Effects of Glucose Oxidase on the Properties of Synthetic Honey

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Abstract

Honey is a very popular product all around the world. It is a sweet and sugary solution that improves the taste of tea and most desserts. It also has a variety of health benefits such as anti-inflammatory and antibacterial properties that improve the lives of people all over the world. Honey is mainly produced by a variety of species of bees, many of which have been domesticated in some form. This domestication has reduced the population of bees in the wild due to inferior genetic fitness. Thus, the purpose of our project and this report was to determine a new and simple way to synthesize honey. This was done by making synthetic nectar at a temperature of 60^{o}C and adding the enzymes invertase, glucose oxidase, and catalase. The mixture was then left to boil at 220^{o}C until a final volume below 70 mL was left. From there, the mixture was analyzed for its water content, pH, sugar content, and viscosity. First, as the amount of glucose oxidase increased, the water content in the honey decreased from 21% at its highest to 12.5% at its lowest. Conversely, as more glucose oxidase was added, the amount of glucose, the viscosity, and the pH of the honey all increased. These results demonstrate that honey can be made synthetically from sugars, and that glucose oxidase plays a distinct role in the changes in the properties of honey. Finally, it is recommended that future projects employ a taste test of the samples with store-bought honey to determine if taste changes with increased glucose oxidase concentration.

Acknowledgments

The project group would like to acknowledge Dr. Susan Roberts for being a great advisor and mentor for this project and the current Ph.D. student Antonio Sassano, who helped us a great deal throughout the length of the project. We would also like to thank Timothy Woodard for helping us get the results related to liquid chromatography. Finally, we would like to thank Tiffany Royal for her help in getting our sugars and enzymes for the project, as the logistics seemed to be against us. We would also like to thank all other members of the Roberts lab who were so welcoming and made the space available for us.

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1. Introduction and Background

1.1 Introduction

As we move to a more sustainable future, it is important to think about the parts of our daily lives that can be made better. While many scientists and researchers have developed better ways to reuse and recycle materials, many others focus on the food industry and the many ways to improve human nutrition while maintaining a green future. With the climate changing and many species around the world feeling its effects, one species has felt its side effects and also has the potential to change the current lifestyle of most humans around the world: the honeybee. This species lost their habitats and, with their overall population dwindling over the years, it is especially concerning to everyone since these bees are responsible for a large quantity of pollination of many fruits worldwide, which is directly linked to the plant's survival. And while, as chemical engineers, we cannot replace the honeybee, we can work to substitute its by-products in a clean, safe way that does not harm the environment and helps reduce the potentially abusive farms many honeybee nests find themselves in. This project aims to recreate the honey from honeybees, straight from the nectar of the flowers or synthetic nectar, using an enzymatic pathway instead of utilizing the labor of bees.

1.2 Background

Before the state of the industry can be understood, the process upon which honey is made needs to be understood. As described by the Honey National Board, “Honey starts as flower nectar collected by bees, which gets broken down into simple sugars stored inside the honeycomb. The design of the honeycomb and constant fanning of the bees' wings causes evaporation, creating sweet liquid honey.”[1] Honey is the product of multiple bees’ constant journey into the wilderness, where they visit different flowers and gather nectar, before heading back to the hive, where honey production can be started. The bee uses some of the nectar to produce the wax where the honey will sit, but the rest of it is deposited into the hexagonal wax molds, where the mix of nectar, bee gut bacteria, constant fanning by the bees’ wings, and the bees’ body heat produce the honey. Under these conditions, the honeybees produce the honey, which humans then harvest, and either consume locally or sell where it is packaged and transported to its final destination. This honey-making process takes a lot of time and effort on the bees’ part, and there are a lot of factors that could go wrong. According to the Bee Informed Partnership, a non-profit organization that tracks the survival rate of bee colonies across the US and in different environments, there has been an increasing trend over the past couple of years in the amounts of beehives that have been lost.[2] While this percentage varies by season, the general trendline is ever-increasing, which makes the venture into beekeeping, a risky economic move. If the industrial economic incentive of beekeeping were gone, a lot of the honeybees’ hives would

go away. While it could prove to be good for the local communities that rely on this income, it would also mean that a lot of other industries that rely on the bees for pollination of their trees and flowers would be jeopardized. According to Clemson University, “The greatest economic impact of honeybees is through pollination of agricultural crops. Production of about one third of the human diet requires insect pollination, and honeybees perform the majority of pollination for these cultivated crops. Globally, three out of four species of cultivated crops are animal pollinated, and honeybees are able to pollinate most of these crops. In the United States, honeybees contribute an estimated $20 billion to the value of U.S. crop production annually.” From this quote, it can be understood that without the bees pollinating the crops, not only the US but many other countries would have their food industry compromised. Therefore, it is of utmost importance that the honeybee population is not only kept alive, but healthy, as a lot of our needs and commodities currently rely on it. There have been some strides to that end, however, more significant steps need to be taken.

Figure 1: A small part of industrial beekeeping is pictured in the image. Each box in the picture hosts a different hive that competes for the region's resources with the others.[3]

The process of synthetic honey has been considered for a very long time. While there have been many local recipes around the world that were successful in creating sugar syrup, the first official one with some scientific relevance came just after the turn of the 20th century. This recorded process of the artificial synthesis of honey dates back to the early nineteen hundreds and credits Professor Herzfeld of Germany with the at-the-time revolutionary process[4]. He created this synthetic honey through his procedure, utilizing common substances that were available around him at the time and noting that by adding tartaric acid to an inverted sugar-water mixture and heating it to 110ºC for 45 minutes, a honey-like substance could be created. Although the professor claimed to have made honey, what he had created instead was just a more

complex sugar syrup solution that was made up of simple sugars with no regard to the actual proportions related to the honey produced by the honeybee. This process did, however, have some good points concerning the synthetic synthesis of honey, as Professor Herzfeld commented on the fact that the flavor of the honey changes based on which plants the honeybee visited, which explains why the German professor decided to use inverted sugar in his recipe. This 1907 line of action leaves a lot to be desired when compared to contemporary modern science practices, but it does suggest that society has been considering artificial honey for a long time and the results can be used as a starting point for future research.

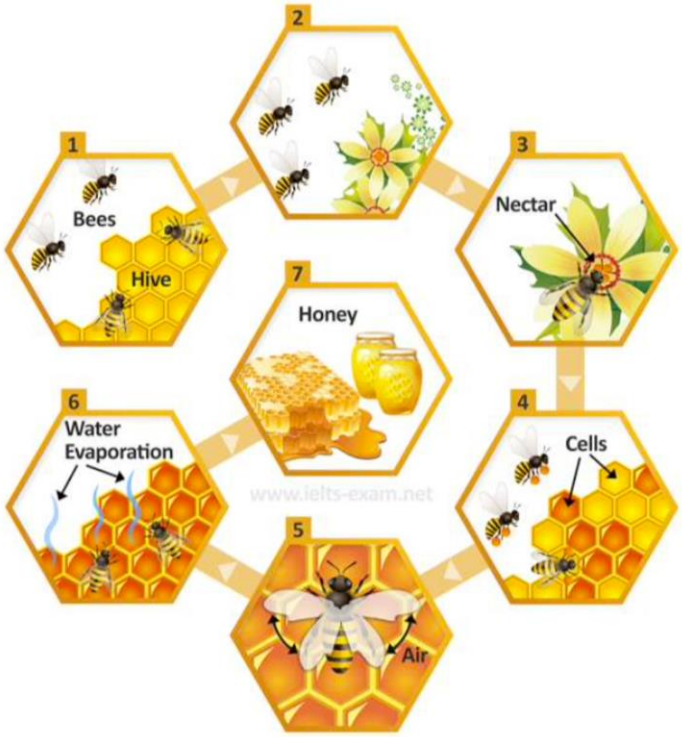

Figure 2: How bees make honey in nature.[5]

