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An Unprecedented Research Project into the Alcohol Retention Variation of Flavored Spirits

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AN UNPRECEDENTED RESEARCH PROJECT INTO THE ALCOHOL RETENTION VARIATION OF FLAVORED SPIRITS

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By

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DEDICATION

This thesis is dedicated to my husband Thomas McFadden for all of the love and support he gives me, without whom I could not have done this. To my parents, John and Denise Pennington and Dan and Lori McFadden, who were the whispers of confidence in my ear, even when I did not want to hear it. To my grandparents, who loved and believed in me, but could not be here to witness this day. To all of the other students and friends who have trekked this journey with me, but in particular, Bailey Gill, who has literally been by my side every step of the way. I could not possibly have completed this without every single one of you. Simply put, thank you.

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I would firstly like to thank my research mentor, Dr. Darrin Smith, for his guidance during this entire process. I would secondly like to thank Dr. Judy Jenkins for all of her support, enthusiasm, and understanding through presentations and thesis writing. Furthermore, Brewing and Distilling Analytical Services, without whom there would be neither research project nor funding. In particular, I would like to thank Amber Weygandt who oversaw my project and was my personal guide into the research. Finally, Dr. Margaret Ndinguri for being a part of my committee.

ABSTRACT

Accurate determination of alcohol by volume (ABV) is necessary, but previously used techniques are proving inaccurate with new flavored spirits. Specifically, control experiments showed that increasing concentrations of sugar led to increasingly inaccurate ABV determination. We hypothesize the intermolecular forces present in these beverages are significantly altered by the presence of sugar, which in turn leads to the observed inaccuracies in ABV measured through distillation. We used additives such as NaCl and NaOH to strategically and systematically vary intermolecular interactions and the influences of these additives on ABV were tested through distillation, densitometry, and nuclear magnetic resonance spectroscopy (NMR). Given the results based on NMR data and NaCl additions, intermolecular hydrogen bonding is not the direct cause of the ethanol retention. However, a direct correlation between increasing pH and increasing accuracy exists in some cases, suggesting that intramolecular forces may be the more dominant interactions affecting ABV determination. The final chapter of this work contains ideas to better understand the fundamental chemistry of these interactions, eventually leading to more robust measurements for ABV determination.

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LIST OF ABBREVIATIONS

Alcohol by Volume.....ABV
Alcohol and Tobacco Tax and Trade Bureau.....TTB
Milligram.....mg
Milliliter.....mL
Alcohol by Weight.....ABWt
Sodium chloride.....NaCl
Sodium hydroxide.....NaOH
Deuterium oxide.....D_2O
Chloroform-D.....$CDCl_3$
Grams.....g
Brewing and Distilling Analytical Services.....BDAS
Molarity.....M
Infrared.....IR
Near-infrared.....NIR
Nuclear Magnetic Resonance.....NMR
International Alcoholometric.....OIML
Quantum number.....I
Magnetic Field.....B_o
Magnetic moment.....μ
Gyromagnetic ratio.....γ
Frequency.....ν
Temperature.....T
Energy.....E
Potassium chloride.....KCl
Silver.....Ag
Silver chloride.....AgCl

Chloride.....Cl^-

CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1. Research Objectives

Nearly 87% of people consume alcohol in their lifetime and all of the alcohol must be tested for quality control and quality assurance purposes.[1] One of these tests is the determination of alcohol by volume (ABV) and/or proof, which is important for several reasons. Taxes for spirits are adjusted, and depend upon, the percentage of ethanol in the spirit.[2] Furthermore, when drinking the product, the consumer has a right to know the concentration of ethyl alcohol in their beverage. In order to determine the ABV, the Alcohol and Tobacco Tax and Trade Bureau (TTB) regulates the spirits must be distilled and restored to the original volume and temperature.[2] However, Brewing and Distilling Analytical Services (BDAS), a beverage testing facility in Lexington, Kentucky, determined that flavored spirits do not consistently distill precisely or accurately, demonstrating the appearance of alcohol retention. Research has not kept up with the growing popularity of these flavored spirits, and because of this, correlations between ethanol retention and the concentration or type of sugar, concentration of ethanol, and other flavoring agents have not been thoroughly studied, leaving the following questions unanswered: Does the presence of sugar in a sugar/water/ethanol solution cause differences between the known ABV and measured ABV when determined via distillation? Are these differences dependent on

the concentration of sugar or are they random? Can additives be chosen to manipulate intermolecular forces in sugar/water/ethanol solutions to facilitate accurate ABV determination by distillation? Can ABV be accurately determined based on the density of the solution?

1.2. Definitions

According to the TTB, the term “distilled spirit” or “spirit” refers to ethyl alcohol from distilled spirits, including all dilution and mixtures thereof for nonindustrial use.[2] The term spirits throughout includes such alcoholic beverages as, but not limited to, vodka, whisky, rum, gin, brandy, liqueur, and tequila.[3] The term “flavored spirit” will refer to any spirit that has a purposeful addition of sugar and/or flavoring after the distillation process.

From governmental regulations, a variety of terms are utilized when working with spirits. The ABWt is the alcohol by weight where ethanol weight is divided by the total weight of the beverage while ABV is the alcohol by volume where ethanol volume is divided by the total volume of the beverage. The proof is twice the ABV, whereas, the proof gallon is a gallon of liquid at 60 °F that contains 50 % ethanol by volume, which is used for taxation purposes. The apparent ABV is the ABV measured directly from an alcoholic beverage using a TTB accepted instrument, while the true ABV is the actual ABV in a sample, as determined by distillation for spirits. Lastly, obscuration corrects the percent ABV when dissolved solids interfere with optical measurements.[2] The specific rules and regulations involve specifications based on solid content and can be found at the TTB website (27 CFR Part 30).[2]

1.3. Liquor History in the United States

When settlers arrived in the present day United States, the alcoholic beverage of choice was beer flavored by molasses, tree barks, fruits, and vegetables. From beer, the pilgrims created wine, mead, metheglin, and cider, which were then followed by liquors. The first liquors utilized a variety of ingredients, including, berries, plums, potatoes, apples, carrots, and grain. The most common liquors at the time were peach brandy and applejack.[4]

The true evolution of liquor began with rum. During the middle of the 1600s, sugar and molasses were exported from the West Indies to New England in the Trans-Atlantic "triangular" trade. However, in 1808, the U.S. prohibited the importation of slaves from Africa, causing the triangular trade to cease. The country would now have to learn how to make and enjoy another alcoholic beverage, whisky.[4]

During the 1800s, immigration to the United States started to boom, including immigrants from areas such as Scotland and Ireland. The Scottish and Irish helped lay the foundation for the modern liquor because they were skilled craftsmen in distillation: distillation and aging of spirits had been occurring in Scotland and Ireland for many generations.[4]

Learning to make whisky was also advantageous for the farmers. Excess corn that could not be sold could be turned into a drink and shared with friends and family or sold to strangers for a higher price than raw material. Shortly after the end of the Revolutionary War though, Alexander Hamilton proposed the country should pay off its debts by taxing a variety of items, including spirits. The rates varied, but small

distillers often paid double what a large distiller would pay.[2] It can be noted this taxation resulted in the Whisky Rebellion, which is the only time in history that U.S. troops have been deployed against American citizens. Thus, this was the beginning of taxation on alcoholic products within the U.S.[4]

1.4. Current Taxation

Throughout the years, the laws on how alcoholic beverages should be taxed have changed. The general rule from the TTB is the proof of spirits shall be determined to the nearest tenth degree, which shall be the proof used in determining the proof gallons. If the spirit has less than 400 mg of solid per 100 mL, the true or apparent proof can be determined; if the spirit has 400 to 600 mg of solid per 100 mL, the true proof must be determined by the apparent proof plus the obscuration; if the spirit has greater than 600 mg of solid per 100 mL, the true proof must be determined by distillation. The proof of the beverage must be within 0.25% and cannot be above the ABV listed on the label for beverages containing greater than 600mg of solid per 100mL (27 CFR Part 5). The current national tax on a proof gallon is $13.50 and is adjusted based on alcohol content. The current tax on a 750 mL bottle is $2.14 and is also adjusted based on alcohol content.[2]

Determining ABV is important for taxation purposes, but it is also important for consumers. If less alcohol exists in a product compared to the label, the consumer is simply paying for alcohol that is not present in the bottle. If there is more alcohol in a product than the label states, the consumer could be at a potential health risk and the government is not paid the proper amount of taxes.

1.5. Fermentation

By definition, fermentation is the anaerobic extraction of energy from food by microorganisms.[5] This is accomplished by the breakdown of complex sugars, such as

starch, into simpler sugars, releasing energy. The cell captures this energy, and in turn, the cells create byproducts. In particular, yeast in the presence of sugar creates ethanol, CO_2, and other acids.[5]

Side reactions can occur during the fermentation process, especially if the temperature is high or if high concentrations of products are present. For example, as the concentration of ethanol increases over time, there is an increased probability that ethanol will interact with enzymes, causing the ethanol concentration to decrease. Furthermore, some of these side reactions create long chain alcohols, acids, and esters, most of which are attributed to unpleasant flavors, typically called fusel alcohols or congeners. The congeners created depend upon the sugar source, but the most common are isopentanol, 2-methyl-1-butanol, isobutanol, propanol; however, there are other esters, aldehydes, and alcohols in smaller concentrations.[5] Some spirits, like vodka contain few flavor compounds and are primarily composed of ethanol and water; still, most beverages are going to contain aldehydes, ketones, aromatics, acids, esters, and alcohols. As an example, methanol is not a by-product of yeast fermentation, but originates from pectin when fruits are macerated; thus, one would expect gin to have higher methanol content than whisky. Aromatics are generally obtained from the barrels that the alcohol is stored in, and thus, whiskys are going to have different volatile compounds because of their different storage /aging processes.[6]

1.5.1. General Requirements for Alcoholic Beverage Creation

All fermentations have certain requirements: a sugar that has lower and upper concentration limits, extreme cleanliness and sanitation requirements, an aerobic environment during the first few days of growth, an anaerobic environment after the first few days in order to create ethanol, and the production of heat and CO_2 (the latter are only problems in large scale).[5]

The yeast is the biggest contributor to the final flavor of the product. Therefore, careful selection of the yeast is necessary and the treatment of the yeast strain during fermentation needs to be meticulous as well. Each yeast strain will produce different congeners and will have different oxygen, nutrient, and temperature requirements. Furthermore, each yeast strain will produce and die at different ethanol levels. To properly care for the yeast, proper nutrition is required, such as vitamins, minerals, and amino acids. Buffers are also necessary to prevent the pH from falling out of the 3.4 to 4.0 range. This is necessary to prevent stress on the yeast and to decrease the growth of unwanted organisms that create off flavors.[5]

The amount of sugar to create the desired ABV in beer should be calculated. It takes 17g of sucrose per 1L to create 1.0% ABV. Thus, to determine the amount of sugar necessary for fermentation, one should follow Equation 1.1.[5]

$$Desired\ ABV\ x\ 17 = g\ of\ sugar\ necessary \quad (1.1)$$

Lastly, the equipment and process used during distillation is very important. All stills need a boiler: a well-sealed container that heats with an outlet for vapor. The

heating can be direct or indirect. Direct heating is when the heat is close to or actually in the boiler while indirect heating is when heat is produced elsewhere and then transferred to the boiler. The condenser is also vital. The heat transferred away from the liquid is directly proportional to the area available for the heat to pass through. The transferred heat is also directly proportional to the thermoconductivity of the material utilized in the condenser, for example, copper transfers heat more efficiently than glass because copper is more thermoconductive.[5] The thermal conductivity for copper is 385.0 (W/m K), whereas the thermal conductivity for glass is only 0.8 (w/m K).[7]

Several different types of stills for distillation are available. A pot still has the simplest design: a boiler is attached directly to a condenser. A whisky still involves a neck that has a small angle between the boiler and condenser to create better fractioning of volatile components. A fractioning still has an actual fractioning column to create an even better separation of volatile components. Lastly, a compound still has not only a fractioning column, but also refluxes at the top. Each one of the stills has an increasing ability to separate the volatile components, but also creates an increased time for distillation, respectively.[5]

Distillation is employed for the separation of volatile components. In particular, the most volatile components, generally toxic, are distilled first and are referred to as foreshots. The next compounds to distill are known as the heads, which contain some compounds necessary for flavor of certain drinks, like whisky, brandy, and rum. Only a small amount of ethanol distills with the heads. The majority of the ethanol distills

after the heads and is the bulk of the distillation. The last component to distill is the tails composed of an increased amount of water and less volatile compounds. These less volatile substances can flavor certain beverages, but are also responsible for hangovers. When creating a drink like whisky, brandy, or rum, the heads and tails will be cut into the ethanol in small amounts to create rich, unique flavors.[5]

1.5.2. Whisky, Bourbon, and Moonshine

Beer is created from cereal grains that contain starch[5] – a polymer sugar composed of amylose and amylopectin.[8] Starch itself is not fermentable, but when the grain is allowed to sprout, enzymes are created that can break the starch into smaller pieces called dextrins.[5] Malting is the process of controlling sprouting to maintain the desired enzymes, and afterwards, this malted grain, and any other grains desired for the recipe, are milled in the first step of brewing beer. This mixture is called mash if it contains the grains, but it is called wort if the liquid portion has been separated. The mash or wort is then fermented, creating beer. When the beer is distilled, grain neutral spirit results. It can also be noted that beer will be made with mash when grain neutral spirit is the final product goal because the distillation process removes undesirable byproducts. If beer is the final product goal, wort will be utilized.[5]

The major difference between whisky, bourbon, and moonshine is the aging process. Moonshine is taken directly from the still and sold as is, whisky has to be aged in container, and bourbon has to be aged in charred new oak containers.[2] While these are not the only criteria that differentiate these products, specific product definitions, and the legal criteria can be found at ttb.gov.[2]

1.5.3. Vodka

Vodka is created from a very similar process to that of whisky. Enzymes create dextrins from starch, the microorganisms create ethanol as a byproduct during anaerobic respiration, and the ethanol produced in the beer-like product is purified through distillation.[5] The main difference is that vodka must be treated with charcoal or other material to prevent any character, aroma, taste, or color.[2]

1.5.4. Flavored Spirits

Until recently, the processes described for the creation of whisky, bourbon, moonshine, and vodka were the only processes that one would expect for the creation of distilled spirits, which did not affect the distillation process necessary for ABV determination. Flavored spirits are crafted through fermentation before being "flavored" with additives such as sugar and vanilla extract. Flavored spirits are growing ten times faster than regular sprits, mainly in vodka and whisky. Specifically, Pinnacle is growing the fastest of all the spirits, driven by its flavored products.[9] The flavored products do not distill properly during the ABV determination, resulting in the appearance of a lower ABV. The reason for this alcohol retention during distillation is currently unknown and will be the focus of this work.

1.6. Carbohydrates

Carbohydrates, also be referred to as sugars, are high-energy biomolecules. These sugars can either be polyhydroxy aldehydes or polyhydroxy ketones. All sugars have multiple chiral centers and can be designated as "D" or "L," depending upon the

chirality of the anomeric carbon. When looking at any sugar in the Fischer projection, “D” sugars have the hydroxyl group on the right of the anomeric carbon and “L” sugars have the hydroxyl group on the left of the anomeric carbon. However, most sugars found in nature will be of the “D” configuration; and, therefore would be called D-sugars.[8] In particular, fructose is a ketohexose, meaning it is composed of six carbons and a ketone, and has three chirality centers.

