Coastal Carolina University CCU Digital Commons

Honors Theses

HTC Honors College

Fall 12-15-2023

Investigation of zinc and iron in wildflower honey

Savannah Simpson Coastal Carolina University, srsimps2@coastal.edu

Follow this and additional works at: https://digitalcommons.coastal.edu/honors-theses

Part of the Analytical Chemistry Commons

Recommended Citation

Simpson, Savannah, "Investigation of zinc and iron in wildflower honey" (2023). *Honors Theses*. 462. https://digitalcommons.coastal.edu/honors-theses/462

This Thesis is brought to you for free and open access by the HTC Honors College at CCU Digital Commons. It has been accepted for inclusion in Honors Theses by an authorized administrator of CCU Digital Commons. For more information, please contact commons@coastal.edu.

Investigation of zinc and iron in wildflower honey

By

Savannah Simpson

Department of Biology

Submitted in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science In the HTC Honors College at Coastal Carolina University

Fall 2023

Louis E. Keiner Director of Honors HTC Honors College Drew Budner, Ph.D. Associate Professor Department of Chemistry Gupta College of Science

Abstract

Honey acts as a valuable food source for both animals and humans. When found in contaminated environments, there is an increased likelihood that honey samples will have high metal concentrations. It has been discovered that honey concentrations in metals can be different based on location. This experiment aims to determine the amount of two valuable metals, zinc, and iron, in various honey sources. These metals can be important to the human body because they help boost immunity and allow the body to carry out the functions required for survival. However, if these elements are present in copious quantities, then it can lead to future health complications. These two metals are also prevalent in the environment and would be transferred into honey. By using an atomic absorption spectrometer, the amount of both zinc and iron can be determined from various honey sources. The concentrations of these metals in honey can be used to confirm safety but also it provides an indicator of locations which can help authenticate honey origins.

Introduction

Honey is commonly collected from plant nectar by bees during pollination. This nectar is carried by bees to honey hives where it can then be used as a source of food and then collected by honey harvesters. This substance is naturally sweet and is often combined with the specific bee components found in honeycombs (Tibebe et al., 2022). In honeycombs, this honey is "deposited, dehydrated, stored, and left in honeycombs to ripen and mature" (Tibebe et al., 2022). Beehives are in a variety of locations which can cause the metal concentration found within honey to vary. Metal concentrations are likely to be higher in polluted or contaminated areas. Plants harboring nectar sources coinciding with these tainted areas will have high metal concentrations. The most common reason for large metal contents is due to "…contaminated water and agricultural fertilizers…" that are absorbed in plant tissues (Tibebe et al., 2022). Other contaminated honey can come from the air and soil alongside industrial factories and human populated roads (Chafik and Adnène, 2022). When this is picked up by the bees, it can then lead to the impurity of the obtained raw honey source. Not only can metal presence in honey be due to pollutants but also due to improper harvesting techniques. Accumulation of poor metals in

honey from improper storage techniques can lead to "...contamination during the fumigation, extraction, and storage of honey..." (Bartha et al., 2020). This will make certain honey sources dangerous for human ingestion by increasing metal content and decreasing honey quality.

Honey contains metals that in trace amounts can be helpful for the human body, but in generous amounts could prove to be dangerous. Understanding metal amounts contained within each honey source can allow experimenters to decide which honey types are best for human consumption. Zinc and iron are two metals commonly found within liquid honey that have human health benefits but may become toxic to the body if present in vast amounts (Kubala, 2022). To investigate the benefits produced from honey, it is vital to understand what this liquid substance is, where it comes from, and how metals contained in honey can contribute to human health. Knowing where these honey sources come from can also allow for identification of highly polluted or contaminated areas in which plant sources contain considerable amounts of honey. Honey processors can then avoid collecting honey from hives located near these polluted areas since they have high metal concentrations. Avoiding these highly polluted areas can then ensure that processed honey only comes from areas that have minimal metal amounts.