The second important process to come around is presented in a 2012 article by Sonali Bhawsar, that details an update to the 1907 recipe for honey. The main update from the prior recipe is the change from tartaric acid to lemon juice or citric acid[6]. This change theoretically improves the flavor, as the subsequent substance takes the flavor profiles of the citric fruit or acid that has been added to it. This effort denotes a key improvement from a recipe that was more than 100 years old at the point of publication: the flavor profile was important to the consumer and acids can break down the sucrose into its main parts of glucose and fructose. While this application is interesting, and of importance to evolution of the field, it leaves a bit to be desired on how to replicate the substance, as no details besides the ingredient list are given. This lack of detail can mean that the recipe is unchanged, save for the acid as mentioned earlier, or that the recipe has been altered in ways that are not discernable. Either way, the acid process was not the focus of this current research, as the current goal of the field is not to completely

replace honey production, but rather complement the already established honey farms and dwindling bee population using synthetic approaches.

Moving from the previous recipes and getting into more technical procedures, the next topic of importance is the 2019 Israeli project where artificial honey was synthesized in a laboratory utilizing enzymes produced by a bacterium, *Bacillus subtilis*[7]. The Israeli team managed to focus their research on synthesizing artificial honey by discovering an enzymatic pathway that converted a nectar solution to a sugar solution that closely resembles the honey found in the wild. The enzymatic pathway that was found utilizes invertase, for breaking down sucrose into glucose and fructose, glucose oxidase, which breaks down glucose into gluconic acid, and catalase, which breaks down the hydrogen peroxide product into water and oxygen. This research highlights one of the last forays into this field of major importance, since at the time of writing this report, the research conducted by the students at the Israel Institute of Technology proves the possibility of synthesizing artificial honey through synthetic means by only using enzymes. While their focus was geared towards finding the enzymatic pathway to generate honey with a basis of a sugar-water mixture to mimic nectar, it is still an important step in the right direction of what can be accomplished in the field, especially as this research could prove to be pivotal in small scale applications and could help honey farmers by having another avenue of honey production. The research presented in this paper focuses on the same enzymes as the ones that were presented by the Israeli team.

The last breakthrough in the field regarding artificial honey comes from lab-grown honey, which came to light in the last couple of years. This method of approaching the problem is done by going one step further from the simple enzymatic process: it is done by using synthetic bee stomachs, which can replicate the honey found in the wild without harming any animals in the process.[8] This idea is very exciting for the future of honey production, however, this type of breakthrough focuses a lot more on the supplying of safe-to-eat honey and replacing natural honey production with artificial synthesis. While the purpose of this paper is not to criticize the legitimacy and long-term effects of solely relying on artificial honey, it is concerning that such research is aiming to replace the bees rather than simply aiding the current ones to recover from their compromised situation in the different ecosystems they find themselves in. But regardless of the environmental consequences that different research might have on the bee population and the honey industry, the development of synthetic bee stomachs that can reproduce the honey process is an important discovery and marks the final update on the field currently.

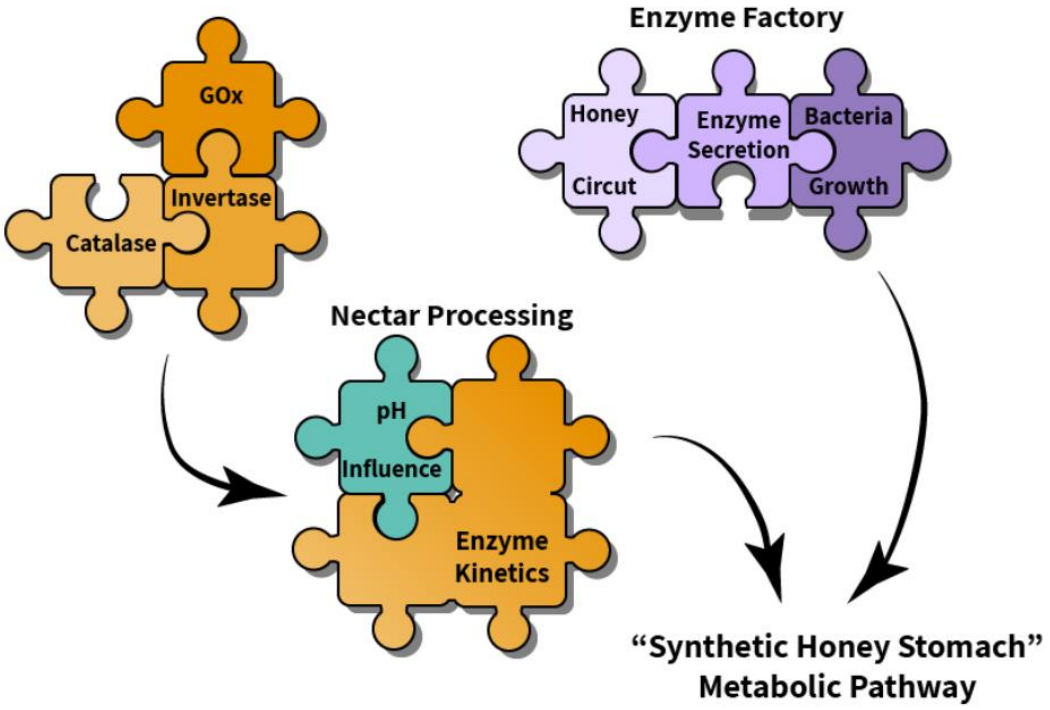

Figure 3: Employment of an enzymatic pathway to create honey using bacterial cell cultures as the main source of enzymes.[7]

1.3 Original Idea

This project was originally tied into a robotics project, where the development of a robotic bee was going to be designed. The purpose of this project and research was to bridge nature and robotics. By developing an enzymatic process to synthesize honey artificially, and by simplifying and streamlining this process, whenever artificial bees and beehives are developed, this research could help solidify the presence of these robotic companions on the fields that could help mitigate the effects of an ever so dwindling bee population. With these core ideas in mind, the current state of artificial honey can be understood.

2. Objectives and Hypothesis

As synthetic honey has been developed in the past via synthetic honey stomachs and an enzymatic pathway with aid from bacteria, the project focused on three main objectives: creating a lab procedure with already prepared enzymes; analyzing the effects of the glucose oxidase enzyme on the final synthetic honey synthesis and comparing it to a store-bought honey option; and calculating a small-scale packed bed reactor that could be fitted inside of a drone to be used for nectar collection and honey processing on the field.

In a list form, this project will have the following objectives:

1. Synthesize honey in a lab setting utilizing enzymes;
2. Analyze the effects of glucose oxidase on the system;
3. Compare synthetic honey to bee-made honey;

Taste is dictated by sugar and gluconic acid concentration. Sweeter artificial honey can be made by altering the amount of Glucose Oxidase (GOx) in its artificial production.