Sugars can undergo cyclization in aqueous environments. This cyclization is the direct result of the lone pairs on the oxygen attacking the carbonyl group to form a ring. From the cyclization, α or β forms of the sugar can be formed. The α-sugars have the hydroxyl group facing downward where the β-sugars have the hydroxyl group facing upwards, as seen in Figure 1.1. The equilibrium formed between the α- and β-sugars is called mutarotation and is accelerated in the presence of an acid or base. Because fructose is a ketose, it is capable of forming both the furanose (5-membered ring) and the pyranose (6-membered ring); however, the furanose form is dominant in nature.[8]

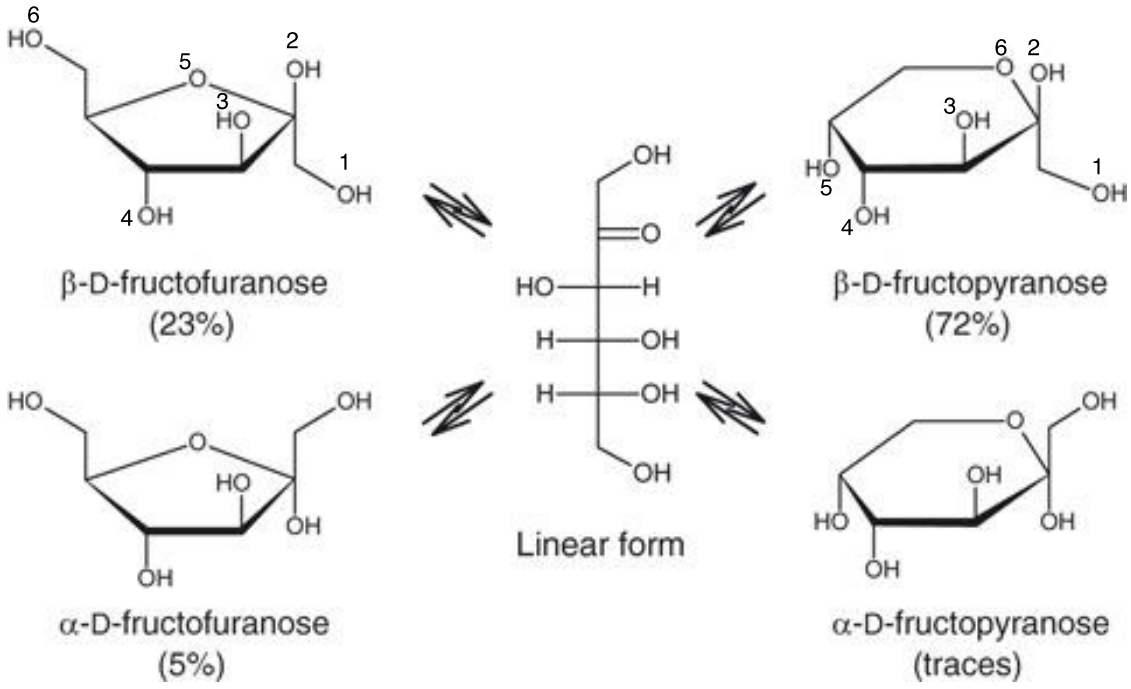


Figure 1.1. Demonstration of the mutarotation of fructose. The furanose rings are on the left and the pyranose rings are on the right. The orientation of the hydroxyl group is denoted as per α- and β- labeling of the sugar. The hydroxyl groups are also numbered, which will be necessary in later chapters.[10]

1.7. Scope of Project

Accurate determination of ABV is necessary, but previously used techniques are proving inaccurate with new flavored spirits. To investigate the cause of the inaccuracy, this project was based on the hypothesis that an increase in hydrogen bonding with increasing sugar concentration would result in a drastic increase in boiling point, beyond what would be seen with colligative properties, resulting in ethanol retention. To test this hypothesis, alcohol/sugar/water solutions were prepare and distilled according to TTB regulations. The solutions consisted of constant 40 % alcohol by weight (ABWt) and varied sugar concentrations from 0 to 35 % w/w in

increments of 5 %. The experimentally determined ABWt from the distillation was compared to the known ABWt, and percent error was calculated. To investigate any changes in the extent of hydrogen bonding as a function of sugar concentration, the boiling point of each solution was calculated, NMR studies were conducted, and distillations were performed. Additives such as NaCl and NaOH were added to the solutions to strategically disrupt intermolecular forces. Sugar concentration was hypothesized to increase solution boiling point based on the resulting intermolecular interactions as described through colligative properties. In the NMR studies, a downfield shift was expected for the hydrogens of ethanol and sugar, explained in further detail in Section 3.3.2 in Chapter 3. The NaCl additions were expected to break hydrogen bonding, discussed further in Section 2.4.2 in Chapter 2 and Section 3.3.3 in Chapter 3. When no differences in boiling points were detected, no differences in the extent of hydrogen bonding were measured via NMR, and NaCl addition did not afford improved distillation accuracy, NaOH was added to determine whether possible interactions (intramolecular) could be broken in the solution to decrease percent error, discussion in Section 3.4.2 in Chapter 3. Lastly, NaOH additions were tested on real samples to see if the reduction in percent error was replicable in all real-world samples, discussed in Section 3.4.3 in Chapter 3. This work demonstrates that the addition of sugar to ethanol/water mixtures creates a more complex solution with intramolecular interactions requiring changes in how to accurately quantitate ABV.

CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction

This chapter focuses on the quantitation of ethanol utilizing distillation and densitometry. Additionally, boiling point determination was completed with a hot plate and thermometer, solution density was determined utilizing a pycnometer and two different densitometers, and pH determination was performed utilizing a pH probe.

2.2 Reagents Utilized

The ethanol was purchased from a liquor store and was 95% ABV, the Invertose high fructose corn syrup (95% purity) was donated by Ingredion, and the distilled water was purchased from Kroger. Buffer solutions of pH 4, 7, and 10 for the pH probe were purchased from Fischer Chemical and were certified to be between 3.99-4.01, 6.99-7.01, and 9.99-10.01, respectively. The 0.1 M NaOH solution was purchased from LabChem. Commerical table salt, NaCl, was purchased from a Meijer. For NMR studies, $CDCl_3$ (purity 99.8%) was purchased from Sigma-Aldrich and D_2O (purity 99.8%) was purchased from Acros. The unnamed vodka (35% ABV), bourbon (45% ABV), and moonshine (30.15% ABV) were purchased from a local liquor store.

2.3 Sample Preparation

1000 g solutions containing forty percent ABWt ethanol and varying water and sugar concentrations from 0 % sugar to 35 % sugar were prepared using 533 mL (430 g) of 95% ABV vodka, 53 g – 368 g (5 – 35 % by weight,) – 368 g (35 % by weight) of high fructose corn syrup, and enough DI water to afford a final mass of 1000 g. Equation 2.1 – 2.2 demonstrate the calculation for ethanol addition. Equation 2.3 demonstrates the calculation for high fructose corn syrup addition. Table 2.1 shows the composition of all solutions.

$$400\ g\ ethanol\ x\ \left(\frac{1\ mL}{0.789g}\right) =\ 507\ mL\ of\ pure\ ethanol \qquad 2.1$$

$$\frac{507\ mL\ of\ pure\ ethanol}{0.95} = 533\ mL\ of\ 95\%\ vodka \qquad 2.2$$

$$\frac{50\ g\ of\ high\ fructose\ corn\ syrup}{0.95} = 53\ g\ of\ high\ fructose\ corn\ syrup \qquad 2.3$$

Table 2.1. Solution Compositions.

Solution (all 40 % ABWt)	Ethanol mass (g)	Sugar mass (g)	Water mass (g)
0% Sugar	435	0	566
5 % Sugar	426	53	522
10 % Sugar	428	104	469
15% Sugar	429	156	421
20% Sugar	428	208	363
25% Sugar	427	259	315
30% Sugar	428	314	257
35% Sugar	428	364	208

2.4 Distillation

2.4.1 Distillation Theory

Distillation is a common method of separation and/or purification of solutions based on the difference in boiling points and volatilities of the substances in a mixture being separated. The boiling point is defined as the temperature at which the vapor pressure equals the external pressure acting on the surface of the liquid. In solutions at room temperature and ambient pressure gaseous molecules and molecules in the liquid phase exist in equilibrium. The higher the vapor pressure, the more gaseous molecules present near the liquid surface; the substance with higher vapor pressure is more volatile and will exhibit a lower boiling temperature than the substance with the lower vapor pressure. Two factors affect the volatility of a substance. First, the mass of the compound is a factor; the more mass a compound has, the less volatile it is. Secondly, are the intermolecular forces between molecules including ion-dipole being the strongest intermolecular force, followed by hydrogen bonding, dipole-dipole, and then London dispersion. Thus, if a solvent has extensive hydrogen bonding, it will take more energy for the vapor pressure to equal the pressure acting on the surface of the liquid.

At a given, constant temperature, the compound with the lower boiling point will have more molecules in the gas phase than the higher boiling point compound. Thus, if the vapor phase were collected and condensed into a liquid, there would be more molecules of the lower boiling point compound than the higher boiling point compound. This separation of solution components based on volatility can be used to separate and/or purify mixtures as is done in distillation.[11]

Despite having a larger mass, ethanol has a lower boiling point than water due to the stronger hydrogen bonding network occurring between water molecules. Thus, as the temperature of a water/ethanol mixture rises, disproportionately more ethanol molecules are present in the vapor. In a distillation apparatus, these gaseous molecules are directed to a cooled condenser, where the vapor is condensed into a liquid, allowing the ethanol to be separated and collected from the rest of the sample.

2.4.2 Boiling Point Elevation

If solute and solvent are mixed together, the solvent will experience a boiling point increase (ΔT_b) based on Equation 2.4,[12]

$$\Delta T_b = iK_b m \qquad 2.4$$

where *i* is the Van't Hoff factor, K_b is the molal boiling point constant for water, and *m* is molality of the solution. The factor most strongly contributing to the boiling point elevation is concentration of solute, not identity of the solute. This boiling point elevation results from an increase in the energy necessary for the vapor pressure to equal the pressure on the surface of a liquid.

If the intermolecular forces in a solution are complicating separation of similar components, additives may disrupt these interactions enough to afford separation.[13] For instance, salts dissociate in aqueous solutions, creating negatively charged anions and positively charged cations. Ions interact with polar molecules in solution, thereby disrupting intermolecular forces and diminishing the impacts of these forces on the solution components' boiling points. A decrease in the attraction between molecules may increase the volatility of a substance (and decrease the boiling point) of a given substance.

2.4.3 Distillation Method

In order to complete a distillation according to the Brewing and Distilling Analytical Services (BDAS) method, it is necessary to have 4-100 mL volumetric flasks and the corresponding caps, 2-250 mL round-bottom flasks, 2 three-way-adapters, 2 condensers and appropriate tubing, a hot plate, and a water circulator per sugar/ethanol/water solution or commercial beverage.

One (1) inch more than 100 mL of sugar/water/ethanol solution, or commercial beverage, was measured with a volumetric flask and was equilibrated to 20 °C by placing the flask into a Lindberg/Blue Waterbath for 20 minutes. After 20 minutes, the solution was vigorously shaken and then excess solution was removed by pipette to bring the volume to exactly 100 mL. The solution was transferred into a 250 mL round-bottom flask. The volumetric flask was rinsed with 50 mL of distilled water that was also transferred into the round-bottom flask with the alcoholic solution. The round-bottom flask was attached to the distillation unit depicted in Figure 2.1. The distillation

unit consisted of a hot plate, the round-bottom flask, a three-way adapter, a condenser, a volumetric flask, and Keck clamps securing the glass pieces together. Once the round-bottom flask was added into the distillation unit, the heating mantle and water circulator were turned on. The solution was allowed to boil. The vapor was condensed and collected the previous volumetric flask. When the distillate volume came approximately one half-inch under the mark, the volumetric flask was removed and quickly capped to prevent any loss of ethanol. It is assumed that all alcohol molecules from the original solution as well as some water molecules were collected as part of the condensate because ethanol's boiling point is sufficiently lower than water. It is imperative that the flask volume not exceed 100.00 mL because when the solution is warmed to 20 °C, the liquid will expand. Thus, in order to prevent the solution from expanding over the 100 mL mark, the distillation is stopped when the distillate volume is approximately one half-inch below the 100.00 mL mark. Next, the round-bottom flask, containing the residual (everything in the original solution except ethanol and some water), was removed from the distillation unit, quickly poured into a separate 100 mL volumetric flask, and capped. Both the distillate and the residual solutions were placed into the water bath for another 20 minutes. After 20 minutes, 20 °C deionized water was added to each solution until the solution accurately measured 100.00 mL.

Three samples were then prepared for analysis via the Anton Paar: the original undistilled sample (also called the direct), the distillate, and the residual. The direct was shaken vigorously and split between two 50 mL plastic Alcolyzer sample holders

and capped immediately. The distillate and residuals were prepared in the same manner. These samples were placed onto the Alcolyzer autosampler carousel, the memory was cleared, and the start button was pressed. Sample analysis is decribed in Section 2.5.

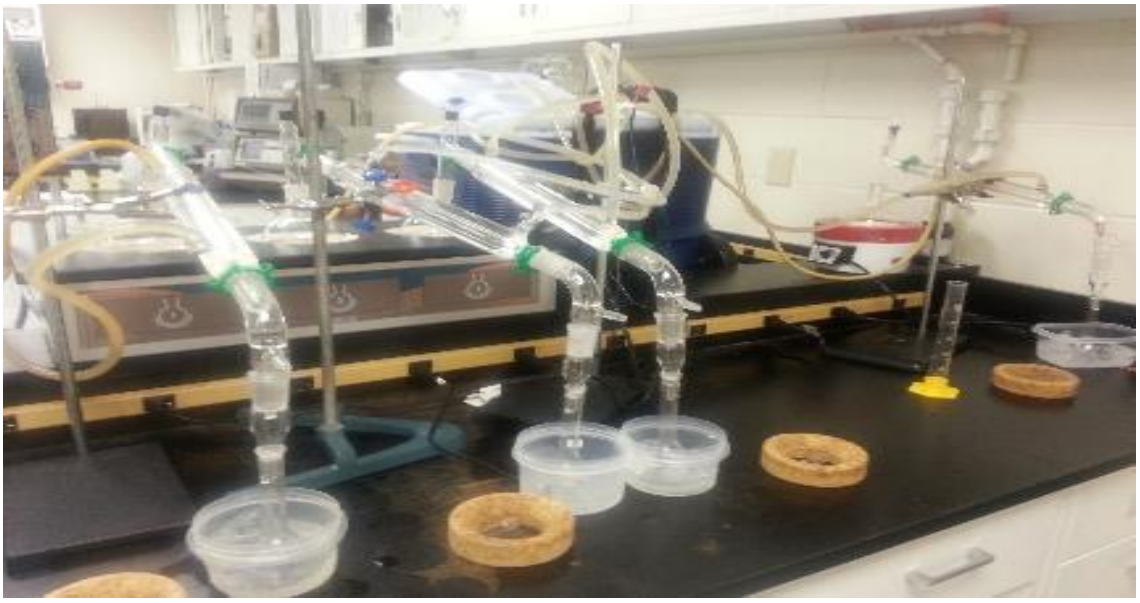


Figure 2.1. Distillation apparatus used at the BDAS facility.[14]

2.4.4 Additives to Modify Distillations

The impacts of salt on the accuracy of ABV determination (described in Section 2.4.2 in Chapter 2) were tested by systematically adding NaCl (1 g – 30 g) to the prepared sugar/water/ethanol solutions prior to distillation.

Similarly, the impact of pH was examined by systematically adding 0.1 M NaOH dropwise to the prepared sugar/water/ethanol solutions prior to distillation. The pH of the resulting solution was measured with a pH probe to achieve a pH of 8.5, 9.5, or 10.5. Once the desired pH was achieved, the pH probe was thoroughly rinsed with distilled water over the round-bottom flask containing the distillate. The small volume added is assumed to have negligible effect on the measured ABV.

2.4.5 Method for Boiling Point Determination

Due to the nature of the distillation set-up, boiling point could not be determined during distillation of the prepared standard solutions (0 % w/w to 35 % w/w), so the boiling points were measured outside of the distillation apparatus. To determine the boiling point of these standard solutions, approximately 100 mL of the standard solution was placed into a large beaker on a hot plate. The hot plate was turned on and the solution boiled. Once the solution began to vigorously boil, a thermometer was placed in the solution with a thermometer and allowed to equilibrate. Once the temperature was steady, the temperature was recorded.