Honey can be classified as monofloral or polyfloral based on the pollen source. Monofloral honey is known to come from one nectar source while polyfloral honey comes from multiple nectar sources (Bartha et al., 2020). It is more likely that impurities will be present in honeys with multiple nectar sources due to a broadened area region and increased contact with contaminated areas. In many instances polyfloral honey sources have proven to be higher in metal concentrations than monofloral honey sources. Recent studies refer to polyfloral honey sources as having toxic metal levels that can cause consumer ramifications. When honey is collected from multiple sources there is a higher probability that metals will be picked up from plant tissues and transferred to beehives. Polyfloral honey sources cover a wider geographic range, giving a higher opportunity for plants to be exposed to contaminated or polluted habitats in contrast to stationary honey sources (Bartha et al., 2020). However, if the main monofloral source being collected by bees is near the source of polluted areas then higher concentrations of metals may be found. This means that when determining which type of honey will result in higher accumulated metal concentrations, it is important to consider both the geographic region and honey source.

To detect metal content in honey sources a variety of methods can be used. This includes, "flame atomic absorption spectrometry, high-performance ion chromatography, neuron activation techniques, flow injection analysis, coupled with atomic spectroscopy, inductively coupled plasma mass spectrometry, atomic absorption spectrophotometry, and inductively coupled plasma optical emission spectrometry" (Tibebe et al., 2022). Each of these analysis techniques can help to measure different honey contents to determine honey's different physical and chemical properties. For example, gas chromatography can be used to "determine the minor and major metals that can be found in honey" (Pita-Calvo et al., 2017).Using a variety of these methods, the highest metal concentrations were found to be calcium, magnesium, calcium, and phosphorus while smaller concentrations include iron, copper, zinc, silicon, sulfur, manganese, fluorine, molybdenum, chromium, and iodine (Pita-Calvo et al., 2017). These metal concentrations may differ depending on the honey being analyzed.

In this experiment, the metals under investigation include zinc and iron. Zinc and iron were chosen because they possess potential human health benefits when available in trace amounts. These two metals are also the two most prevalent metals found within your body (Kubala,2022). However, when present in enormous amounts these metals can pose potential

health complications to humans. Determining which honey sources are low in harmful metal contents can help to ensure human safety and provide insight into which areas contain a low level of pollutants. Zinc can be very important in trace amounts to individual functioning. Zinc is a mineral that can be found in many food and plant sources and within the human body. Throughout the human body, zinc can be found in "...tissues, blood cells, bone, and teeth" (Jeejeebhoy et al., 2009). It is important to get regular doses of zinc through food sources since the body cannot store this mineral and produce it itself. Ingesting zinc in trace amounts allows the body to fulfill everyday genetic functions that are essential for individuals to perform basic functions. Examples of these genetic functions include genome expression, DNA replication steps/processes, and forming vital proteins that provide humans with vitamins that boost their immune system (Kubala, 2022). If individuals are deficient in zinc, then it can lead to potential health problems and negative consequences. These potential health problems can include the "... stunting of growth and sexual immaturity..." (Jeejeebhoy et al., 2009). Other side effects of zinc deficiency also include "... diarrhea, decreased immunity, thinning hair, impaired taste or smell, dry skin, fertility issues, and impaired wound healing" (Kubala, 2022).

All these unforeseen consequences due to low zinc levels in the human body prove that zinc is an essential element in properly functioning individuals. Alternate advantages of zinc include burn, wound, and acne treatment. As well as the ability to decrease the development of diseases that can have life-threatening consequences as you age (Kubala, 2022). Supplemental zinc amounts can help to strengthen the cells, protecting your body from infection. In fact, "elemental zinc may decrease the incidence of infection in older adults by nearly 66%" (Kubala, 2022). However, in high levels, zinc can be toxic to the body and have side effects. This could include negative consequences that will make individuals feel unwell and sick with unforeseen

consequences. These consequences include "nausea, vomiting, diarrhea, abdominal cramps, and headaches" (Kubala, 2022). When zinc is present in large quantities it becomes toxic to the body and does more harm than good. For example, overabundance of zinc can cause a mineral imbalance in the body, making individuals more prone to infections due to a decrease in immune system function. More severe side effects brought upon by mineral imbalances in the body due to zinc overabundance involve "iron deficiency anemia, sideroblastic anemia, and neutropenia" (Meixner, 2023).