3. Experimental Plan

3.1 Nectar Formulation

Nectar concentration differs between flowers of different varieties. These differences can be due to a variety of reasons, but most differ due to varying pollination techniques by different animals. Thus, it was important to determine the correct flower to emulate the lab-synthesized nectar on, as fresh flowers with large quantities of nectar were unavailable to use. The nectar chosen to emulate was from the *Ribes magellamicum*, whose overall nectar concentration and individual sugar concentration were described by Chalcoff et. al.[9] The overall nectar concentration percentage was 16%, and within the nectar, the concentration percentages for sucrose, fructose, and glucose were 54.5%, 23.3%, and 22.2% respectively.[9]

To emulate this in 500 mL of synthetic nectar, 420 mL of tap water was mixed at 350 rpm at 20ºC with 18.6 g of pharmaceutical grade D-(-)-Fructose (ThermoScientific), 43.6 g of ACS grade Sucrose (Research Products International), and 17.8 g of D- (+)-Glucose (Sigma-Aldrich). The chemicals were mixed until the sugars dissolved into the solution and the liquid became clear.

3.2 Enzymatic Pathway

Once the nectar was made and the sugars dissolved, the enzymatic pathway required to make honey was emulated. As mentioned earlier, the enzymatic pathway that bees use naturally to convert sucrose in nectar to the sugars necessary to compose honey goes through two main enzyme steps: an enzyme to break down sucrose and an enzyme to turn glucose into gluconic acid and hydrogen peroxide. Bees generally secrete α-glucosidase to break down sucrose, but invertase was used since it works equally well to do the same function while cleaving the sucrose from the fructose end of the compound instead of the glucose end. Bees also use amylase to hydrolyze starches in plant nectar into glucose molecules, but since the nectar made in the lab is synthetic, this step was not needed. Glucose oxidase breaks down the glucose into gluconic acid and hydrogen peroxide, which in both bees and synthetic nectar acts as an antiseptic for honey. Finally, catalase is used to break down the hydrogen peroxide into water and oxygen to give oxygen fuel for the conversion of glucose into gluconic acid (Figure 4).

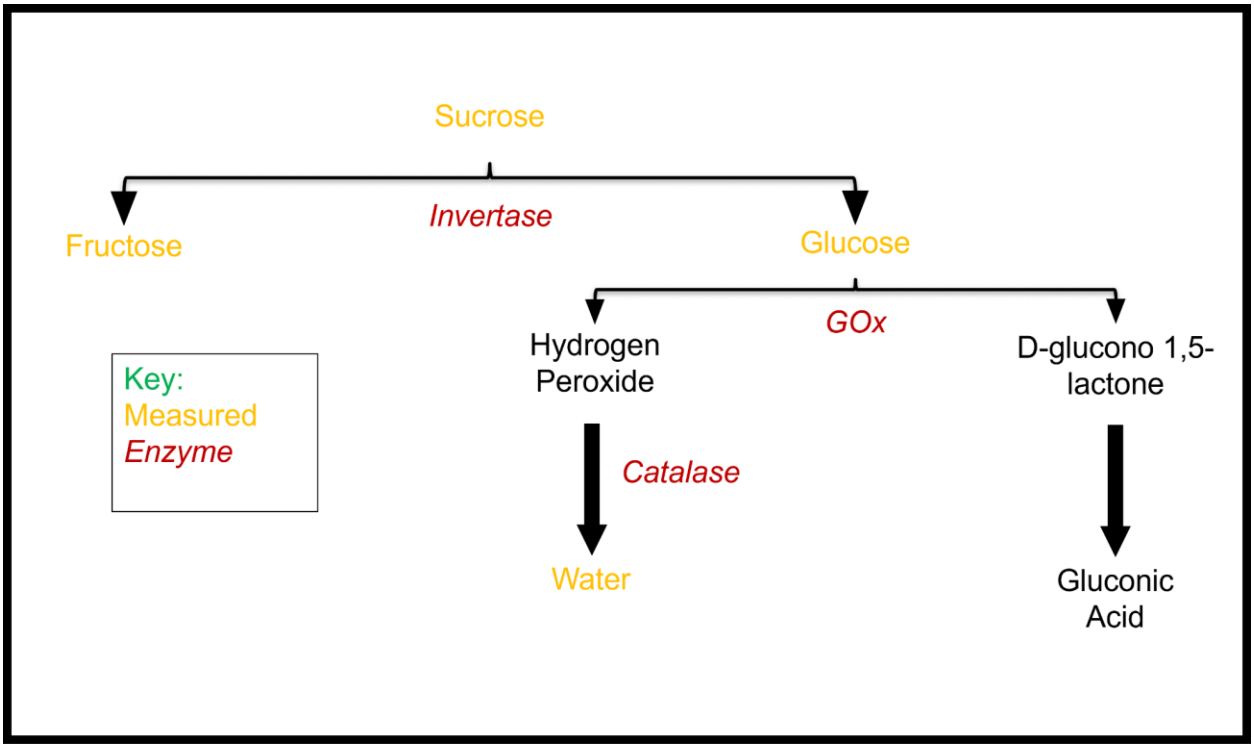

Figure 4: Enzymatic pathway used in the synthesis of artificial honey.

For the conversion of nectar into honey, the nectar solution was heated to 60°C and the pH was adjusted from ~9.3 to an optimal pH between 5.0 and 5.5 using acetic acid and 1M NaOH.[7] These conditions were determined through research as the optimal temperature and pH for the enzymes to catalyze their respective reactions. Once the conditions were met, 7.314 g of invertase was added along with 3.6 mg of catalase. Glucose oxidase was added in increasing intervals of 0.8 mg per trial, starting with 0.9 mg and increasing to 2.5 mg. These values were calculated using Michaelis-Menten kinetics (Appendix A). The reactions were allowed to run for 5 minutes, at which point the temperature was increased to 175°C to induce boiling. After 1 hour of boiling, allowing for the enzymes to settle to the bottom of the solution, the solution was vacuum filtered using a Buchner funnel. This step allowed for the solid enzymes to be removed from the solution, which removed some of the impurities and reduced the overall boiling temperature. After filtration, the solution continued to boil at 220°C until the water content was 15-20% of the entire solution. This percentage was determined physically using a refractometer.

3.3 Analytical Methods

3.3.1 Refractometry and Density

A refractometer was used to determine the water and sugar content of the honey. The samples were pipetted in droplets onto the refractometer prism to coat it in its entirety. The sample was then covered with the illuminator and exposed to light. Next, the sample was measured by looking through the lens and reading the measurements of water percentage and Brix (sugar content) associated with the level of fluid (Figure 5).

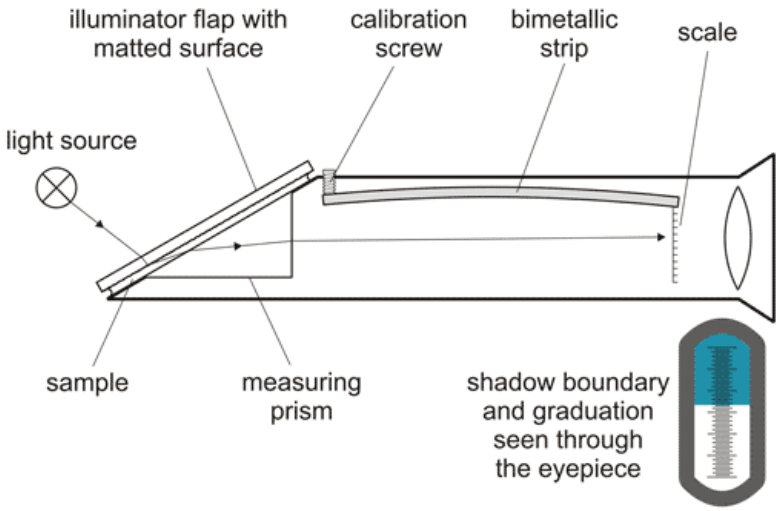

Figure 5: Image of a refractometer, the device used by beekeepers to determine the sugar and water content in honey.[10]

Density was calculated by measuring the mass and the volume of a set amount of synthetic honey. Once these values were recorded, the density was calculated using the following formula:

$$\rho = M/V$$

where M is mass and V denotes volume. A sample calculation can be found in Appendix A.