2.5 Vibrational Spectrometry for ABV Determination

2.5.1 Vibrational Spectrometer (Alcolyzer)

The Anton Paar Alcolyzer is an absorbance spectrophotometer whose light source is in the infrared region (IR) of the electromagnetic spectrum. The block diagram in Figure 2.2 includes a NIR light source that irradiates the sample, a dispersion element that splits the transmitted light into its separate wavelengths, and a photodiode to detect this transmitted light.[15]

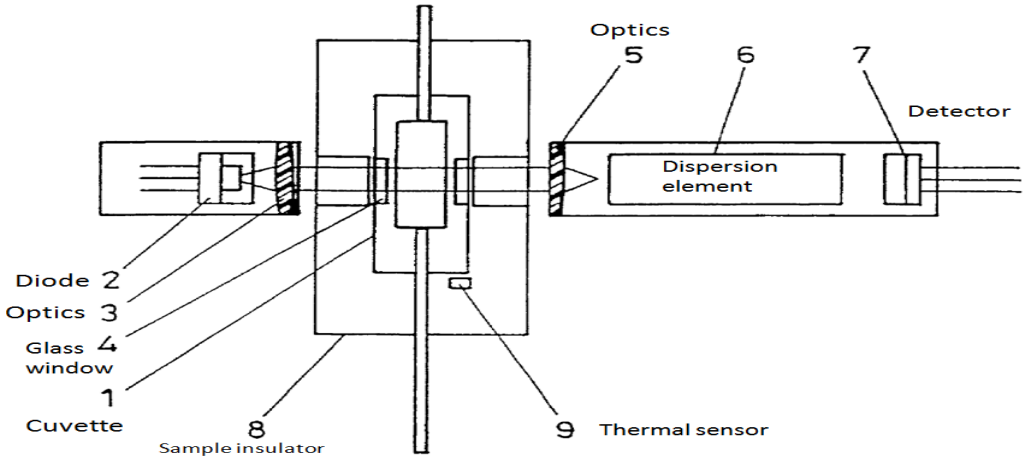


Figure 2.2. A block diagram of an Alcolyzer is similar to the unit used for the reported data.[15]

2.5.2 Molecular Vibrations

To understand vibrational spectroscopy being utilized with the Alcolyzer, it is important to understand that energy (*E*) and frequency (*v*) are different, but are directly related based on Equation 2.5, where *h* is Planck's constant.

$$E = hv \qquad 2.5$$

Molecules are constantly vibrating, but vibrational spectroscopy measures the energy necessary to excite molecules so that the amplitude of the stretching and bending vibrations are larger.[16] These motions include symmetric and asymmetric stretching, rocking, scissoring, wagging, and twisting, as illustrated in Figure 2.3.[17] In order for the absorption to be possible in the IR region, the light must match the natural vibration state of the molecule and the molecule must undergo a dipole change.[16]

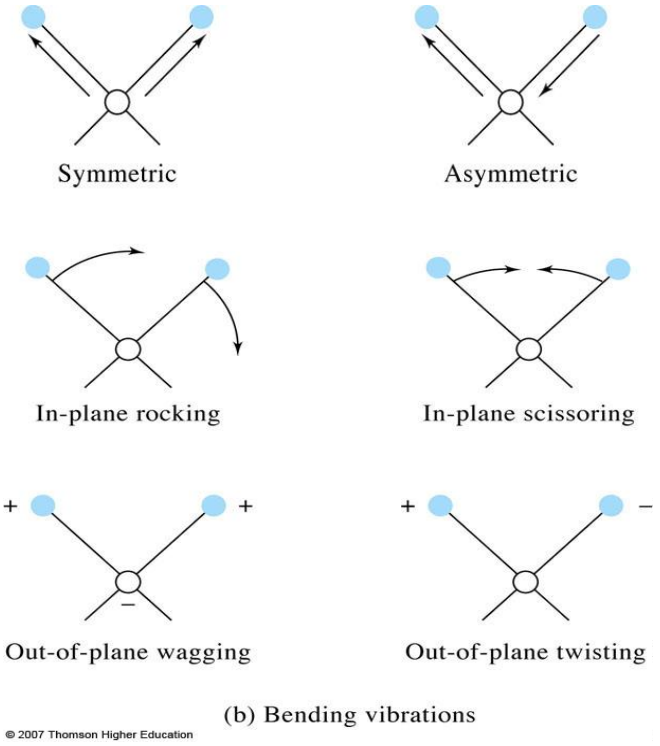


Figure 2.3. Possible bending and stretching motions after IR absorption for a generic molecule. The arrows denote the direction in which the atom is moving. The (+) denotes that the atom is coming out of the page and the (-) denotes that the atom is going behind the page.[16]

When absorption occurs the molecule is excited to a higher energy state. The molecule can be excited from the ground state to the lowest energy excited state or the molecule can be excited from the ground state to an even higher energy state. Respectively, these absorptions are referred to as fundamental frequencies and overtones on a spectrum and can be visualized in Figure 2.4.

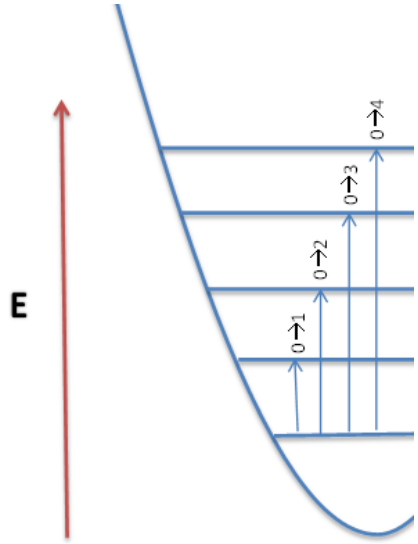


Figure 2.4. An energy diagram illustrates the fundamental frequencies and overtones. The transition from 0 to 1 represents a fundamental frequency. All other transitions represent overtones.[18]

Overtones are whole number multiples of the fundamental frequency.[16] As an example, if a C=O fundamental frequency is located at 1700 cm^{-1}, the overtones could be located 3400 cm^{-1}, 5100 cm^{-1}, etc. Furthermore, combinations can be seen on IR spectra, which is the creation of a peak when two fundamental frequencies are combined.[16] For example, a peak is possible around 3000 cm^{-1} for conjugated C=O bonds from the C-O stretching frequency at 1300 cm^{-1} and the C=O stretching frequency at 1700 cm^{-1}.

Fundamental frequencies occur at different energies depending upon the bond strength and the types of atoms in the bond, based on Equation 2.6,

$$\nu = 4.12\sqrt{\frac{k}{\mu}} \qquad 2.6$$

where *v* is frequency, *k* is the force constant, and μ is the effective mass. The frequency is directly related to the force constant, k. The force constant is based on the strength of the bond and is approximately 5 x 10^5 dynes/cm for a single bond, 10 x 10^5 dynes/cm for a double bond, and 15 x 10^5 dynes/cm for a triple bond. A stronger the bond will require more energy to create a vibration within the bond. The frequency is indirectly related to the effective mass, μ. The effective mass is calculated from Equation 2.7, where M_1 is the mass of the first atom involved in the bond and M_2 is the mass of the second atom involved in the bond.

$$\mu = \frac{M_1 M_2}{M_1 + M_2} \qquad 2.7$$

Larger mass atoms will generate a greater μ. If μ is large, or the atoms involved in the bond are heavy, the frequency will be smaller based on Equation 2.6. This also logical because heavier atoms would be more capable of creating vibrations within a bond compared to smaller atoms. This would require less energy to vibrate the bonds, lowering the frequency.

In order for IR light to be detected, IR light must be shined on the sample. Some of the IR light will be absorbed by the sample, while some light will be transmitted. A ratio between the intensity of the light transmitted to the initial light intensity can be calculated at all wavelengths. Afterwards, absorption can be plotted. The work of Engelhard, et al demonstrates the NIR spectra of the wavelengths measured by the Alcolyzer, seen in Figure 2.5.[17] These peaks are composed of overtones and combinations from different stretching and bending modes in water. Water absorbed

IR light exciting the molecule and causing the bonds to vibrate with larger amplitudes. The energy absorbed lead to excitation to the lowest energy excited state for some molecules, while other molecules were excited to even higher energy states. For example, the peak at 1000 nm has an overtone of the symmetric stretching mode of water combined with the asymmetric stretching mode of water. However, some of the light was transmitted through the water without absorption and was split into its component wavelengths and hit the detector. Once the light hit the detector, a current was created and the absorption was plotted on a graph.

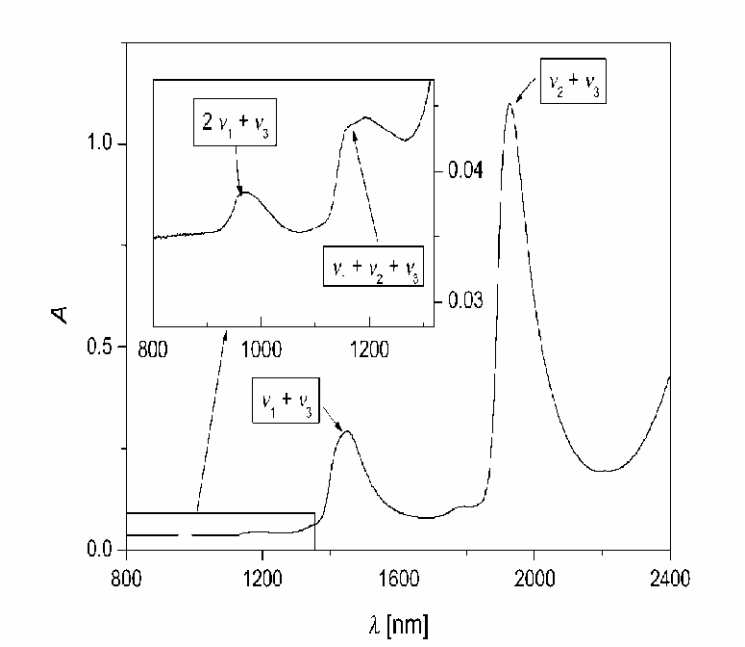


Figure 2.5. The NIR spectrum of water, where v_1 is the symmetric stretching mode of water, v_2 is a bending mode of water, and v_3 is the asymmetric stretching mode of water. There overtones and/or combinations in the NIR are utilized for quantifying neat water on an Alcolyzer.[17]

2.5.3 Quantitating ABV with the Alcolyzer

The Alcolyzer shown in Figure 2.2 utilizes two (2) wavelengths of excitation light to quantitate ABV. The software associated with the instrument calculates the difference between the absorbance of the ethanol peak and the water peak.[17] In order to identify useful spectral features for ABV quantification, the spectrum of water was compared to the spectrum of the alcoholic beverage, as shown in Figure 2.6. This difference spectrum is enlarged to show features of interest near 1700 nm, as these alcohol absorbances do not change in shape or intensity when water-ethanol hydrogen bonding interactions occur.[17]

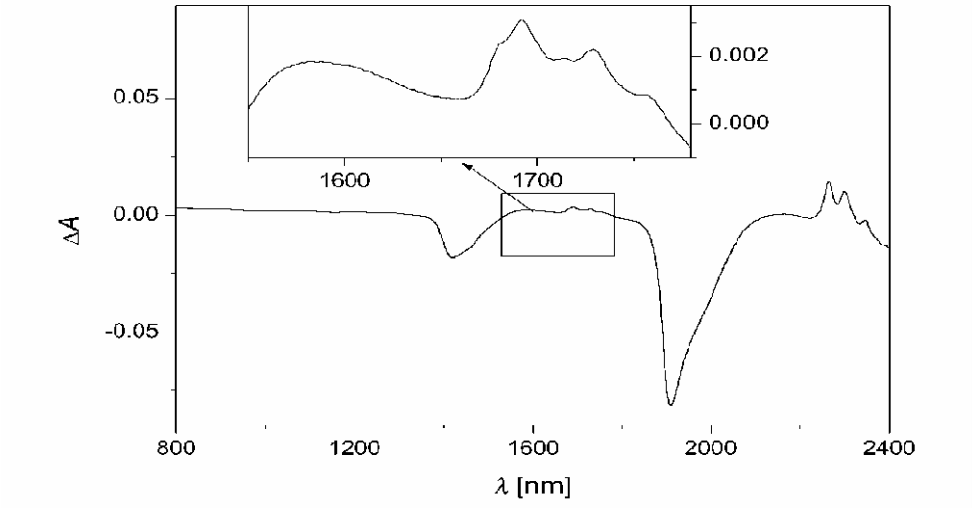


Figure 2.6. An entire spectrum plotting the difference in absorbance between the alcoholic beverage and water is shown. Following this measurement, two specific peaks are utilized for quantitation of ABV. [17]

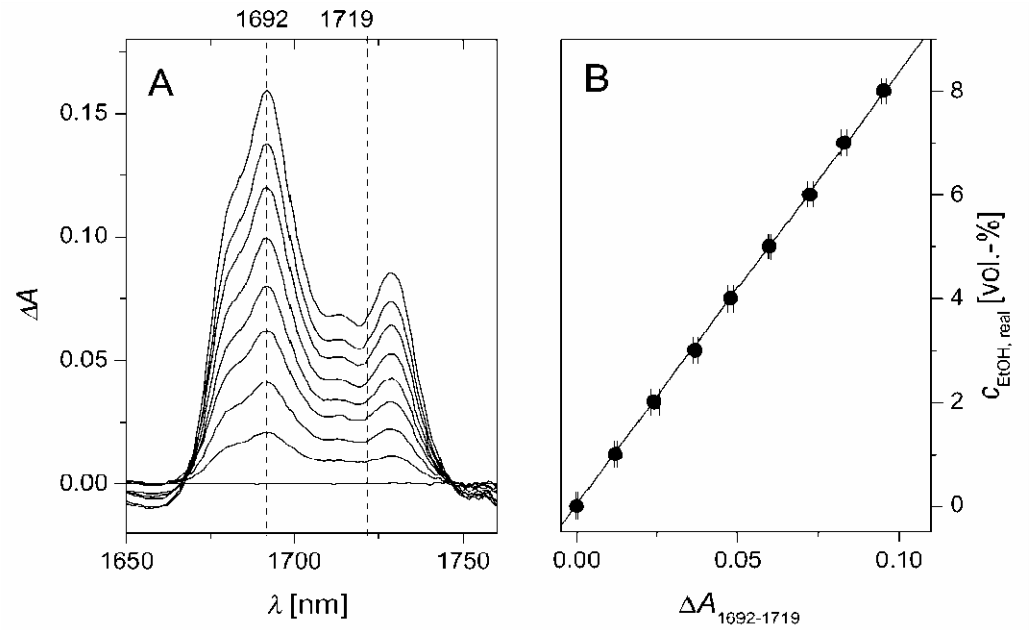


Figure 2.7. Part A shows the two peaks that are utilized for the ABV quantitation. Both peaks are around 1700 nm and are attributed to the CH_2 stretching vibrations of ethanol. Part B shows a plot of ΔA vs. concentration of ethanol. This linear regression is used for interpolation to determine ABV.[17]

Two frequencies are utilized to calculate the ABV, as shown in Figure 2.7A. From the collected absorbance measurements, instrument software creates multiple linear regressions from plots of ethanol concentration vs. ΔA at these frequencies, and then utilizes the interpolation to determine the amount of ethanol present in a sample, as shown in Figure 2.7B. The limit of linear regression linearity occurs at approximately 10 % ABV. Thus, the ABV concentration must be less than this or the measurement will not be valid without prior dilutions.[17]

Because the flavored spirits of interest to this work typically have more components than just ethanol and water, corrective calculations account for any peak shifting or broadening associated with the interactions between ethanol and other

compounds. In particular, the instrument utilizes the Tabarie relationship as one of its corrective calculations. This mathematical equation relates the specific gravity of a sample to the distillate and the extract, as seen in Equation 2.8,[19] $SG_{beer} = SG_{alcohol} + SG_{extract} - 1$ 2.8

where SG_{beer} is the specific gravity of the sample, $SG_{alcohol}$ is the specific gravity of the distillate, and $SG_{extract}$ is the specific gravity of the residual. Without this correction, the peak broadening of ethanol could result innaccurate higher ABV values. However, this relationship falls off around 10% due to the fact that the relationship between specific gravity and alcohol concentration are not linear past this range.[19,20]

The Alcolyzer is both precise and accurate, even in the presence of other substances, as shown in Figures 2.8A and 2.8B. Figure 2.8A shows the two peaks that are utilized for ABV determination and Figure 2.8B plots the linear regressions of the determined ethanol concentration vs. the real ethanol concentration, demonstrating how selective the Alcolyzer can be. Up to 4 % maltose in a beer sample does not affect the accuracy of the ABV determination. [17]

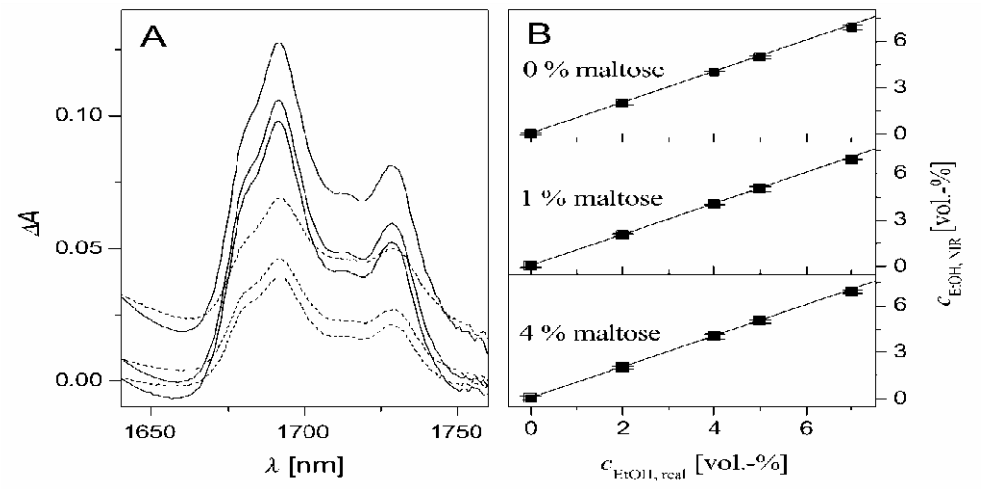


Figure 2.8. Part A shows the two peaks that are utilized for the ABV quantitation. Both peaks are around 1700 nm and are attributed to the CH_2 stretching vibrations of ethanol. Part B shows a plot of determined alcohol concentration vs. real alcohol concentration.[17]