Aside from zinc being prevalent in the human body, iron is another abundant trace metal. Iron is the most abundant trace metal in the human body that can prove to have health benefits when present tin the human body in proper amounts. (Kubala, 2022). Iron can be found in food and plant sources and dietary supplements. Through ingestion, iron can be introduced into the bloodstream producing hemoglobin and myoglobin, which can aid in the oxygenation of red blood cells and muscle cells (Lefton, 2022). Since iron is found in red blood cells, it can be used to prevent anemia in individuals. In fact, "iron deficiency is the most common cause of anemia, occurring in 5% of women and 2% of men" (Lefton, 2022). Myoglobin essentially provides oxygen to the muscle cells of individuals. This oxygen allows muscle cells to function and can be essential for athletic performance. When present in adequate amounts, iron can help prevent not only anemia and enhance athletic performance, but also prevent exhaustion, restless leg syndrome, and improve awareness (Lefton, 2022). If iron is not present, then it can result in health consequences that stem from deficiencies. Some of these deficiencies include "fatigue, shortness of breath, dizziness, headaches, pale skin coloring, brittle nails, and difficulty concentrating" (Lefton, 2022). Most of the time if iron is present in substantial amounts, then it will just be absorbed in the bloodstream and saved for when iron levels are depleted. However,

iron overload can pose potential health problems for individuals with genetic disorders. An example of this is known as hemochromatosis. Hemochromatosis is when the body stores the iron from food in the body. Other problems from iron overload include "internal bleeding, seizure, coma, and even death" (Lefton, 2022). As previously mentioned, both zinc and iron have been found to be present in honey sources. It is important to understand the balance needed to maintain adequate levels of zinc and iron in the body without causing harm to the individual up taking this sugary substance. Given this information, it is better to have higher levels of iron in the body than zinc due to the potential health problems that can occur when levels are high.

In the past, the main methods used for honey analysis include both Inductively Coupled Plasma Optical Emission Spectrometry (ICP) and Atomic Absorption Spectrometry (AA) to evaluate for the presence of metals. Plasma Optical Emission Spectrometry has been useful in detecting elemental concentrations in substances. This is made possible by a combination of three different steps. The first step involves the use of high energy plasma. This plasma is generated by using either "... high-power radio frequency signal or through microwave irradiation..." (Levine, 2021). Once this is done the gasses that exist within the plasma can then ionize and allow for charged particles to coexist in the "...plasma matrix..." (Levine, 2021). The plasma matrix and the sample will then interact so that the sample can be aerosolized. Once this process is completed, the sample will be degraded into the individual elements that are obtained within the sample. To understand the categorization of each element, the wavelengths of each are separated (Levine, 2021). A detector is then used to confirm the "final sample composition" (Levine, 2021). This conclusion is made by using wavelength determination and ionization techniques. These two characteristics allow for the sample and plasma matrix to interact so that the elements that make up the overall sample can be determined. Prior to this experiment, this

was used to collect metals in honey. An example of this is seen when avocado honey types were analyzed using ICP. When these honey types throughout Spain were analyzed high concentrations of different metals were found. With the help of ICP the safety of honey areas could be determined. Prominent levels of potassium, sodium, and phosphorous were found to be present in excessive amounts, magnesium and calcium were present in moderate amounts, and low levels of cadmium and lead were observed (Terrab et al., 2004). Since these metal concentrations were relatively low, the ranges that honeys were selected from have proven to be low in both pollutants and chemical exposure. Zinc and iron were also both present during honey collection but had no notable levels that could impact consumer health.