3.3.2 Viscometry and pH

The viscosity of the solution was analyzed using a viscometer. The synthesized honey was introduced into the size 3 viscometer[1] in a quantity of 15 mL. Then, the vent hole was covered, and a pipette was used to pull the honey to the other side of the viscometer to the tabbed bulb. Once the honey reached the elevation of the higher tab on the viscometer, the honey was released, and a timer was started. The timer was stopped once the honey slid to the second tab, and this time value was multiplied by a known constant to determine the viscosity in centistokes.

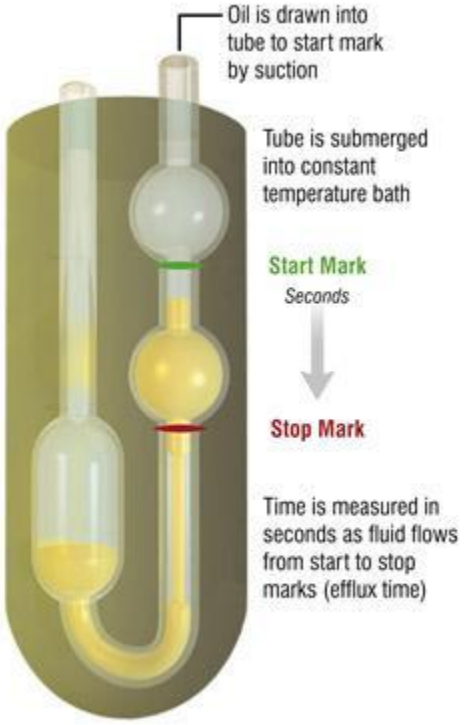

Figure 6: Image of the viscometer used to measure the viscosity of a fluid.[11]

The pH of the solution was also taken after refractometry was performed. The pH probe was calibrated at pHs of 4.00, 7.00, and 10.00. Once calibrated, the pH of the solution was taken and recorded.

[1] The size required for a viscometer is based on the range of viscosities that are tested. For example, the more viscous a fluid is, the larger the size needs to be so the fluid is not compressed to a point where the viscosity changes.

3.3.2 Liquid Chromatography

To determine the concentrations of each sugar in the final honey product, liquid chromatography was done. First, standard curves were created using control samples for each sugar at different dilutions. From there, the honey sample was diluted by a factor of 100 to reduce the viscosity of the samples and ran through the HPLC unit to obtain the data of individual sugar concentrations. After dilution, 1 mL of synthetic honey was sampled and transferred to an HPLC vial. The samples were analyzed using a Shimazdu Nexera 40 Series HPLC with a Rezex Roa Organic Acid column and refractive index detector (RID) to analyze sugar content. The mobile phase was 5 mM sulfuric acid, the mobile phase flowrate was 0.6 mL/min, and both the column and detector were kept at a temperature of 35^{o}C. Finally, the data was gathered in Excel and analyzed further.

4. Results and Discussion

4.1 Effects of Glucose Oxidase

Several parameters were measured and compared to store-bought honey. The parameters that were selected for testing were pH, water composition, density, sugar composition, and viscosity. To test these parameters, three batches were made, each with an increasing amount of glucose oxidase in them. These batches had the same proportions starting nectar proportions, as seen in Section 3.1, with the only difference between them being the varying amounts of glucose oxidase present in the system, as described in Section 3.2. To reiterate, the glucose oxidase in the batches changed from 0.9 mg in batch 1 to 1.7 mg in batch 2 to 2.5 mg in batch 3. With these steady increases of 0.8 mg between the batches, the following results were gathered as a characterization of the batches.

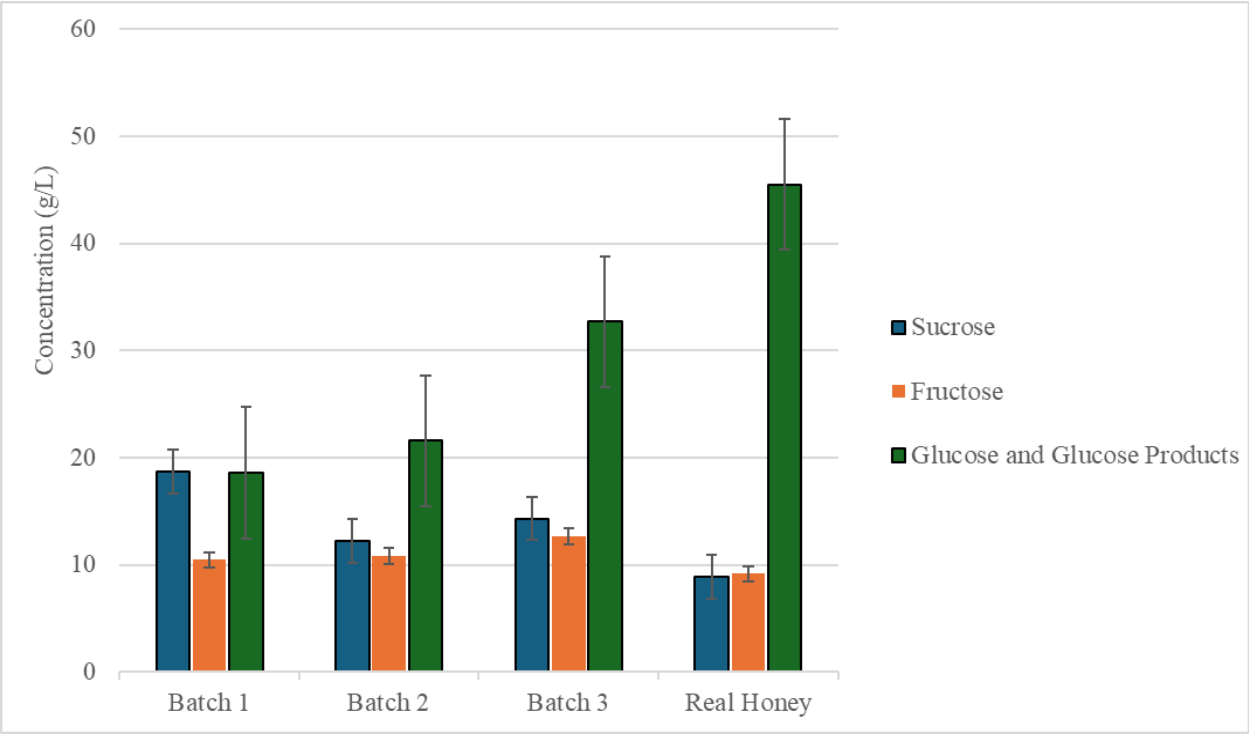

Figure 7: Comparison of the sugar concentrations of the synthetic batches and the sugar concentrations of natural honey. The error bars depict the standard error of the concentrations across each sugar. The error is slightly larger across the glucose and glucose products due to the increased variance in the data, which was brought about by the fact that only four samples were tested.