2.5.4 Data Processing from Alcolyzer

Two reports are generated for each sample analyzed with the Anton Paar instruments: one contains the ABV at 20°C, which is listed as International Alcoholometric (OIML), and the other reports density, but only the density printout is necessary. This density report is combined with independent calculations to determine ABV. In order to complete the calculations, the density of the direct was taken from the printout. From density, the specific gravity (*SG*) of the direct is determined by Equation 2.9.

$$\boldsymbol{SG_{direct} = \frac{Density}{0.998201}} \qquad 2.9$$

The ABV at 20 °C can be found listed as OIML on the density printout. These ABV values were utilized to determine the true ABWt utilizing Equation 2.10.

$$\boldsymbol{True\ ABWt = \frac{Average\ ABV\ (20°C)x\ 0.7907}{SG_{direct}}} \qquad 2.10$$

After the true ABWt was calculated, the percent error was determined for the sample following Equation 2.11.

$$\boldsymbol{Percent\ Error = \frac{Known\ ABWt - Calculate\ True\ ABWt}{Known\ ABWt}\ x\ 100\%} \qquad 2.11$$

2.6 Density Theory

Because the Alcolyzer cannot measure above 10 % ABV without dilutions, density must be utilized to determine ABV. At the atomic/molecular level, density is how tightly packed atoms/molecules are and on the identity of the sample. As an example, certain elements contain more neutrons and protons, subatomic particles that have a large impact on mass, but a negligible effect on size. The large increase in mass, but little difference in size, makes these atoms denser. Density is most commonly measured in g/mL. Water has a density of 0.998 g/mL at 25 °C, while ethanol has a density of 0.789 g/mL. This means that water is able to pack more tightly, such that more mass is in a certain area.

2.6.1 Density Instrumentation

The following sections are a discussion of the density determination methods used for this study.

2.6.1.1 Densitometers

Densitometers can be utilized to quantitate ABV at all levels, but are limited to binary systems such as ethanol and water. In this work, a DMA 5000 densitometer was used, which consists of a tube, frequency oscillator, magnet, and coil, depicted in Figure 2.9. The cell is filled with sample and subjected to electromagnetic force.[21]

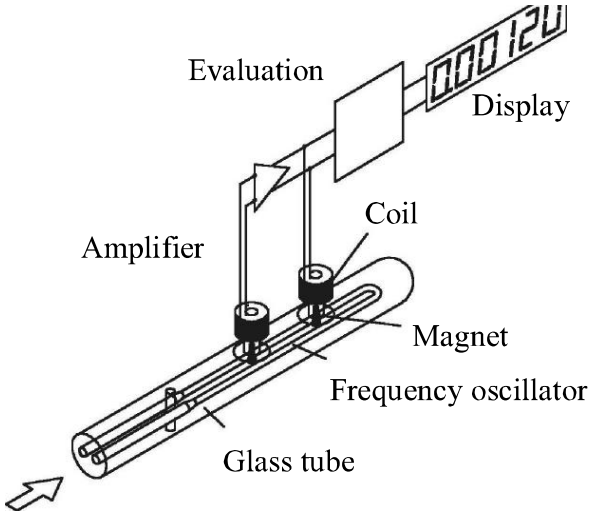


Figure 2.9. The internal components of a standard densitometer.[21]

The density is determined from the DMA using Equation 2.12, where τ is the oscillation period, ρ is the density of the liquid, v is the volume of the cell, m is the mass of the cell, and C is the spring constant. The volume, mass, and spring constant are known values, so when the oscillation period is measured, the density of the liquid can be determined.[21]

$$\tau = 2\pi\sqrt{\frac{\rho v+m}{C}} \qquad 2.12$$

2.6.1.2 Pycnometers

Pycnometers are also used to measure the density of distillates such that ABV can be quantified. Pycnometers have a set mass and volume. The mass of the pycnometer can be measured when it is completely dry and again when it is completely filled with a solution, allowing the mass of the solution to be determined.

Because the pycnometer has a set volume, the density can be determined by taking the mass divided by the volume, as seen in Equation 2.13, where the m_2 is the mass of the full pycnometer, m_1 is the mass of the dry pycnometer and v is the volume of the pycnometer.[22]

$$d = \frac{m_2 - m_1}{v} \qquad 2.13$$

2.6.2 Method for Density Determination

The densities of the standard sugar/water/ethanol solutions (0 % to 35 % w/w sugar) were determined by three different methods. The first method involved utilizing the ST Instrument Inc. eDrometer densitometer. To use, water was first pushed through the tubing, utilizing a syringe, to ensure that the densitometer was clean and working. Then, a syringe was filled with the standard solution and pushed through the tubing. To ensure that the density was correct, no air bubbles were visibly present in the tubing. Once the density equilibrated, the density was recorded.

The second method of density determination employed the densitometer attached to the Alcolyzer.

The last method utilized a pycnometer. To prepare the sample, 100mL of solution was placed into a centrifuge tube and equilibrated to 20 °C in a water bath for 20 minutes. During this time, the mass of the dry pycnometer was determined. After the 20 minutes, the solution was poured into the pycnometer and the pycnometer mass determined again. Based on math previously discussed in Section 2.6.1.2, the density of the solution was determined.

The differences between the densities measured by each method were calculated. Measured densities were also plotted onto OIML charts to compare the percent ethanol that would be calculated based on density. These density determinations were not done in replicate.

2.7 Nuclear Magnetic Resonance (NMR) Spectroscopy

As discussed previously, NMR is a useful tool for determining if hydrogen bonding is present in a solution; thus, it is important that NMR be understood.

2.7.1 NMR Theory

All nuclei have a property known as spin: the nuclear movement of an atom that creates a magnetic moment along an axis of rotation. In order for a nucleus to possess this property, the atom must have an odd mass number and/or an odd atomic number because this results in a spin angular momentum and a magnetic moment.[16]

The number of possible spin states that an atom can possess is determined by the quantum number, I, the sum of the spins of uncoupled protons and neutrons. If the atom has an odd mass, I is equivalent to ½ + n, where n is a whole number multiple. If the atom has an even mass and an even atomic number, I is equivalent to zero. If the atom has an even mass and an odd atomic number, I is equal to whole number multiples greater than one.[16] As an example, ^{2}H has an I equal to one because it has an even mass, but an odd atomic number. In order to determine the number of possible spin states, Equation 2.14[16] is utilized. One can also determine the number of

spin states by counting from $-I$ to $+I$ in whole number increments.[16] For example, ^{1}H has I = 1/2. Utilizing the equation, it can be determined that the number of spin states is 2. However, if one were to count -1/2 to +1/2 in whole number multiples of one, they would also determine that there are only two spin states: +1/2 and -1/2.

$$2I + 1 \qquad 2.14$$

In magnetic field (B_o), the magnetic moment (μ) can either be aligned with B_o or against B_o. When μ is aligned with B_o, μ possesses lower energy than when μ opposes B_o. An increase in the applied field strength causes an increase in the energy gap between spin states, shown in Equation 2.15.[16] Each atomic nucleus has a different ratio of magnetic moment to angular momentum called the gyromagnetic ratio, γ, which affects the sensitivity of nucleus detection, as seen in Equation 2.16.[16] For ^{1}H NMR, the constant is 267.53 radians/Tesla. Furthermore, γ can help determine the frequency of radiation that a nucleus will absorb (ν).[16]

$$\Delta E = h\upsilon = B_o \gamma \frac{h}{2\pi} \qquad 2.15$$

$$\upsilon = \frac{\gamma}{2\pi} B_o \qquad 2.16$$

NMR occurs when energy absorption causes a change in the spin orientation. In an applied magnetic field a nucleus will precess, or spin, about an axis in the direction of B_o. The frequency of this precession is called the Larmor frequency (ω). In order for a spin change to occur, the ν must match ω and couple.[16]

The energy necessary to transition from one spin state to another is very small, approximately, 2.39 x 10^{-5} kJ/mol, allowing any given hydrogen nucleus to have both spin states occupied almost evenly; however, there will be a slight excess of nuclei in the lower energy spin state.[16] The Boltzmann ratio of nuclear spins allows the number of excess nuclei in the lower energy state to be determined, where N_{upper} and N_{lower} refer to the number of nuclei in the higher and lower energy states, respectively, k is 1.380 x 10^{-23} J/K, h is 6.626 x 10^{-34} J/s, T is the temperature in Kelvin, and υ is the operating frequency of the instrument, as seen in Equation 2.17[16]. As the operating frequency increases, the excess nuclei in the lower energy state increases.

$$\frac{N_{upper}}{N_{lower}} = e^{-\Delta E/kT} = e^{-h\upsilon/kT} \qquad 2.17$$

2.7.2 NMR Instrumentation

In general, the NMR is able to collect data by the following process. At any given time, the nuclei are precessing. Once a pulsed magnetic field is applied, the nuclei are excited to higher spin states and relax with time. The detector senses the fluctuation of the magnetic field by the precessing nuclei as they relax and the fluctuation will be at different frequencies depending upon the chemical environment where the atom is located.[16]

More specifically, the pulse is a powerful short burst of energy that contains a wide range of frequencies. As the nuclei relax, electromagnetic radiation is emitted. Because most molecules contain different nuclei, many different electromagnetic

frequencies are emitted simultaneously, creating multiple overlapping signals; therefore, Fourier Transform is necessary.[16]

2.7.3 Interpreting Spectra of ^{1}H NMR

Proton frequencies are capable of being shifted downfield (left on the spectrum) or upfield (right on the spectrum) depending upon the chemical environment that surrounds the hydrogen and the chemical environment resulting from the neighboring atoms. Specifically, hydrogens are shielded by the electron density that surrounds them, resulting in an upfield shift. This shift is possible because valence electrons are caused to circulate in a specific manner in an applied magnetic field so as to generate a counter magnetic field opposing the applied magnetic field. Thus, the greater the electron density around a nucleus, the greater the induced field of the electrons will be, diminishing the effect of B_o. When the magnitude of B_o experienced by a nucleus is smaller, the nucleus precesses at a lower frequency; the shift will be closer to the right of the spectrum because the energy involved in the emission is smaller.[16]

When different amounts of electrons are present near a given nucleus, there will be different chemical environments, resulting in different radiation absorbed and different resonance frequencies. Therefore, all nuclei in chemically identical environments are chemically equivalent and will have the same chemical shift, whereas, chemically distinct nuclei will appear at different chemical shifts. These different chemical shifts are in the range of parts per million (ppm, δ), as described by Equation 2.18.[16] The unit of ppm is actually a ratio of the shift from TMS to the

spectrometer frequency, such that the chemical shift for a given nucleus will not depend on the spectrometer frequency. [16]

$$\delta = \frac{Shift\ from\ TMS\ (Hz)}{Spectrometer\ Frequency\ (MHz)} \qquad 2.18$$

The area of a signal at a given chemical shift is proportional to the number of ^{1}H nuclei in a given chemical environment.[16]

When interpreting a NMR spectrum, a key factor to determine the identity and/or structure of a compound is the chemical shifts corresponding to each nucleus or each set of chemically equivalent nuclei. Electronegativity directly affects the electron density around a given nucleus, which will affect the chemical shift of the signal corresponding to that nucleus. Thus, if a large nearby dipole exists caused by a very electronegative atom, less electron density will be present around the observed nucleus and the peak will be shifted downfield. This effect increases as the number of neighboring electronegative atoms increase, and this effect decreases as the distance from the electronegative atoms increases. Hybridization also affects the chemical shift of atoms; in particular, a proton on a sp^2-hybridized carbon will have a larger downfield shift compared to a proton on a sp^3-hybridized carbon. Elements that can undergo hydrogen bonding, such as nitrogen and oxygen, often display broadened peaks due to hydrogen bonding.[16]

A third consideration, is spin-spin splitting. This is the result of nearby spin-active nuclei affecting the chemical shift of a signal. For an example, seen in Figure 2.10[23], given a proton with a neighboring hydrogen on an adjacent carbon, if the spin

of the nucleus of the hydrogen attached to the adjacent carbon is aligned with the magnetic field, the observed proton is de-shielded, causing a downfield shift. However, when the adjacent hydrogen nucleus is aligned against the magnetic field, the proton is shielded, resulting in an upfield shift. Both spin combinations are equally likely to occur, resulting in two peaks in the signal for the observed proton, a doublet. Different spin combinations will affect the splitting pattern of the signal, enabling the determination of how many neighboring hydrogen atoms there are for a given proton.

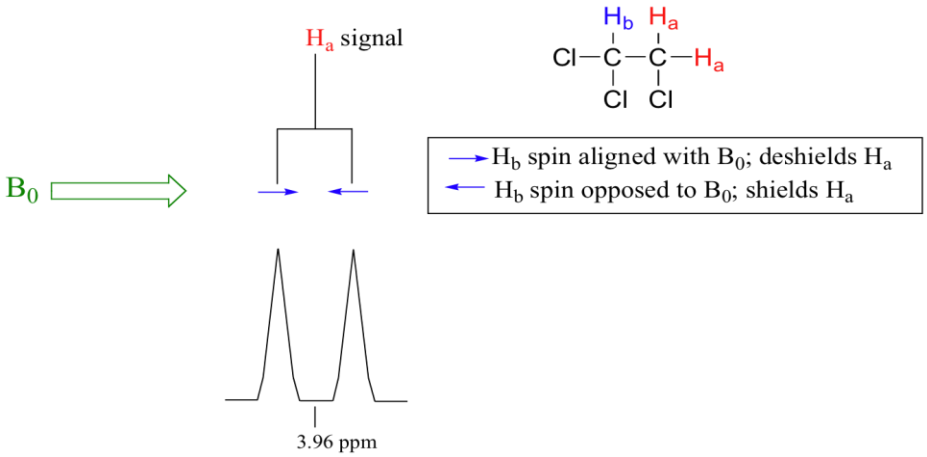


Figure 2.10. This figure illustrates peak splitting of H_a caused by the alignment of the neighboring H_b. When the nucleus of H_b is aligned with the magnetic field, H_a is deshielded and the peak is shifted downfield. When the nucleus of H_b is aligned against the magnetic field, H_a is shielded and the peak is shifted upfield.[23]

2.7.4 1H NMR Parameters

A JEOL ECS-400 NMR was utilized to test the hydrogen-bonding hypothesis due to its ability to look directly at hydrogen atoms and the chemical environments surrounding them. The parameters included a pulse attenuation of 79 dB, a pulse width of 1 μs, and a scanning region from -2 ppm to 12.5 ppm. The measurements were completed at ambient room temperature.

2.7.5 ^{1}H NMR Method for Sample Preparation

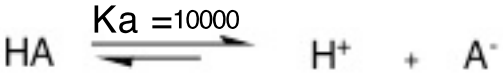
Approximately 1mL of sample was added to an NMR tube along with 1 mL of the desired solvent ($CDCl_3$ or D_2O). The NMR tube was shaken vigorously and placed into the spinner.

2.8 pH and pK_a

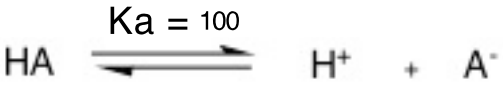
2.8.1 pH and pK_a Theory

The pH of a solution is the negative log of the hydrogen ion activity. The activity of the hydrogen ion is defined as the hydrogen ion concentration multiplied by an activity coefficient, which takes into account the interaction of the proton with other species in the solution. However, the activity coefficient is typically neglected in dilute solutions and the pH is simplified to be the negative log of the hydrogen ion concentration.[24] In highly acidic solutions, the hydrogen ion concentration is large. Conversely, in highly basic solutions, the hydrogen ion concentration is small and the hydroxide concentration is large.

