*, Academic Press, 2017, pp. 495–512. https://doi.org/10.1016/B978-0-12-802104-0.00025-1

(103) "Sodium Citrate." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/Sodium_citrate#section=Solubility.

(104) "Monosodium Glutamate." *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/ Monosodium_Glutamate #section=Solubility.

(105) No DCR. Saccharin and its salts. 2th Ed 1987

(106) “Sodium Benzoate.” *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/sodium_benzoate#section=Solubility.

(107) “Sodium Lactate Product Ingredient for Lactic Acid.” *DrugBank*, www.drugbank.ca/salts/DBSALT001765.

(108) “Sodium Bicarbonate.” *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/ sodium_bicarbonate#section=Solubility.

(109) “Sodium Phosphate Monobasic.” *Chemical Book*, www.chemicalbook.com/ChemicalProductProperty_EN_CB6219354.htm.

(110) “Sodium ascorbate.” *National Center for Biotechnology Information. PubChem Compound Database*, U.S. National Library of Medicine, pubchem.ncbi.nlm.nih.gov/compound/sodium_ascorbate#section=Solubility.

{111) “Citric Acid Monohydrate.” *Sigma-Aldrich*, www.sigmaaldrich.com/catalog/product/sial/c1909?lang=en®ion=US.