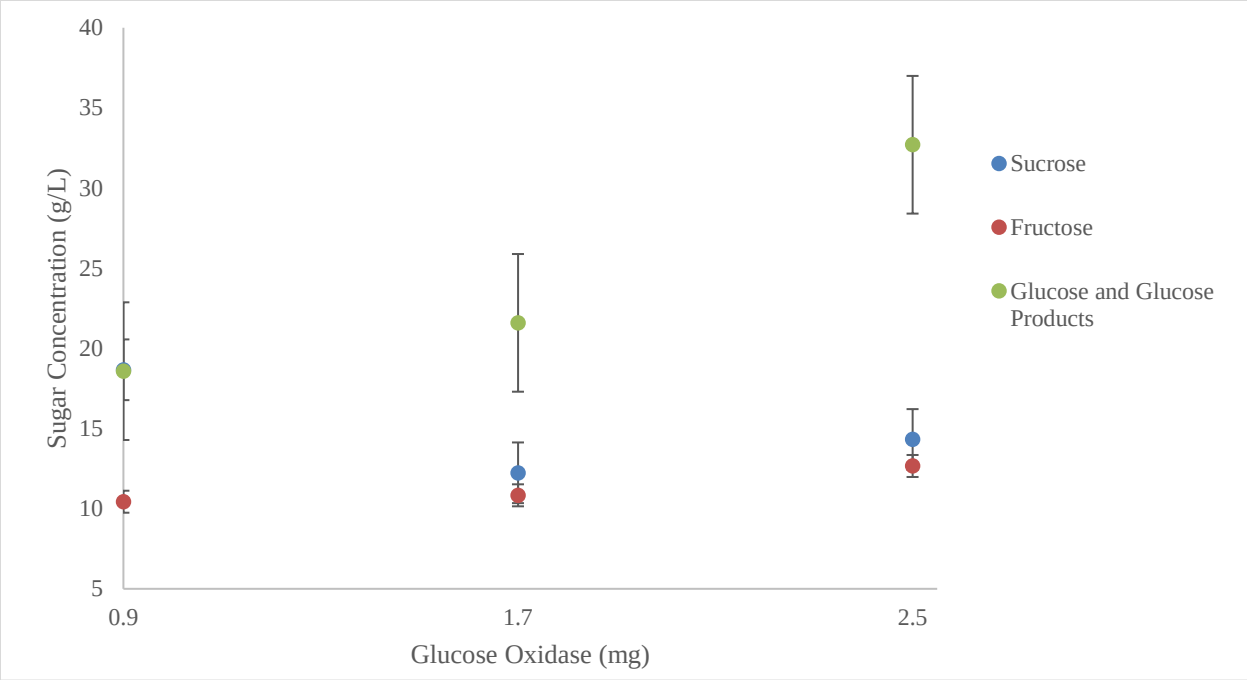

Figure 8: Concentrations of sucrose, fructose, and glucose products in each batch where the mass of glucose oxidase was varied. The error is slightly larger across the glucose and glucose products due to the increased variance in the data, which was brought about by the fact that only four samples were tested.

These data demonstrate that the addition of glucose oxidase into the system affects the concentration of different sugars. As more glucose oxidase is added to the mixture, the batches increased the number of glucose-related products. While it is easy to separate and measure sucrose, fructose, and glucose in a mixture using liquid chromatography, separating the glucose into the more specific D-glucono 1,5-lactone and gluconic acid molecules is more challenging. Therefore, to get results to demonstrate the relationship between the glucose oxidase and the sugars, we measured both glucose-related products as a measure of the total glucose.

Figure 9 describes the relationship between increasing glucose oxidase and the water composition in the three different batches.

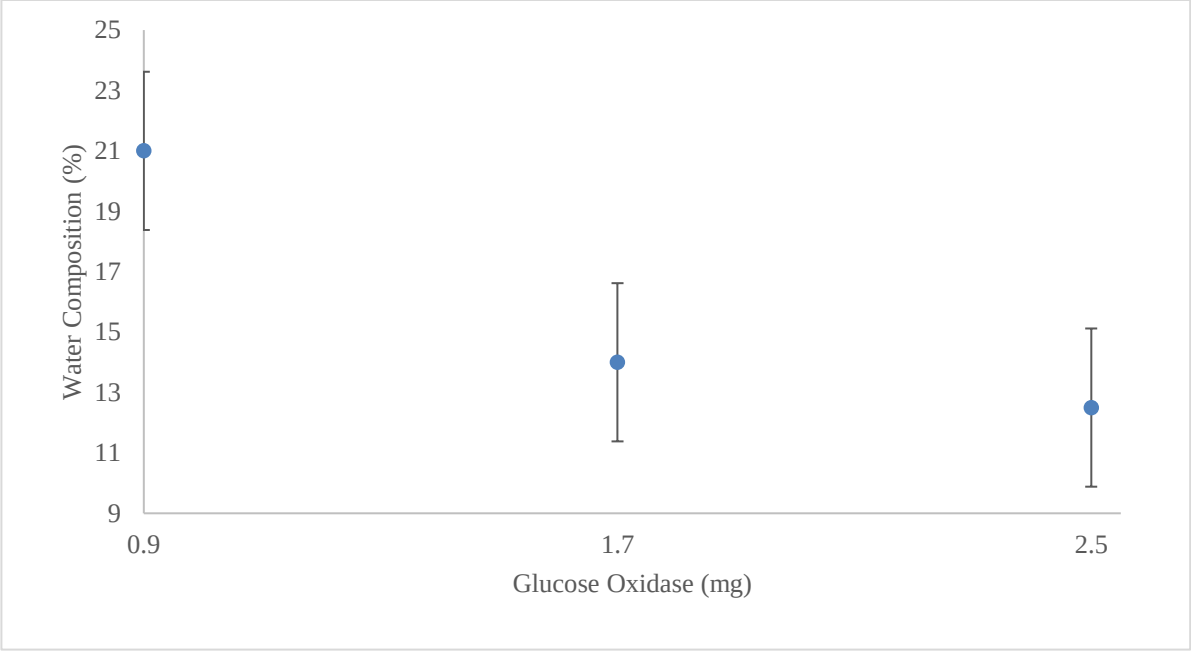

Figure 9: Comparison of the water composition of each batch to the mass of glucose oxidase in each batch. The error is large across in this graph due to the increased variance in the data, which was brought about by the fact that only four samples were tested.

As can be seen from the graph, the increase in glucose oxidase lowers the overall water percentage in the batch. This is a unique relationship since adding glucose oxidase and catalase to the system breaks down the glucose oxidase product of hydrogen peroxide into the water product of the catalase. However, this can be explained through the different final volumes accrued between the batches. While doing the procedure, the water was boiled off for 6 to 8 hours, and while keeping it at a constant temperature throughout, uneven heating up and cooling down times may have resulted in the evaporation of more water than expected. Additionally, there is uncertainty in the data as only one batch per measurement was done, resulting in a relatively high standard deviation. Even with these factors, the batches reported between 13%-21% water, which is around the median water percentage of real honey, which measures between 15%-25% water. These data show that there is a decreasing relationship between glucose oxidase and water measurements, but even with the strong association between the two, the water percentages of the batch are still around the expected range when compared to bee-made honey.

Figure 10 compares the amount of glucose oxidase in the batches to the sugar composition.