The acid ionization constant, K_a, is the equilibrium constant for the ionization of an acid. Thus, a larger K_a indicates that the substance is more acidic because it is able to release hydrogen ions more readily into solution. Shown in Equation 2.19a, this generic substance would be more acidic than the different generic substance in Equation 2.19b because its equilibrium favors dissociation. By taking the $-\log K_a$ to equal pK_a, then the opposite must be true of the pK_a; a large pK_a indicates that the substance is a weak acid because it does not dissociate as easily, preventing the release of hydrogen ions into solution to lower the pH.[12]



2.19a



2.19b

In a molecule with multiple labile hydrogen ions, dissociation constants usually differ. Simply, the stability of the conjugate base can be used to predict the relative magnitude of dissociation constants.

In this work, the dissociation constants of hydrogen ions on fructose (Figure 2.10) are of particular interest. At higher concentrations, fructose will act as an acid and the hydroxyl in position 2 is the most acidic. This is shown in Figure 2.10a and Figure 2.10b (for numbering see Figure 1.1). It can be seen that the equilibrium favors deprotonation of the second hydroxyl group (Figure 2.10a) compared to the

equilibrium for the deprotonation of the first hydroxyl group (figure 2.10b). This is logical because the conjugate base in Figure 2.11a has the negative at a tertiary location compared to a primary location in Figure 2.11b. The conjugate base in Figure 2.11b is less stable than the conjugate base in Figure 2.11a, making the hydroxyl at position 2 more acidic because deprotonation is more favorable. The basicity and acidity of fructose is discussed in more detail in Section 3.4.1 of Chapter 3.

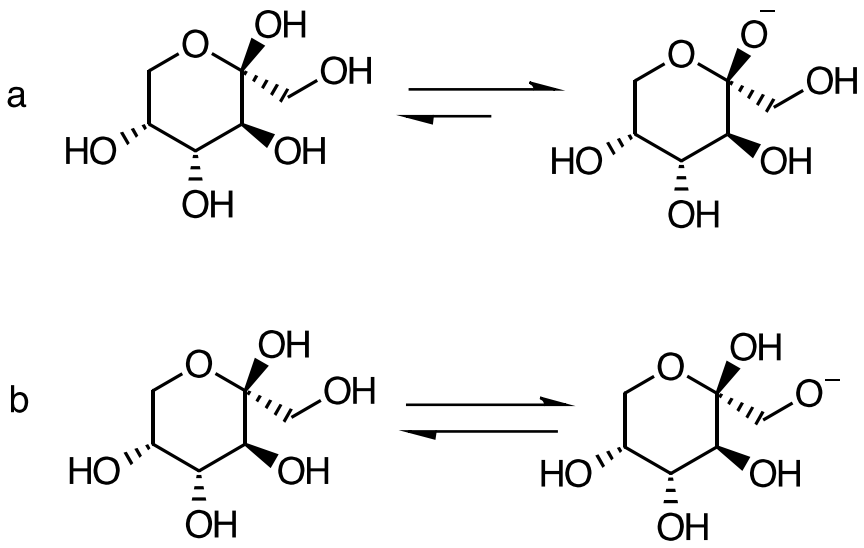


Figure 2.11. Figure 2.11a demonstrates that deprotonation of position 1 is favorable compared to deprotonation of position 2 in Figure 2.11b.

2.8.2 pH Instrumentation

pH measurements are based on a pH electrode and a reference electrode. The pH electrode is composed of an inert glass tube with a hydrogen ion sensitive glass membrane tip. The inside of the glass tube is filled with a solution of known pH. The difference in hydrogen ion concentration inside the tube and in the solution creates a potential across the glass membrane is utilized for pH determination. The reference electrode is composed of an internal element of Ag/AgCl, an electrolyte fill of KCl/AgCl, and a liquid junction. Electrical contact must be maintained in order for pH measurements to be able to occur. This means that diffusion of ions between the reference solution and the process solution must be possible. The KCl is an ideal fill solution because K^+ cations diffuse through water at the approximately the same rate as Cl^- anions. Because these ions move at approximately the same rate and have the same magnitude of charges, a net zero charge at all points within the liquid junction would be present. This allows the reference electrode to maintain a constant potential at any temperature, whereas the pH electrode develops a potential proportional to the pH of the solution.[24]

The pH is measured as the difference in millivolts between the potential of the pH electrode and the reference electrode. Based upon a slope of mV/pH, with a known mV concentration, the pH can be determined, as seen in Figure 2.12.[24]

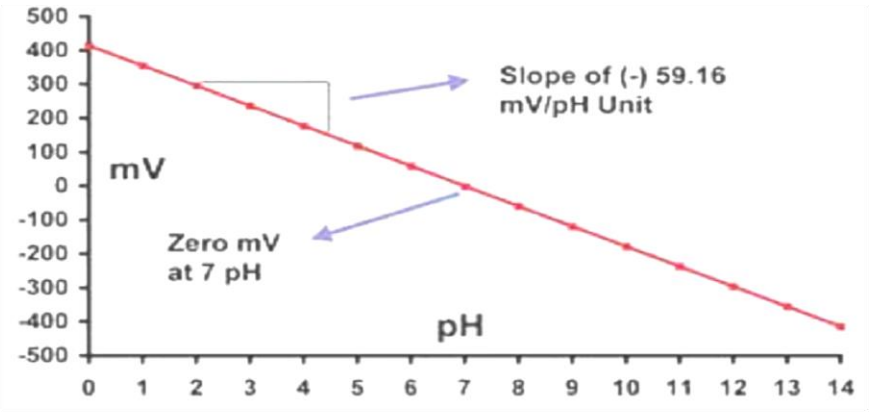


Figure 2.12. This figure shows the direct correlation between pH and the potential (mV). Based on the slope and y-intercept, the pH of a solution can be determined if the potential difference is known.[24]

Temperature affects hydrogen ion dissociation constants and therefore must be accounted for in pH adjustments.[24] The Van't Hoff equation correlates temperature and dissociation constant as shown in Equation 2.20.[12] *K* is the equilibrium constant, ΔH^O is the standard enthalpy change, *R* is the gas constant, *T* is the temperature, and ΔS^O is the standard entropy change.

$$\ln(K) = -\frac{\Delta H^\circ}{R}\left[\frac{1}{T}\right] + \frac{\Delta S^\circ}{R} \qquad 2.20$$

As the temperature increases in endothermic reactions, acids will dissociate into more ions, increasing *K*. This increase in dissociated ions is a direct result of Le Chatelier's Principle. When the temperature increases in an endothermic reaction, the equilibrium will try to offset the increase by creating more products. As the number of products increase (the ions), the *K* also increases. At higher temperatures, dissociation would increase, causing a solution to appear more acidic; at lower temperatures, there

is less dissociation, causing a solution to appear less acidic. Thus, the measured pH needs to be adjusted so that all pH measurements can be comparable, regardless of temperature.

2.8.3 Method for Determining pH

The pH of prepared solutions was determined (Denver Instrument UltraBasic pH Meter). Calibration with standard buffer solutions (listed in section 2.1) preceded all pH measurements. First the probe was rinsed with water and then the probe was inserted into the standard pH 7 buffer solution. The standardize button was pressed and the linearity was recorded. This process was repeated for the pH 4 buffer solution followed by the pH 10 buffer solution. If the linearity was above 96.0, the pH probe needed no further calibration; the pH probe always fell within these calibration limits.[25]

To test the pH of the prepared standard solutions (0 % sugar to 35 % w/w sugar), approximately 150 mL of the standard solution was poured into a large beaker. The pH probe was inserted into the solution for 15 seconds, removed, rinsed, and re-inserted into the solution for an additional 30 seconds before the pH was recorded. This procedure was not completed in replicate for the standard solution.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Density Results

All of the densitometers gave density values very similar to one another, in fact, all three densities for each of the standard solutions (0 % to 35 % w/w) are within thousandths of the other densities (g/mL). However, the ethanol concentration derived from these density values based on International Alcoholometric (OIML) Tables did not yield such similarity. As can be seen in Appendix A, the ethanol concentrations do not fall within the required 0.25 % alcohol by volume (ABV) of each other, as dictated for accurate and consistent alcohol measurements by Brewing and Distilling Analytical Services (BDAS). As an example, the density values of the 0 % w/w sugar solution were 0.93483, 0.92833, and 0.93198, as determined by the DMA 5000, the eDrometer, and the pycnometer, respectively. However, the ABV determined from these density values are 47.04, 50.93, and 49.10, respectively. Clearly these ABV values are not similar and because the ABV for each solution fall outside of the acceptable 0.25 ABV range, these inconsistencies necessitate further investigation. Based on the known ABV of the solutions, the densitometer is the most accurate, which is highly desirable because most alcohol testing facilities will utilize the DMA. However, these experiments were not performed in replicate so the results are not conclusive and should be tested in greater detail in future work.

3.2 % Sugar vs. % Error

Figure 3.1 demonstrates the % error of the alcohol by weight (ABWt) as a function of sugar concentration. All of these solutions were prepared to be 40 % ABWt ethanol, and ABWt was measured using the distillation method as described in Section 2.4.2 of Chapter 2. When no sugar was added, the average percent error was 0.27 % ± 0.21 %. When sugar was added, the average error ranged from 1.54 ± 0.15 % to 2.62 ± 0.36 %. The acceptable percent error dictated by the Alcohol and Tobacco Tax Trade Bureau (TTB) is 0.53 %. Thus, the only standard solution to fall within the acceptable TTB range is the 0 % w/w sugar solution. Addition of sugar at any level causes an accuracy issue, but not in a direct linear fashion. In fact, a slight downward trend is noticeable from 5 % to 35 %; however, this trend only had a correlation coefficient of 0.51. Sugar concentrations beyond 35 % need no investigation since greater than 35 % sugar would not be utilized in any alcoholic beverages.

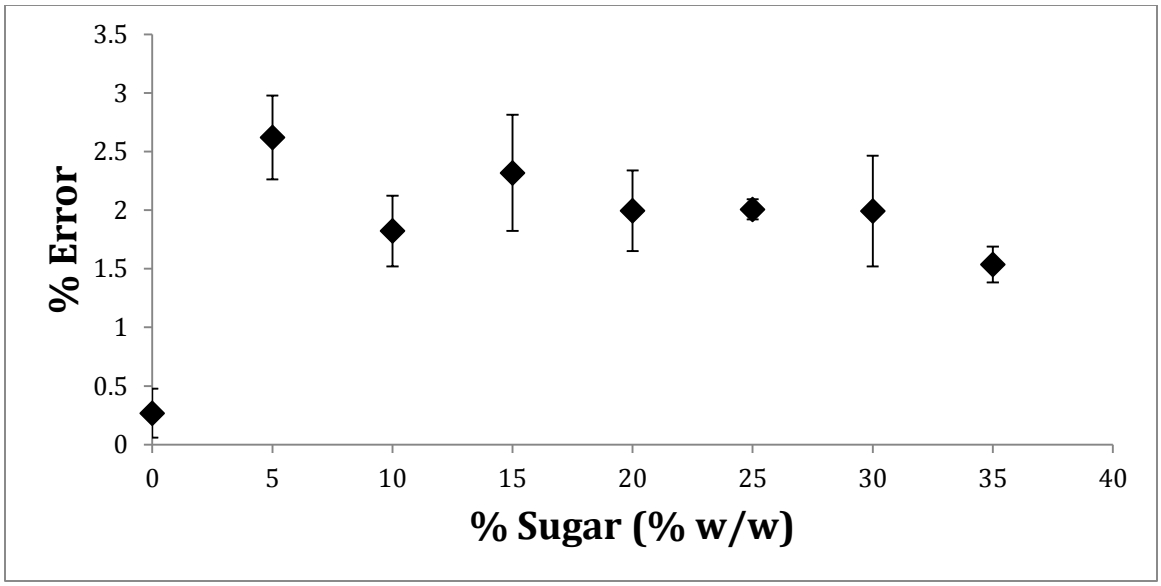


Figure 3.1 The comparison of the percent sugar (w/w) versus percent error. As illustrated from the plot, the percent error is well above the allowable 0.53% error when sugar is added (in any amount) to the solution.

The first hypothesis to explain this phenomenon was that hydrogen bonding interactions between the sugar and the ethanol, resulting in a drastic increase in boiling point beyond what is expected from colligative properties. If the boiling point of ethanol approaches the boiling point of water, the distillate may not contain all of the ethanol in solution as is assumed. This will be discussed in detail in the following sections of the chapter and this hypothesis was investigated with boiling point determination, ^{1}H nuclear magnetic resonance (NMR) spectroscopy, and systematic NaCl additions. The second hypothesis that could explain this phenomenon is a glycoside reaction between the sugar molecules and the ethanol molecules. If sugar and ethanol were reacting, the ethanol would be retained in the residual, resulting in the apparent decreased ABV. However, once the pH is adjusted, the alcohol could be

removed from the sugar, allowing all of the ethanol to be distilled; thus, eliminating the alcohol retention. It is also plausible that other intermolecular interactions occurring, such as ion-dipole interactions, may contribute to the observed ethanol retention. The effects of pH on sugar/ethanol/water reactions and on other intermolecular forces were probed by systematic addition of NaOH. This will also be discussed in detail later in Section 3.4.

3.3 Hydrogen Bonding Hypothesis Data

Hydrogen bonding is a particularly strong dipole-dipole interaction between polar molecules in solution. In order for hydrogen bonding to occur, at least one molecule must have a hydrogen atom bound to an electronegative element such as oxygen or nitrogen. In the resulting polar bond, the hydrogen atom is electron deficient (partially positive) and the other atom is electron rich (partially negative). Hydrogen bonding is the attractive force between a partially positive hydrogen atom and a partially negative oxygen, nitrogen, or another electronegative element. Hydrogen bonding is pivotal to many of life's functions including, but not limited to, the bonding in a DNA helix, the structure of proteins, and the properties of water. Sugars, such as fructose, have five possible sites for hydrogen bonding per molecule. This means, theoretically, that five ethanol and/or water molecules could interact with one fructose molecule. As the ethanol molecules interact with the sugar molecules, this strong interaction could increase the boiling point of ethanol. In reality, the sites available for hydrogen bonding will vary based on the conformation and concentration

of sugar in the solution due to intramolecular hydrogen bonding and proton transfers, as discussed in Section 3.4 (for numbering on the β-pyranose molecule see Figure 1.1). As an example, a proton transfer from 1 to 6 on β-pyranose is shown in Table 3.1. This would leave hydroxyl 6 with two protons, making it less likely to need another proton in hydrogen bonding, but hydroxyl 1 with no protons, making it more likely to need another proton in hydrogen bonding. However, based on what is known about fructose, these positive and negative charges can be distributed throughout the whole molecule without the need for intermolecular interactions.

Nose, et al., determined in water-ethanol mixtures, that addition of acid, such as acetic acid, benzoic acid, gallic acids, phenol, or pyrogallol, increased the proton exchange between ethanol and water and the strength of hydrogen bonding between ethanol and water.[26] Hojo, et al., also found that hydrogen bonding structure in whisky was strengthened due to chemical components in the wooden casks, mainly acidic and phenolic compounds or aldehydes, but determined that glucose (up to 2700 ppm) did not have an effect on the hydrogen bonding strength between water and ethanol.[27] However, this value is equivalent to approximately 1.54 mg/ 1 kg water (or 0.000154 % w/w sugar), a significantly lower sugar concentration than those typically found in flavored spirits. As such, further research into these types of interactions are necessary in the glucose concentration range relevant to flavored spirits.

3.3.1 Boiling Point Results

The boiling point of ethanol is known to be 78 °C at standard temperature and pressure.[28] Boiling point elevation occurs whenever there is another substance added

into a purified solvent and is discussed more thoroughly in Section 2.4.1 of Chapter 2. In the 0 %w/w sugar solution, the boiling point should have been approximately 85 °C, as calculated by Equation 2.4. The measured boiling point was 83 °C, as seen in Figure 3.2. This small difference is attributed to the pressure and elevation of the facility in which the boiling point measurements were taken.