Atomic Absorption Spectrometry (AA) is another useful technique for elemental analysis, specifically metals. When Atomic Absorption Spectrometry is used, a flame or graphite furnace is first used to heat the sample. Once this is done the atoms in that sample are exposed to light, moving to an excited state (Visser, 2021). When these atoms move to an excited state, wavelength absorption can then be determined by using radiation energy. Finally, a detector is used to determine the absorption of metal in a substance through light beam intensity (Visser, 2021). This is a useful tactic for analyzing specific metals in different liquids. Comparing the standard absorption of both metals to the metals found in honey samples. This method was previously used to test honey safety in different geographic regions of Turkey. Atomic Absorption Spectrometry was used once honey samples were warmed and made into a liquid component using selected reagents of H₂0₂ and diluted solutions of NO₂ and HCl (Nail et al., 2019). From atomic absorption spectrometry high amounts of Cu, Pb, and Cd were found, indicating high elemental findings in these honey samples. Elevated levels of these trace minerals led experimenters to believe that the environmental pollutants existing in Turkey provinces are relatively high (Nail et al., 2019). This could be due to continued industrialization brought upon by economic growth increasing the number of unwanted metals that enter food substances.

Furthermore, Atomic Absorption spectrometry was used in another study to determine heavy metal concentration in provinces throughout the Zhejiang province in China. This analysis found the highest metal concentration to be in zinc. This level was so high that it was "…near the maximum allowable contaminant level in foods and honey in China (Zhuang et al., 2012)." This notably high amount of zinc was determined to be 1329.5 µg/kg with the help of Atomic Absorption Spectrometry (Zhuang et al., 2012). It is assumed the this occurred from poor harvesting quality from both producers and bees. In this study, the metal concentration differed depending on the location, meaning some areas are better fit for adequate honey processing than others. For instance, Italy, Poland, and Slovenia all had lower concentrations of zinc (Zhuang et al., 2012). In comparison to the Zhejiang province in China, these areas have much lower exposure to heavy metals. This means metal exposure to plants is much lower and producer habits are safer. Understanding which samples are high in metal concentrations can gear consumers to healthy honey habits.

Overall, the goal in this experiment is to analyze the metal concentrations discovered in zinc and iron. These metal concentrations were determined by using an atomic absorption spectrometer (AA). To allow for these metal concentrations to be accurately depicted by the AA, stock solutions were created, and a calibration curve was configured. This would ensure accurate honey concentration averages and allow for the accurate analysis of results. The geographic range of these honey concentrations varied from Minnesota, Illinois, Iowa, Colorado, Virgina, Georgia, North Carolina, and South Carolina. The honey sources were limited to what was easily accessible to the experimenter. After analysis, the average honey concentrations for both zinc and iron in the provided honey samples, these values were compared to a similar study. This study looked at the average honey concentrations of trace metals in the West Mediterranean regions of Turkey. If the observed values matched the expected literature values, it would ensure this experiment was done accurately. Furthermore, the experimenter could conclude if the honey sources are considered safe for human consumption by comparing the observed values to the expected literature values. To further this study, the geographic ranges with high zinc and iron concentrations could be analyzed to determine why they yield high concentration amounts.

Methods and Materials

Honey: In this experiment, 21 available honey sources purchased directly from producers or through commercial sellers are being used. These honey samples were taken from various places throughout South Carolina and were not restricted to a particular area. The range for these honey samples included Minnesota, Illinois, Iowa, Colorado, Virgina, Georgia, North Carolina, and South Carolina. Sample variety was limited by what was easily acquired. The honey amount obtained by the experimenter prior to overnight heating was around 5 grams.

Chemicals: The chemicals used in this experiment were 20% hydrogen peroxide and nitric acid (HNO₃). The supplier for 20% hydrogen peroxide was BICCA. The supplier for nitric acid (HNO₃) was Macron Chemicals. Distilled water was produced using a Milli-Q system and had a resistance >18 M Ω .

Instrumentation: Atomic Absorption Spectrometry was used to generate an average concentration of each honey brand reduced into a liquid form. The AA was a Shimdazu AA-7000.