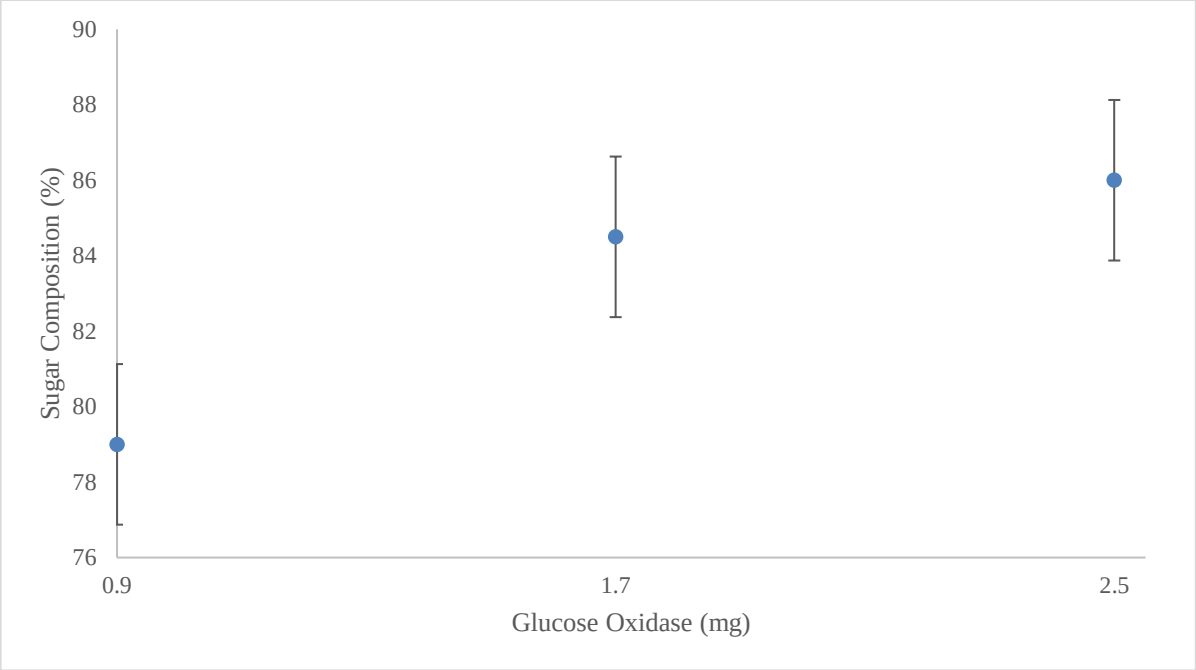

Figure 10: Comparison of the combined total percent of the three sugars for each batch as glucose oxidase amounts are changed. The error is large across in this graph due to the increased variance in the data, which was brought about by the fact that only four samples were tested.

This graph highlights that with an increase in glucose oxidase, the overall sugar composition increased. Gathered from the same refractometer as the water composition percentage graph, these results denote the same trendline of increasing glucose oxidase into the system resulting in both a decrease in water percent and increase in sugar percent. This is also not intuitive, since adding glucose oxidase breaks down the glucose into different components, however, there is a simple explanation for this. Similarly to the different sugar breakdowns, the refractometer assumes that the solution is mostly sugar and water. As the glucose oxidase increases in the following systems, the overall percentages stop adding to 100%, denoting that there are some elements present in the system that are neither water nor sugar. This discrepancy could be explained by a significant increase in the D-glucono 1,5-lactone and gluconic acid molecules, which could have added up to a significant enough percentage to be picked up by the refractometer. When compared to real-life honey, the sugar composition is a bit different. In the traditional way of making honey, there are a lot of inconsistencies with the refractometer data collection, as there are impurities in the honey that are introduced by the bees. While the impurities can be tracked with different analytical methods, we were not able to determine what kind of impurities were present in the store-bought honey that was used in a real-honey comparison. However, the expected percentage of sugar in real honey is predicted to be around 70%-83%, which the three batches follow somewhat closely.[12]

The relationship between the increasing glucose oxidase and the effect on density measurement is shown in Figure 11.

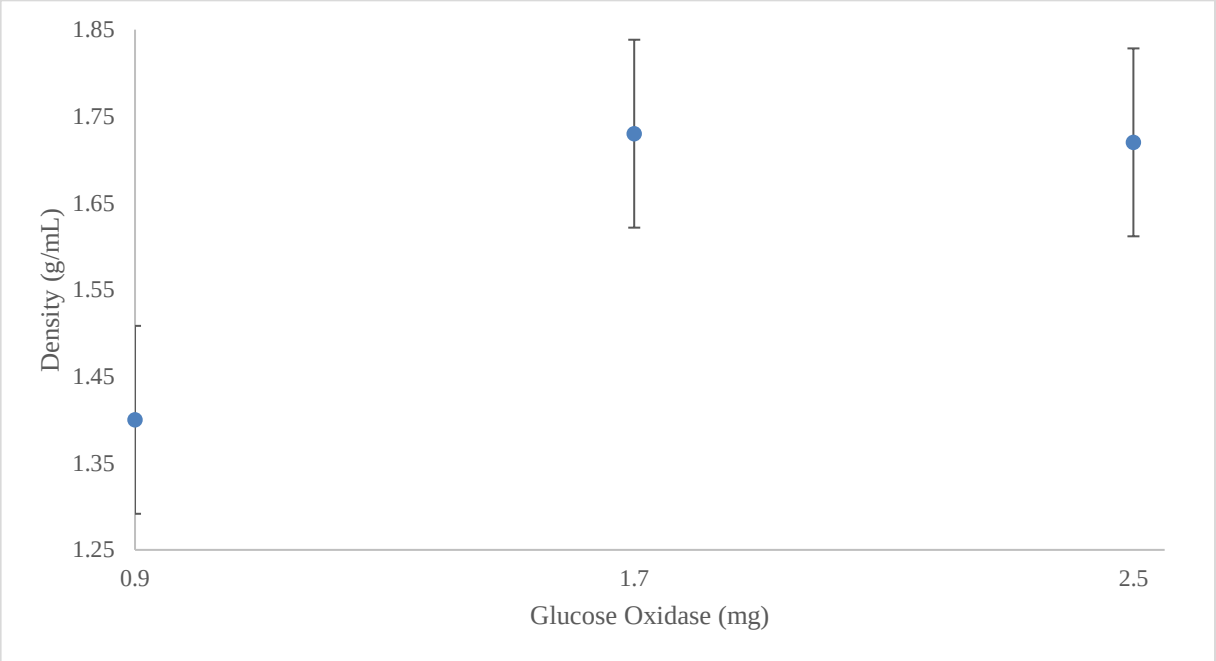

Figure 11: Effect of glucose oxidase on the density of each batch. The error is large across in this graph due to the increased variance in the data, which was brought about by the fact that only four samples were tested.

Figure 11 denotes the glucose oxidase levels and the increase in the density of the honey substance. As glucose oxidase content is increased in the honey-making process, the density also increases. It is interesting to note that there is a sharp increase between batches 1 and 2. While real honey that was tested had a density that was close to the first batch, the higher density of the second and third batches does not come as a surprise. With the products of glucose oxidase being heavier than sugars, as their concentration increased in the latter two batches, the overall density also increased. This result suggests that for synthetic honey to have a density close to bee-made honey, less glucose oxidase would be recommended.

Figure 12 shows the effect of glucose oxidase on pH.