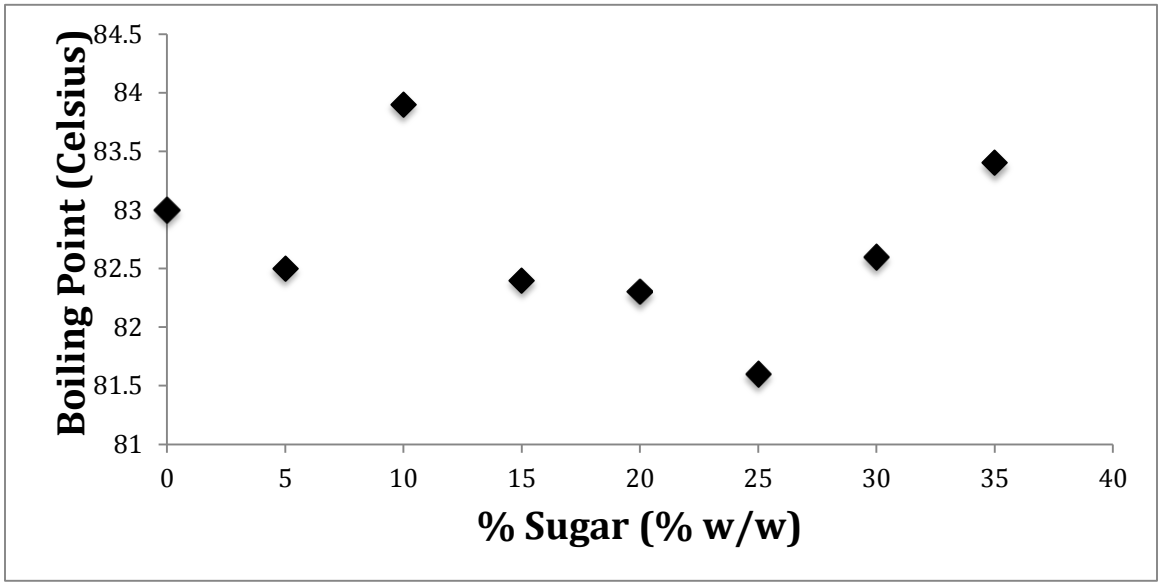


Figure 3.2. Comparison of boiling point versus percent sugar (w/w). This figure shows that the boiling point does not increase as expected based on colligative properties nor does it show a drastic increase of the boiling point expected based on hydrogen bonding.

With the addition of sugar to the mixture, the boiling point was expected to increase. However, the boiling point remained near 83 °C, suggesting that any increase in temperature was too small to be accurately measured with the techniques used here. More precise measurements should be recorded in future work.

3.3.2 NMR Results

The boiling point measurements suggest that hydrogen bonding does not contribute significantly to ethanol retention. However, NMR studies were conducted to investigate the presence of hydrogen bonding with more specificity. Literature suggests that hydrogen bonding between ethanol and sugar gives rise to a downfield trend of the hydrogens in the ethanol molecule.[26] As discussed in Section 2.7.1 in Chapter 2, decreasing electron density results in a downfield shift of the peak. If no hydrogen bonding occurs, a covalently bound hydrogen is only sharing electron density with one other atom, but when hydrogen bonding, this hydrogen shares electron density with two electronegative atoms, causing the hydrogen atom to have less electron density than it would in the absence of hydrogen bonding. Thus, the NMR studies conducted here will be analyzed to determine whether or not a downfield trend occurs with increased sugar concentration.

NMR samples were prepared and the instrumental parameters were as discussed in Section 2.7.4 of Chapter 2 and all NMR data can be found in Appendix B. The water shift (Appendix B.1) demonstrates no downfield trend, as seen by the near-zero slope. This was to be expected because the hydrogen-bonding hypothesis was based on ethanol hydrogen bonding with sugar, not water.

From the many peaks of fructose, seen in Appendix B.2 and Appendix B.3, the hydrogen directly attached to oxygen in ethanol could not be independently resolved. The protons attached to the carbons were investigated instead. For the ethanol CH_2 and CH_3 shifts, no distinct downfield trends were identified. Due to the lack of observed downward shifts, we conclude that hydrogen bonding interactions experienced by ethanol do not change significantly with the addition of sugar.

Appendices B.3 – B.7 show NMR data relevant to the hydrogens of fructose. Each of these shifts results from different hydrogens in different conformations of fructose (see Figure 1.1). The first fructose shift (Appendix B.4) results from the proton at position 5 on the α-fructofuranose ring. The second fructose shift (Appendix B.5) results from the proton at position 4 on the α-fructopyranose ring. The third shift results from the proton at position 2 on the fructose chain (Appendix B.6). The fourth shift results from the proton at position 2 on the β-fructopyranose ring (Appendix B.7). Similar to the results for water and ethanol, there is no downfield trend in the chemical shifts for the fructose hydrogens. Due to the lack of observed downward shifts, we conclude that hydrogen bonding interactions experience by fructose do not change significantly as a function of sugar concentration.

Overall, hydrogen bonding does not appear to increase with increasing sugar concentration, as per the lack of a downfield trend in the chemical shifts of hydrogens associated with water, ethanol, and fructose.

3.3.3 NaCl Modified Distillations

Without salt (NaCl), the percent error in ABV was approximately 1.5 % for a 15 % w/w sugar solution, as illustrated in Figure 3.4. After the addition of 1 g of salt, the percent error dropped to 1.2 %. However, an increase in the amount of salt to 5 g caused the percent error to increase to 1.7 %. Lastly, an increase in the amount of NaCl to 30 g causes the percent error to decrease to approximately 1.5 % again. Because these experiments were not done in replicate, any statistical difference of these values cannot be confidently stated. Ultimately though, the addition of NaCl did not significantly cause the percent error to decrease significantly enough to be applicable for use in avoiding ethanol retention during distillation.

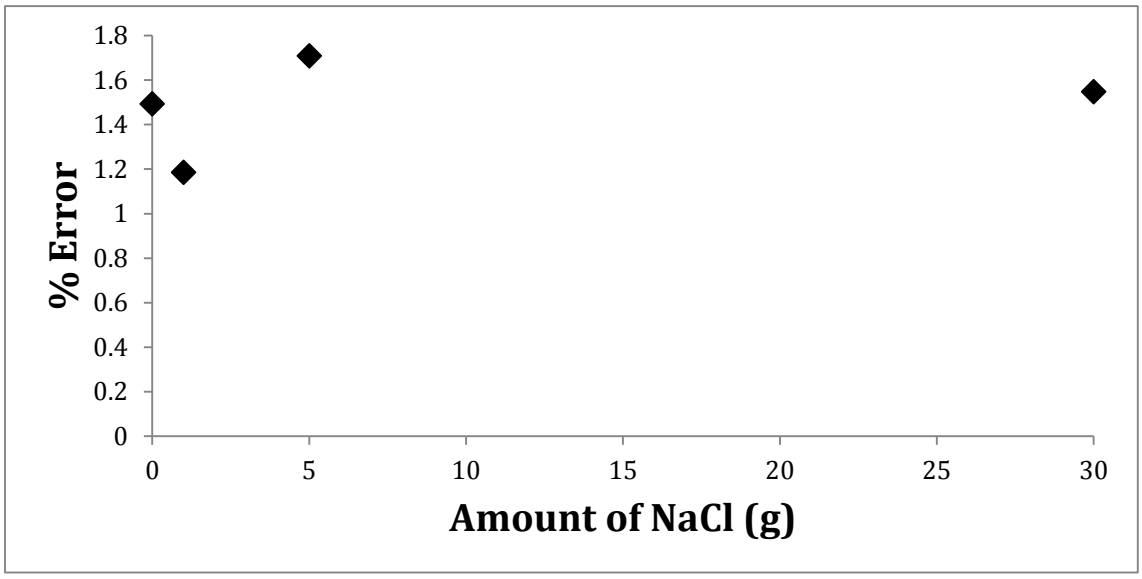


Figure 3.4. Comparison between a series of NaCL additions along with the percent error for ABWt analysis. This figure shows the percent error does not drop below the acceptable 0.53% regardless of the amount of NaCl added.

This data also suggests that the sugar-ethanol hydrogen bonding hypothesis cannot explain ethanol retention during distillation. If the hydrogen bonding was the direct cause of the distillation error, the percent error should have decreased with an increase in salt as discussed in Section 2.4.2 of Chapter 2.

3.4 Glycoside Hypothesis Data

To systematically investigate the effects of varying degrees of protonation on ethanol retention, the pH was altered by the addition of NaOH. Would adjusting the pH affect intermolecular forces within the 15 % w/w sugar standard solution, causing a decrease in percent error?

3.4.1 Intramolecular Forces of Fructose

The most dominant intramolecular force within a fructose molecule is hydrogen bonding between hydrogen and oxygen and depending upon the concentration of fructose within an aqueous solution, the fructose can act as a base or as an acid as a direct effect of hydrogen bonding. At "low" concentrations (below 50 g/100 mL), fructose acts as a base because its proton affinity is greater than that of water. Variation in proton affinity (PA) exists among the same hydroxyls in different conformations, but there is also a larger variation among the different hydroxyls of the same conformation, as seen in Table 3.1 (for numbering of the oxygens, see Figure 1.1).

Table 3.1. Proton Affinities of D-Fructose (Values in kJ/mol)[29]

	Oxygen Number					
Fructose Form	1	2	3	4	5	6
acyclic	1 to 2	817.17	3 to 5	4 to 2	776.68	775.33
β-pyranose	1 to 6	818.62	776.09	878.04	763.26	792.14
β-furanose	1 to 5	818.29	3 to 2	731.96	803.88	6 to 2
α-furanose	1 to 6	816.69	3 to 2	736.81	785.94	807.33

However, these variations are less than 10 % of the total proton affinity value, so protonation of any of the hydroxyls is equally likely in aqueous solutions. However, the PA cannot be calculated for all hydroxyls due to hydrogen bonding mediated proton transfers within the molecule; a fructose molecule can abstract protons from the surrounding solvent, but also from within itself.[29] Furthermore, the stability of the fructose molecule to act as a base is incredibly strong when compared to other alcohols due to its ability to stabilize the charge through multiple surrounding hydroxyl groups.[29]

At “high” concentrations, (above 50 g/100 mL), fructose acts as an acid, despite the greater PA of the hydroxyls compared to water.[29] This observed effect is believed to result from intermolecular hydrogen bonding stabilizing neighboring molecules.[29] Furthermore, intramolecular hydrogen bonding mediated proton transfers occur within the molecule when a hydroxyl group is deprotonated that results in (an unstable molecule conformation).[29]

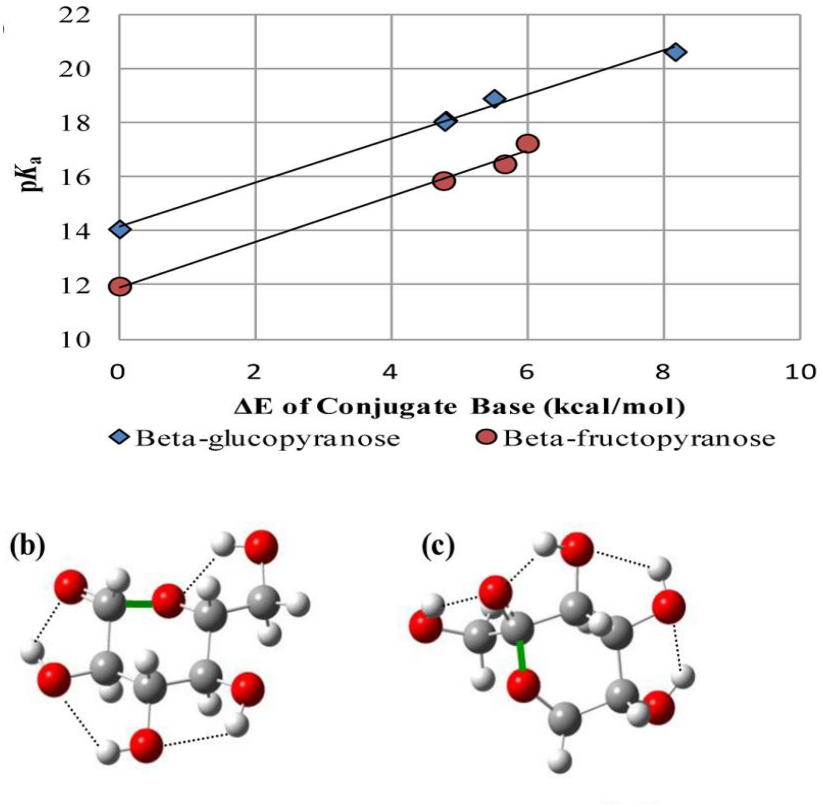


Figure 3.5. This figure shows calculated pK_a values based on the stability of the base. It also shows the conjugate base of glucopyranose (b) and fructopyranose (c), both in optimized geometries, with the dotted line representing hydrogen bonding.[26]

Generally, the most stable conjugate base results in the most acidic proton; therefore, the same can be stated for fructose. If the conformation can maximize hydrogen bonding to decrease the localization of a negative charge associated with deprotonation, there is greater acidity and is shown in Figure 3.5. If the molecular stability difference, as quantified by free energy, between the protonated form and the deprotonated form is small, the pK_a is lower, indicating that the compound is more acidic. As the energy difference becomes larger, the conjugate base becomes increasingly unstable, and the sugar molecules become less acidic. Note that the

anomeric hydroxyl group has the lowest pK_a in all forms of fructose, suggesting this is the most likely site for deprotonation.

3.4.2 pH Data

Figure 3.6 illustrates a pH reduction as the concentration of sugar increases. In particular, going from 5 % to 10 % w/w causes a drop in the pH from 8.0 ± 0.01 to 5.36 ± 0.01. This pH difference of 2.64 is associated with over a 400-fold increase in the proton concentration of the solution from only a 5 % w/w increase of fructose.

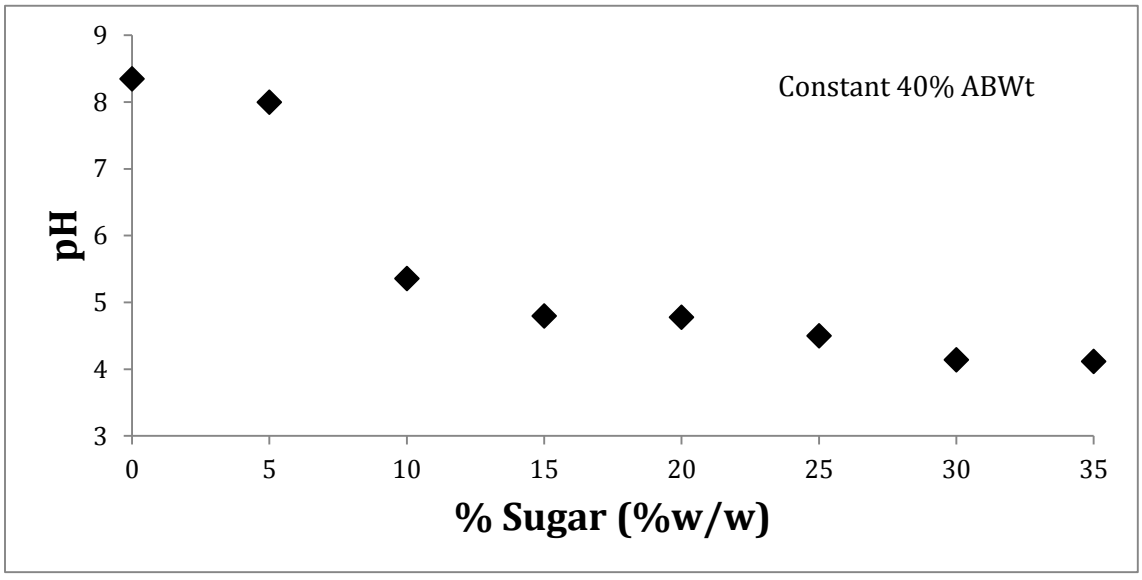


Figure 3.6. Comparison between percent sugar (w/w) and pH. This figure shows the increase of the sugar concentration correlates to significant pH decreases.

Based on the discussion in Section 3.4.1, the measured pH change is logical. As the concentration of fructose increased, fructose acted as an acid. This is the direct result of intermolecular hydrogen bonding stabilizing neighboring fructose molecules. The hydrogen bonding between fructose molecules increased with increasing concentration, resulting in more stable conjugate bases. Furthermore, the conformation of fructose was able to maximize hydrogen bonding within the molecule to decrease the localization of a negative charge associated with deprotonation, thus, increasing stability of the conjugate base. Lastly, based on the pK_a value of the anomeric hydroxyl group, it is most likely that the second hydroxyl group was the site of deprotonation.

3.4.3 NaOH Modified Distillations

A correlation between the decreasing percent error (measured as an absolute value) and increasing pH is shown in Figure 3.7. At a pH of 4.8, the percent error was 1.6 %, more than three times the allowable limit by the TTB. When the pH is adjusted to 8, the percent error drops to under 1 %, only two times the allowable percent error. At a pH of 9.45, the percent error is at 0.4 %; while at a pH of 9.51, the percent error is almost 0 %. When the pH reaches 10.42, the percent error is 0.22 %. Qualitatively, a downward trend occurs for the percent error as pH increases, confirmed by the correlation coefficient of 0.91016. Replicate measurements are needed to determine the best pH for error reduction when measuring ABV by distillation, but the data clearly shows decreased ethanol retetnion as a function of increasing pH.

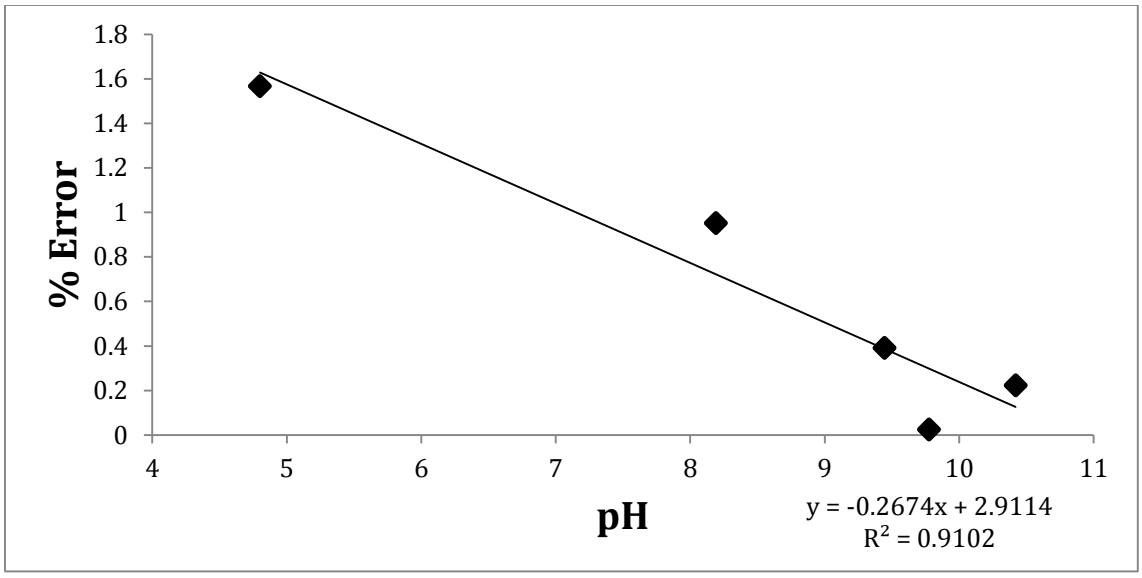


Figure 3.7. Comparison between percent error and pH adjusted by NaOH additions. This figure shows a direct correlation between increasing pH and decreasing percent error.

3.4.4 Reactions of Sugars

Based on the correlation between increasing pH and decreasing percent error, reactions of sugars were investigated. Sugars can undergo a variety of reactions: ester formation, ether formation, glycoside formation, epimerization, reduction, oxidation, chain lengthening, and chain shortening. Glycoside formation was the utmost important reaction to consider to this research. In the presence of an acid, the hydroxyl group can become protonated, forming water, making it a good leaving group. Once water leaves, an alcohol can attack the anomeric carbon position and become deprotonated, forming a glycoside.[8] The reaction results in a racemic product due to the lack of stereospecificity during the alcoholic attack, as seen in Figure 3.8.

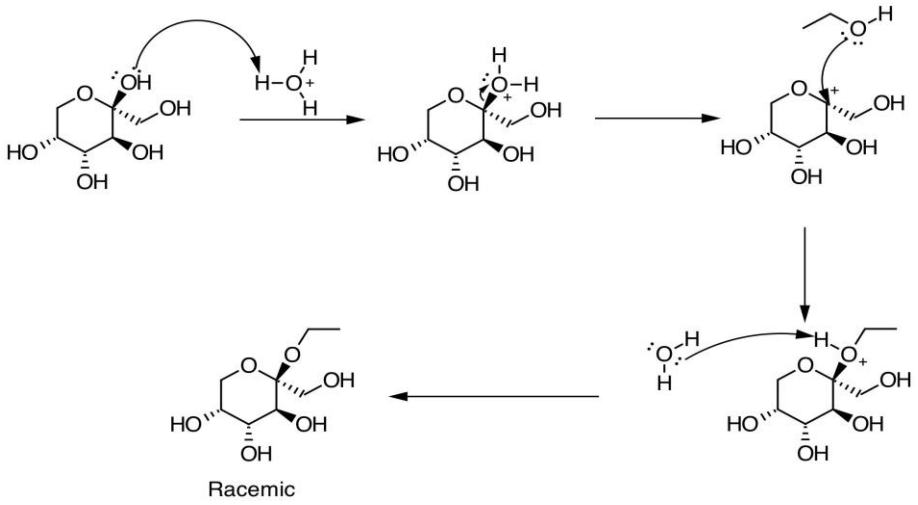


Figure 3.8. Glycoside formation reaction scheme with β-fructopyranose as the sugar.

This decreasing percent error with increasing pH may be a direct result of the reversal of a glycoside reaction, as seen in Figure 3.9. Thus, decreasing the pH would result in hydroxide ions that could attack at the anomeric carbon and release the ethanol.

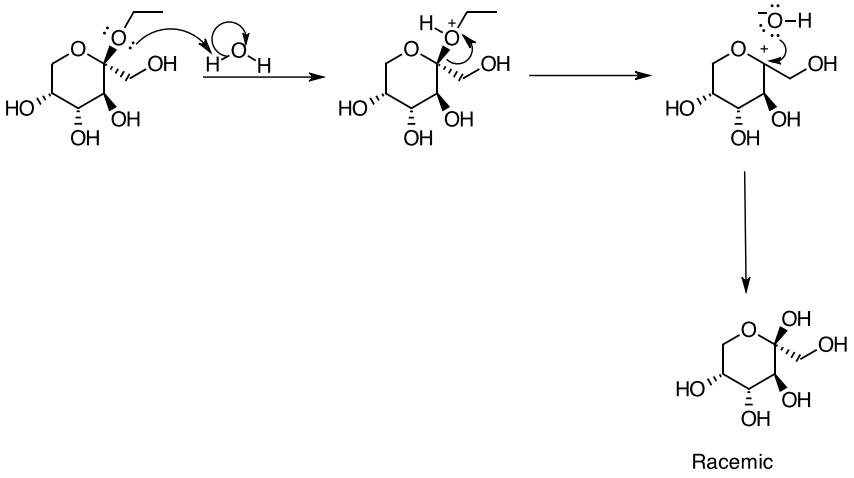


Figure 3.9. Mechanism for glycoside deformation.

The acidity and basicity of fructose was discussed in detail in Section 3.4.1. Until researching the cause of the pH decrease with increasing sugar concentration, the strongest intermolecular force expected to be present in the solution was hydrogen bonding.[12] However, fructose is the acid of the water/ethanol/sugar solution meaning that there would have to be an overall net negative charge on the sugar molecule, even if other surrounding sugar molecules stabilize the charge.[29] With a net negative charge, the strongest interaction would actually be ion-dipole interactions, or possibly even ionic compound formations. These interactions are much stronger than hydrogen bonding and would not be as easily broken by the addition of a salt.[12] However, the addition of a base would reverse the deprotonation of the sugar molecules, restoring the net zero charge of the sugar, eliminating any ion-dipole or ionic interaction between the sugar and ethanol.

3.4.5 pH Conclusions

Increasing pH results in a decreasing percent error, but the exact reason for this decrease was unknown. The mentioned glycoside mechanism was a proposed elucidation; however, intermolecular interactions are just as likely. Both theories account for the decrease in ethanol, but without further research, it would be impossible to state that these are the only plausible explanations or state that one is more correct.

3.5 Real Solutions

Due to the efficacy of reduced pH on ABV determination when measuring prepared sugar/water/ethanol solutions, this strategy was applied to real-world samples. The increase in pH was tested on three different alcoholic beverages: vodka, bourbon and moonshine. The purpose of this experiment was to test if basifying an actual alcoholic beverage also resulted in a decreased percent error; however, the bourbon tested well within TTB guidelines, and thus, will not be discussed.

As shown in Figure 3.10, vodka fell just within the acceptable 0.5 % error allowed by the TTB. However, the large standard deviation shows valid results would not always be achieved. After basification, the percent error of the vodka dropped and the standard deviation became smaller, suggesting that increasing the pH of the vodka via NaOH addition mitigated the apparent ethanol retention caused by the sugar.

When repeating this process with the moonshine, the percent error did not fall within acceptable ranges originally and the standard deviation was quite small. After basification, the percent error almost doubled and the standard deviation increased greatly.

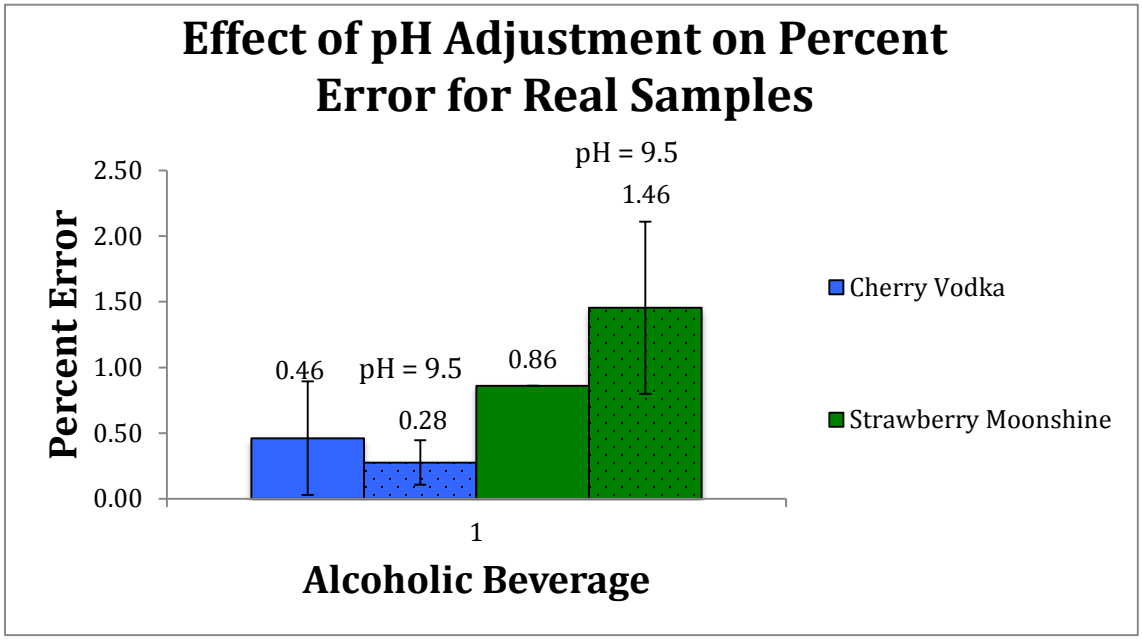


Figure 3.10. Percent error (for ABV determination) and standard deviation of vodka and moonshine samples before and after basification.

One plausible explanation for the decrease in percent error for the vodka, but the increase in error for the moonshine is that vodka is a much more simple solution. As the base was added into the moonshine, other compounds (such as alcohols, ketones, aldehydes, aromatics, etc.) may react; similar reactions are not possible in vodka. These reactions may ultimately increase apparent ethanol retention. Basification may not work for all solutions, but because of the improvement seen in simple solutions like neutral spirits, this method merits further study. Still, not all spirits should immediately be transferred to this basification method. In fact, the vodka originally tested within acceptable TTB regulations; and therefore, did not need basification. If a sample regularly tests within acceptable limits during distillation, no base should be added. If a sample has never been tested before, it should be run accordingly to regular distillation protocols before the basification method is attempted.

3.6 Overall Conclusions

It is now known that the addition of sugar to an ethanol/water mixture causes a percent error beyond what is accepted by the TTB. The original hypothesis for the increased percent error was drastic increase in the boiling point due to increased hydrogen bonding with increasing sugar concentration. However, boiling point determination and NMR studies did not indicate increased hydrogen bonding strength. The addition of NaCl before distillation also did not reduce the percent error enough to be useful for analysis at BDAS. All of the available data indicates that intermolecular

hydrogen bonding is likely not the sole cause of the observed ethanol retention during distillation.

After these results, pH became the focus of the research. With increasing sugar concentration, the pH dropped drastically. At high concentrations, the sugar can act as an acid. After pH adjustments by the addition of 0.1 M NaOH to the 15 % w/w standard solution, the percent error decreased. However, this decrease in percent error was potentially useful for spirit samples, but not replicable on all real-world samples. This decrease could be a result of breaking the glycoside formation or from breaking the intermolecular interactions (a direct result of breaking the pH dependent intramolecular interactions within the sugar molecule). However, more research needs to be done on these theories, as well as the many different variables that could be affecting the distillation. The future direction of this research will be discussed in Chapter 4.

CHAPTER 4

CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Broad Conclusion

The addition of 0.1 M NaOH to the 15 % sugar solution resulted in a decrease of percent error. The exact reasons for this decrease are unknown, but breaking intermolecular interactions and breaking the glycoside formation are two plausible explanations. While one spirit sample showed a decrease in percent error for ABV determination, this decrease was not replicable on all real-world samples. More research needs to be completed on these ethanol/water/sugar solutions in order to better understand the intermolecular interactions that will result in a robust alcohol by volume (ABV) determination method.

4.2 Future Directions

4.2.1 Density

Since the density determinations were not done in replicate, repeating these experiments is necessary before confidently stating that all of the methods cannot be used interchangeably and that the DMA is the best density determination method.

4.2.2 Solution Composition

As determined by the distillation of hand-made solutions with 40 % alcohol by weight (ABWt) and varying sugar concentrations from 0 % to 35 % w/w, the addition of sugar to solutions creates a percent error well outside of the TTB standards. However,

other variables could be contributing to the problem of these beverages. In the future, other sugars and sugar combinations should be tested, such as pure glucose, pure fructose, and various glucose/fructose mixtures. In addition to sugar, various flavoring additions should be tested as an independent cause to percent error, and afterwards, in addition to sugar.

4.2.3 Distillation Apparatus

No variables with the distillation apparatus were investigated. It is possible that the type of condenser would have an affect on these distillations, as well as the angle at which the condenser is placed or the addition of a fractioning column.

4.2.4 Theory Validation

A plausible mechanism for the decreased ABV concentration in these flavored beverages is glycoside formation. However, until further research has been completed, the mechanism cannot be stated as the correct, only listed as a possible explanation. The same can be stated about the possible ion-dipole and ionic interactions. To confirm either mechanism, mass spectrometry studies should be completed on the direct, distillate, and residual samples at all sugar concentrations.

4.2.5 Real Samples

Basification of the cherry vodka resulted in a decreased percent error, but an increased percent error for the strawberry moonshine. This indicates that more complex solutions may have additional side reactions during basification compared to simpler solutions, which results in an increased percent error. This means that addition of a base will not work on all real-world samples and that samples should first be

tested by distillation before the base is added. However, this process needs to be further verified on a wide variety of beverages that have been shown not to fall within the acceptable TTB range before statements are made about its effectiveness.

4.3 Closing Remarks

This body of work presents the opportunity for future research by other students, especially as the flavored alcoholic beverage industry continues to grow. I feel blessed to have been able to participate in so many different forms of research during my time at EKU and the scientist that these experiences have enabled me to become.

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Appendix A

Tables generated from determined densities. The densities were used to determine ABV. Each table represents the density determined from each of the three methods and the corresponding ABV.

40% ABWt/0% w/w Sugar Solution		
Density Method Determination	Calculated Density (g/mL)	Corresponding ABV from OIML Tables
DMA 5000	0.93483	47.04
eDrometer	0.92833	50.93
Pycnometer	0.93198	49.10
40% ABWt/5% w/w Sugar Solution		
Density Method Determination	Calculated Density (g/mL)	Corresponding ABV from OIML Tables
DMA 5000	0.94876	39.53
eDrometer	0.9430	43.03
Pycnometer	0.9474	40.40
40% ABWt/10% w/w Sugar Solution		
Density Method Determination	Calculated Density (g/mL)	Corresponding ABV from OIML Tables
DMA 5000	0.96119	30.81
eDrometer	0.9564	34.42
Pycnometer	0.9599	31.82

40% ABWt/15% w/w Sugar Solution		
Density Method Determination	Calculated Density (g/mL)	Corresponding ABV from OIML Tables
DMA 5000	0.97311	20.52
eDrometer	0.9659	26.93
Pycnometer	0.9702	23.18
40% ABWt/20% w/w Sugar Solution		
Density Method Determination	Calculated Density (g/mL)	Corresponding ABV from OIML Tables
DMA 5000	0.99854	0.46
eDrometer	0.9929	3.65
Pycnometer	0.9957	1.68
40% ABWt/25% w/w Sugar Solution		
Density Method Determination	Calculated Density (g/mL)	Corresponding ABV from OIML Tables
DMA 5000	0.98707	8.05
eDrometer	0.9804	13.72
Pycnometer	0.9838	9.16
40% ABWt/25% w/w Sugar Solution		
Density Method Determination	Calculated Density (g/mL)	Corresponding ABV from OIML Tables
DMA 5000	0.99854	0.46
eDrometer	0.9929	3.65
Pycnometer	0.9957	1.68

40% ABWt/30% w/w Sugar Solution		
Density Method Determination	Calculated Density (g/mL)	Corresponding ABV from OIML Tables
DMA 5000	1.01315	N/A
eDrometer	1.0081	N/A
Pycnometer	1.0108	N/A

Figure A.1. ABV determined values for different sugar concentration (%w/w) solutions from different calculated density determination methods.