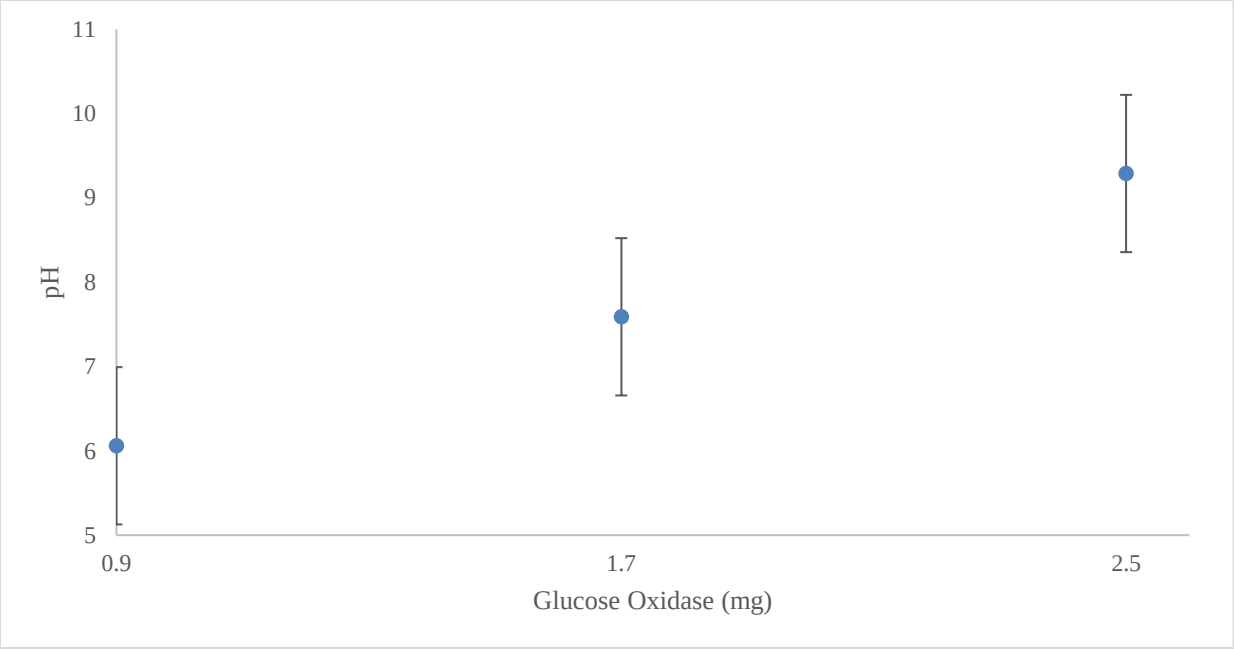

Figure 12: Effect of glucose oxidase on pH for each batch. The error is large across in this graph due to the increased variance in the data, which was brought about by the fact that only four samples were tested.

From Figure 12, it can be seen that pH increases with increasing glucose oxidase. This relationship is also unexpected, as higher amounts of glucose oxidase results in an increase in gluconic acid, which should lower the pH of the final solution. However, this might not be the case due to the decrease in water content that was described previously. With a decrease in water content, there are more sugars present, which drives the final batch to be more basic due to the sugars present being basic in nature. Additionally, water is needed to convert D-glucono 1,5-lactone into gluconic acid, which could decrease the acidity of the solution. Sucrose and fructose sugar contents are somewhat stable and support this hypothesis. There is more sugar present that increases the pH than gluconic acid which lowers the pH. With ideals amounts of water, there should be a higher conversion of gluconic acid, which would show an inverse proportion between glucose oxidase and pH, however, this was not the case through this experiment. Therefore, it is concluded, based on this data, that pH and glucose oxidase are directly related.

4.2 Comparison to Natural Honey

The results shown so far have explored the relationship between the properties of synthetic honey and glucose oxidase concentration, but the results gathered from this project can also be compared to natural honey. Honey is a substance that has a range of different parameters, since the bee honey-making process is very inconsistent, as bees visit numerous different flowers to make a bit of honey, and the honey that is bought from stores has incredible variations. Therefore, there is no golden standard of measurement. To minimize inconsistency and to be able to compare the synthetic honey to real honey the following data tables were created.

Table 1. Sugar and water compositions for each batch.

Samples	Water (%)	Sugar (%)
Batch 1	21.0	79.0
Batch 2	14.0	84.5
Batch 3	12.5	86.0
Control	16.0	82.5

Table 1 shows the sugar and water composition for each batch and the control. The control in this table and all others refers to the bee honey that was bought and tested for this project from Nature Nate's Honey Co. 100% Pure Organic Raw & Unfiltered Honey. According to research, honey has a range of about 17%, while having 70% to 83% sugar composition.[12] Data on the water composition of synthetic honey is comparable to data for bee honey. Batches 2 and 3 have less water than batch 1 and the control. An important detail to note is that certain percentages do not add up to 100%. This is believed to be the presence of other substances, like the D-glucono 1,5-lactone and gluconic acid that are present in the later batches and therefore do not show up in the sugar percentage, and the presence of vitamins and other impurities that are present in bee made honey. All in all, these data support the conclusion that synthetic honey is similar to bee-made honey.

Table 2. pH values for all batches.

Samples	pH
Batch 1	6.06
Batch 2	7.59
Batch 3	9.29
Control	9.29

As can be seen in Table 2, the pH of the batches increased with the addition of glucose oxidase into the system, reaching a peak of 9.29. Interestingly enough, the control also had the same high pH, which deviates from the expected range of pH found in honey, since it is predicted to fluctuate between 3.4 and 6.1 pH.[13] Only batch 1 fell within the predicted range. This could be because the bees that made the store-bought honey visited flowers that had more basic nectar, which led to the creation of honey with an unusually high pH. Future experiments could test a range of store-bought honeys to determine if this was the case.

Table 3: Viscosity results for each batch.

Samples	Kinematic (cm^2/s)	Dynamic (cP)
Batch 1	16.87	2367.37
Batch 2	n/a[1]	n/a[1]
Batch 3	n/a[1]	n/a[1]
Control	47.39	6859.04

[1] Batches 2 and 3 were too viscous for the size 3 viscometer.

Viscosity was difficult to measure for this project based on the available equipment. While collecting data for all the batches, the viscometers that we had access to were too small to measure the viscosity of batches 2 and 3. Qualitatively, they are more viscous than batch 1 and the bee honey that was tested, moving very slowly through the flask and the viscometer. Flow was slow enough to warrant the stop in the data collection phase of these two batches. There is a definite relationship between glucose oxidase and viscosity, but it could not be quantified in this project. What can be concluded from these data is that the viscosity for batch 1 was smaller than the control. Only batch 1 was in the range for the dynamic viscosity of honey, which ranges between 2000-6000 cP.[14] The viscosity of the bee honey was found to be a bit higher than expected, with a value of 6859.04 cP, which could indicate that this batch of honey was not only more basic than expected but also more viscous than expected, which highlights the need for testing a number of different honeys.

Table 4: Density data for each batch.

Samples	Density (g/mL)
Batch 1	1.4
Batch 2	1.73
Batch 3	1.72
Control	1.45

Table 4 describes density, which highlights the effect that glucose oxidase has on the system. The addition of more glucose oxidase increased the batch density significantly. Quantitatively, the values found in the table for batches 2 and 3 are higher than the measured density of honey. Considering that batches 2 and 3 have increased amounts of glucose oxidase when compared to batch 1, this behavior could be due to the increased amount of D-glucono 1,5-lactone and gluconic acid present in the system, which affects the overall density of the

substance. However, to match the density of honey, the amount of glucose oxidase presents in batch 1 would be preferable, since it is closer to the measured results than the bee-made honey.

Table 5: Concentrations of sucrose, fructose, and glucose products in each batch.

Samples	Sucrose (g/L)	Fructose (g/L)	Glucose (g/L)
Batch 1	18.7	10.4	18.6
Batch 2	12.2	10.8	21.6
Batch 3	14.3	12.7	32.7
Control	8.88	9.14	45.5

Table 5 shows the sugar concentrations found in the batches. As can be seen from the different values, sucrose varied a little but still maintained a steady amount throughout all the batches, while fructose changed minimally. Glucose, however, increased significantly through the batches, which indicates that glucose oxidase amount affects the glucose present in the system. What needs to be noted, however, is that glucose oxidase breaks down glucose into the D-glucono 1,5-lactone and gluconic acid molecules, so the increase in glucose does not follow an intuitive way of thinking. Possibly, the liquid chromatography that was used to separate the concentrations of sucrose, fructose, and glucose was not discerning enough to also differentiate between glucose and its products. Therefore, the increase in glucose could also mean an increase in D-glucono 1,5-lactone and gluconic acid in the system, which then explains the increase observed. The bee-made honey, however, had lower amounts of sucrose and fructose, but higher amounts of glucose than any of the three batches. This could be due to impurities in the honey, similar to the D-glucono 1,5-lactone and gluconic acid present in the batches, or because this specific honey is more glucose-heavy. Honey is described as being a sugar solution with fructose and glucose measuring up between 70%-83% of the total sugar composition,[12] this could be an example of a more glucose-heavy honey batch. Overall, this table shows that there is a relationship between sugar concentrations and glucose oxidase, but additional experimentation is needed to clarify the relationship.

Collectively, these data show that synthetic honey is comparable to bee honey. The bee honey that was tested was out of range of what was expected based on literature, highlighting the need to test different bee honey sources. Data also show that the amount of glucose oxidase in the system has a profound effect on the synthetic honey characteristics, suggesting future research should focus on enzyme concentration.

5. Conclusions and Future Work

5.1 Conclusions

Through this work, all of the objectives were achieved. First, it was determined that honey could be produced in a laboratory setting. The process presented in this paper produced results that physically resemble naturally occurring honey. The synthetic honey was of similar clarity and color to that of natural honey, as shown in Figure 13.

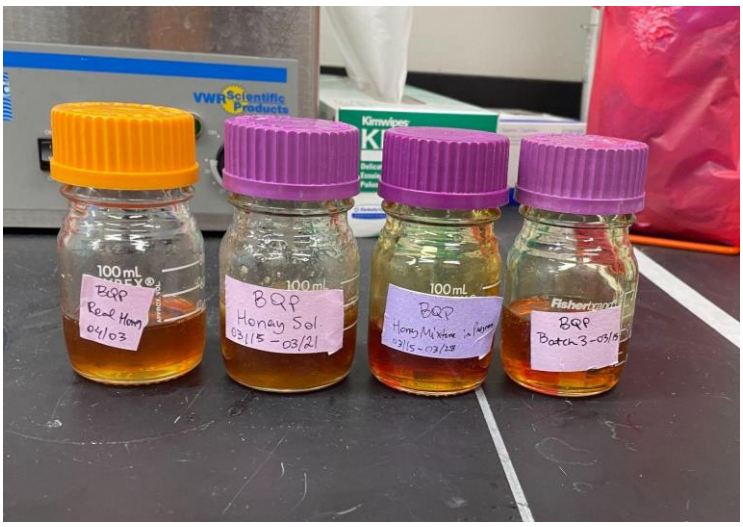

Figure 13: Honey samples produced in the lab. From left to right the samples are as follows: natural honey (real honey), Batch 1, Batch 2, and Batch 3.

As for the second objective, it was determined that there were certain trends within the data taken between the amount of glucose oxidase in each batch and the different properties of honey. For the pH, sugar content, both overall and of just glucose and glucose products, and density, the overall trend was shown to be an increase in the values as the amount of glucose oxidase increased. These trends suggest that as more glucose oxidase was added, glucose was converted to more D-glucono 1,5-lactone, increasing both density and pH. Unfortunately, viscosity data cannot corroborate this trend, as the viscometer used in testing was not suitable for highly viscous samples. Future experiments should identify a viscometer that can measure highly viscous solutions. Further, increasing glucose oxidase concentration resulted in decreased water content, which makes sense due to the fact that the percentage of total sugar in each batch increased as glucose oxidase concentration increased. As for the individual sugars, sucrose and fructose content was essentially constant across all three batches. Fructose was unchanged in the process and sucrose was not completely converted by invertase. Finally, the collective data on the three batches and the store-bought honey show that both natural and synthetic honey can vary based on synthetic or natural nectar source.

5.2 Recommendations and Future Work

This study provided excellent proof of concept for the synthesis of synthetic honey using enzymes. There is significant additional work that could be performed to move this project forward. The first recommendation for future work would be to take more data overall on this process as it pertains to glucose oxidase. Only three batches were made using these specific proportions, and only one batch was tested for viscosity. It would be beneficial to obtain more data on the process by adding or removing glucose oxidase to identify a statistically significant trend in the data using more than three data points. Further, it may be beneficial to change other parts of the process to determine their effect on the overall process. For example, it may be beneficial to determine the effect of increasing or decreasing the amount of invertase from the system. Invertase is a major enzyme in the pathway and its conversion of sucrose is a major determinant of the final product's composition. It would be interesting to see how the amount of invertase present affects this final composition.

Another variable that would benefit from being changed in some way would be the sugar proportions in the initial nectar. The nectar used in this experiment was synthesized due to an inability to obtain large amounts of nectar from natural sources. In a future scale-up of the process, it may be possible to obtain a large enough quantity of flowers to extract enough nectar for the process to work. However, for lab work, it may not be feasible to obtain such a large amount. Using this change, it could be beneficial to determine if this process works for different nectar sugar concentrations from different flowers. New calculations using the sample shown in Appendix A would be needed to determine the number of enzymes needed to make the honey, but the process overall should be the same and could be repeated for other flowers.

Finally, it would be interesting to introduce a taste test into the experiment. As honey is a popular food and is used for a variety of different things, people would like to expect a certain set of flavors from any synthetic honey. It would be useful to perform a taste test to determine how close in taste synthetic honey is to natural honey. Knowing if it tastes different would prove useful for future workers on this project to think about what different compounds could bring the flavor closer to that of natural honey.

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Appendix A: Calculations

This appendix shows examples of the calculations done for this study.

Enzymatic Calculations

To calculate the grams of invertase needed for the reaction, the following data was collected[7] and compiled.

$$V_{max_{Inv}} = 0.13\ M/min\ \rightarrow 0.00216\ M/sec$$
$$K_{cat_{Inv}} = 21.7\ sec^{-1}$$

Knowing that $V_{max} = K_{cat} \cdot E_0$, and that

$$K_M = \frac{(K_{-1} + K_2)}{K_1}$$

Then the $E_{0_{Inv}}$ was found to be

$$E_{0_{Inv}} = \frac{0.00216\ \frac{M}{sec}}{21.7\ sec^{-1}} \rightarrow 9.985 \cdot 10^{-5}\ M$$

The molecular weight of invertase is known to be $75{,}000\ \frac{g}{mol}$, therefore the amount of invertase needed for the reaction to go further, in grams, was found to be $7.489\ grams$.

Similar calculations were done to find the amount needed for glucose oxidase and catalase.

With the amount of invertase known and knowing that it has a 1:1 relationship with glucose through the reaction, then the final amount of moles of invertase would be equal to the starting amount of glucose for the glucose oxidase reaction. Because one of the assumptions of this math is the saturation of sucrose in the system for the invertase to break down, the starting proportions for the synthetic nectar were decided to be:

$$Sucrose: 43.6\ grams$$
$$Fructose: 18.6\ grams$$
$$Glucose: 17.8\ grams$$
$$Water: 420.\ grams$$

For a total of 500 grams of nectar solution to become the different batches of synthetic honey.

Density Calculations

This is the following sample calculation to determine the density of the first batch of synthetic honey made in the lab:

Measured volume: $4.9\ milliliters$
Measured mass: $6.876\ grams$
Calculated density: $\rho = \frac{Mass}{Volume} \rightarrow \frac{6.876\ grams}{4.9\ milliliters} \rightarrow 1.403\ \frac{grams}{milliliter}$