Appendix B

NMR shifting of hand-made solutions for corresponding peaks. Trials were done in triplicate. The average and standard deviation for each shift is shown along with the linear fit.

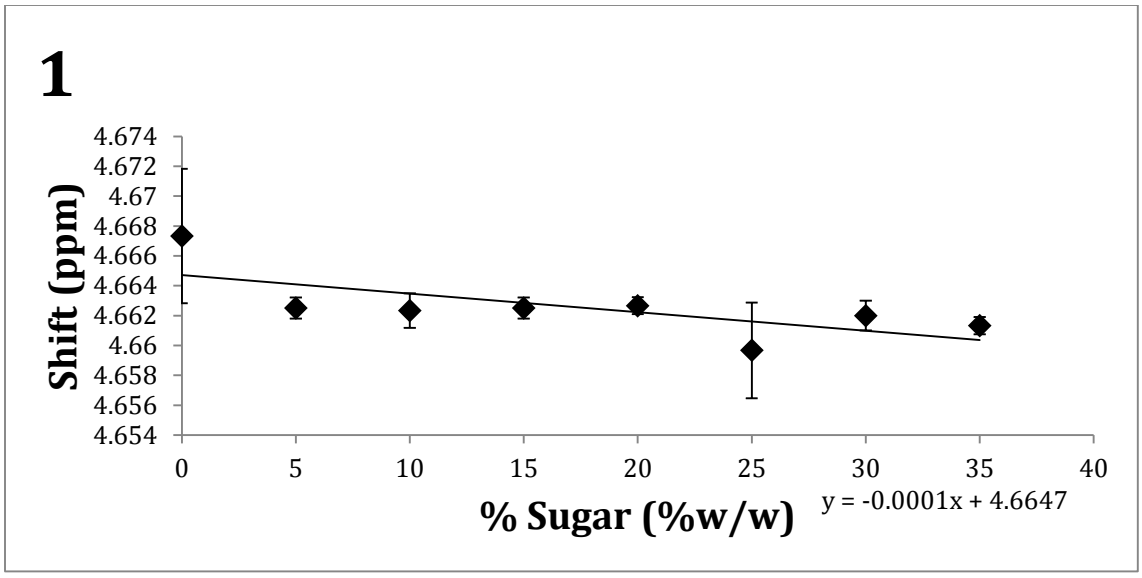

Figure B.1. NMR shift for water at various sugar concentrations.

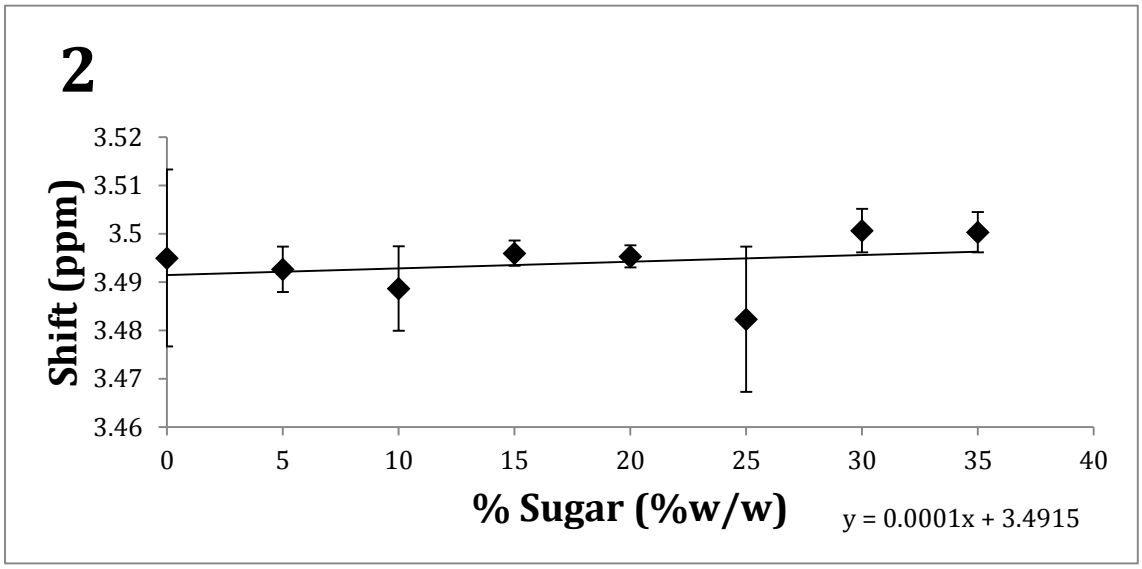

Figure B.2. NMR shift for CH_2 group in ethanol at various sugar concentrations.

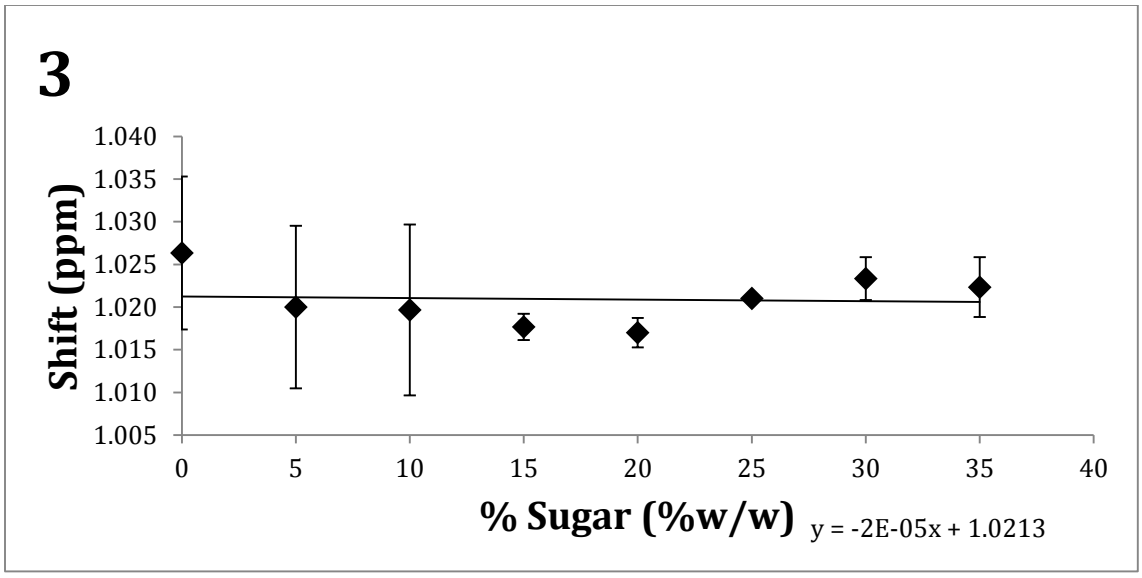

Figure B.3. NMR shift for CH_3 group in ethanol at various sugar concentrations.

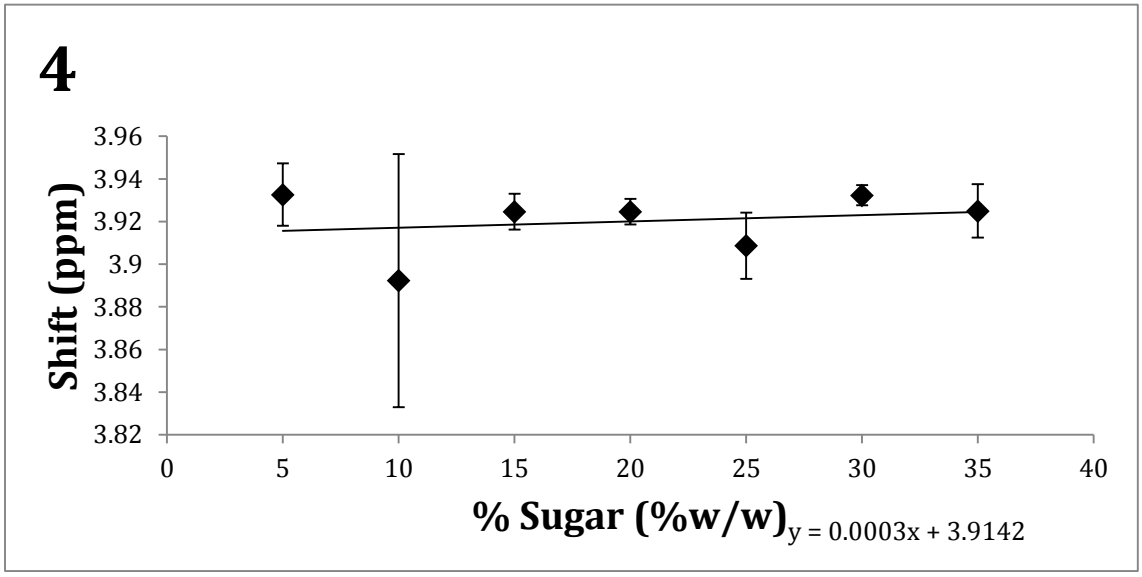

Figure B.4. NMR shift for one hydroxyl group in fructose at various sugar concentrations.

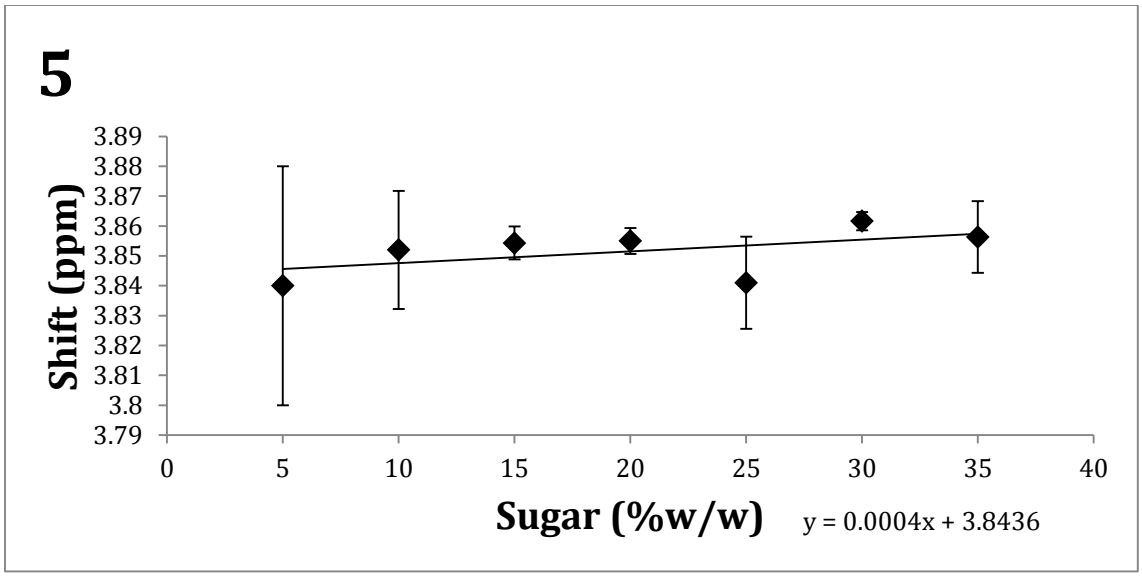

Figure B.5. NMR shift for one hydroxyl group in fructose at various sugar concentrations.

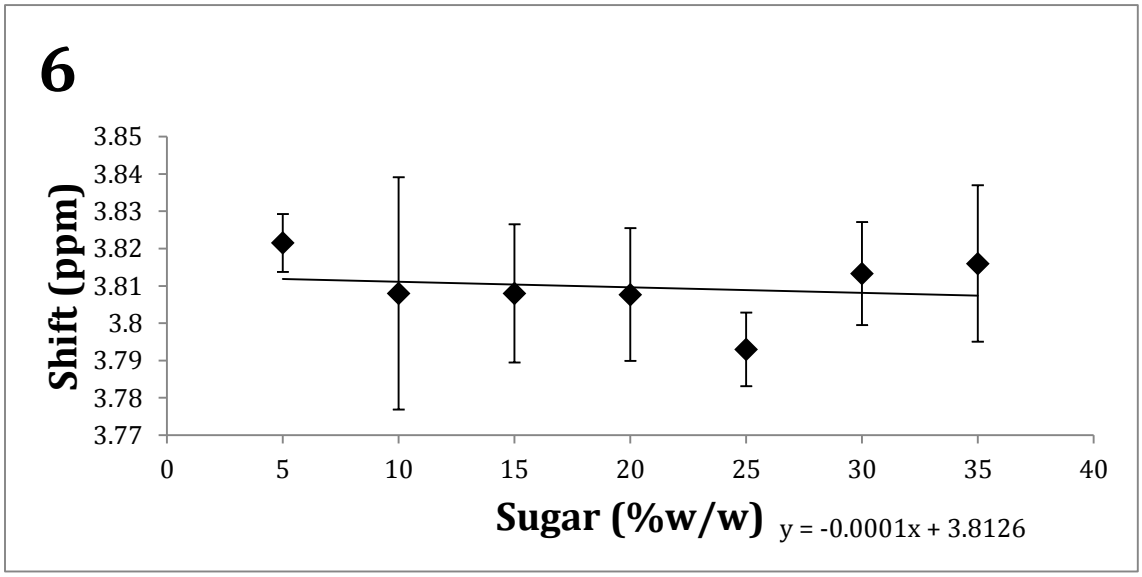

Figure B.6. NMR shift for one hydroxyl group in fructose at various sugar concentrations.

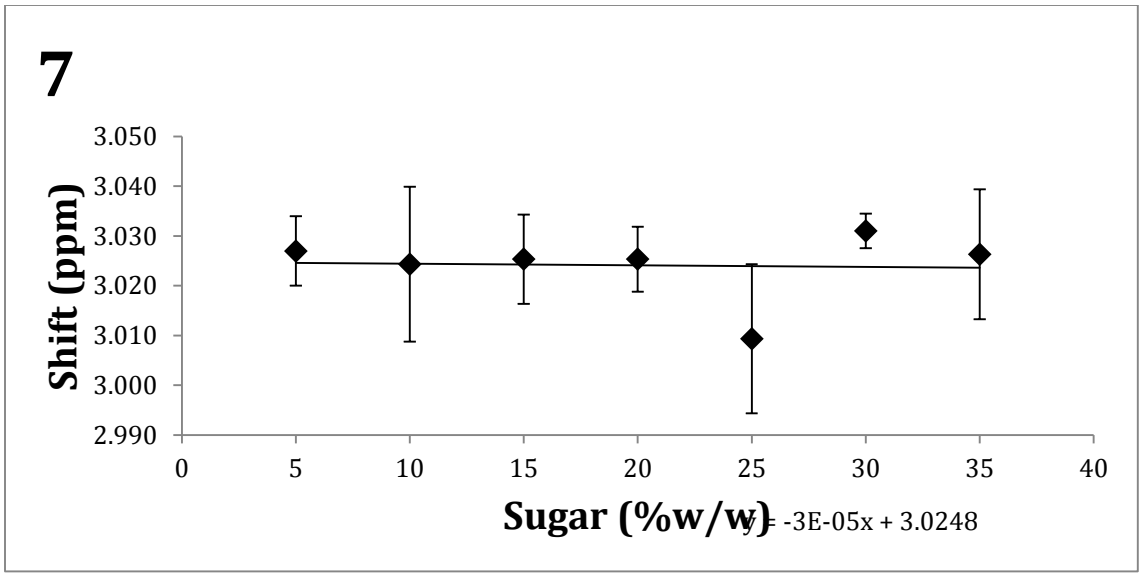

Figure B.7. NMR shift for one hydroxyl group in fructose at various sugar concentrations.