Sample Preparation: Five grams of each of the selected honeys were placed into a crucible. Once the samples were held within the crucible and weighed, they were placed within the muffle furnace at 600°C overnight to separate inorganic metals from the organic substance in honey by ashing. Once heated, the remaining ash content is treated with 2 mL of hydrogen peroxide and nitric acid. These components were collected and poured into the crucible before being moved to the volumetric flask. Diluted water was used to wash the crucible, so that any remaining ash would make its way into the 25 mL volumetric flask. The 25mL volumetric flask was then filled with diluted water. This along with standardized treatments of zinc and iron allow for honey analysis using the Atomic Absorption Spectrometry.

Standards were made for both metals under experimentation. The standards were created by taking out 1 mL from the original metal concentration (1000 ppm) and placing it into a 100 mL volumetric flask. The volumetric flask was filled the rest of the way with distilled water. This allowed for the creation of a stock solution that could then be further diluted out to create standard solutions. The standard solutions for both zinc and iron were different depending on the way that they are absorbed by the atomic absorption spectrometry. The standards that were created from zinc varied from 0.25 mL to 1.00 mL. The standards created for iron ranged from 0.25 to 1.5 mL. For zinc the standards that proved to work the best were 0.25 mL and 0.75 mL. The actual zinc concentrations of these standards were 0.25 mL of 10 ppb and 0.75 mL of 30 ppb. For iron, the standards that proved to be the most beneficial were 0.5 mL and 0.75 mL. The actual iron concentrations of these standards were 0.5 mL of 25 ppb and 0.75 mL of 30 ppb.

Results:

When atomic absorption spectrometry is used, the instrument must undergo calibration to ensure that the results accurately depict the concentrations displayed in the liquified honey sample. To ensure that accurate calibration occurred, the original stock solution had to be diluted, create a concentration that could be read by the spectrometer and act as a baseline for the metal concentrations. The values used for the diluted stock solution varied depending on the metal under investigation. The stock solutions that worked best for zinc were 0.25 and 0.75 (figure 1). On the other hand, the stock solutions that worked best for iron were 0.5 and 0.75 (figure 2). From these stock solutions, a calibration curve could be calculated. In both instances of zinc and iron, the calibration curve turned out to be nonlinear when the concentration was tested against the absorbance. Zinc displayed a higher absorbance than iron. This nonlinearity can be explained by the R² value. The R² value is referred to as a "goodness of fit" measure for linear regression models to determine how well the regression line approximates the actual data (Frost, 2023). When the R^2 value is extremely close to 1, this indicates that the given model provides a good indication of the observed results (Frost, 2023). In this case, the R² value for both zinc (figure 1) and iron (figure 2) were greater than 0.95. Given that these values were close to the line of best fit, this indicates that the regression line accurately represents the observed values (Frost, 2023). The line of best fit for iron was closer to 1 than that of zinc. This indicates that the values provided for iron are slightly more accurate than the average concentrations denoted by zinc.

The honeys analyzed are listed in table 1 and came from a large geographic range. This varied range included Minnesota, Illinois, Iowa, Colorado, Virgina, Georgia, North Carolina, and even South Carolina. The Kirkland honey brand was found in a mixture of unnamed

geographical regions. Although these sources varied in location, experimenters were primarily focused on the honey sources near their current geographical region. The geographical regions of interest during this experiment were South Carolina, North Carolina, and even Georgia. The honey products sourced from South Carolina were Uncle Jims Southern Honey and Heartland Honey. Honey brands sourced from North Carolina, Beach Road Honey, Hubert Lowe, Silver Spoon Alpines, Silver Blossom, Orange Blossom, and Green Swamp Apiary. The last geographic region under experimental analysis was Georgia. The honey brands produced in Georgia included Blue Ridge Honey Company, Savannah Honey, and Bear Hug Honey. These areas are coded by state in table 1. South Carolina is coded yellow, North Carolina is coded light blue, and Georgia is colored a brilliant green.

In table 1, the honey products that exhibited the highest metal concentrations was a tossup between Georgia and South Carolina. The metal concentration for zinc in South Carolina ranged from 4.496 to 4.580 mg/g and the iron concentration ranged from 8.780 to 9.510 mg/g. Georgia also experienced similarly high metal concentrations with the zinc range being from 4.432 to 4.446 mg/g while the metal concentration for iron fell between 7.464 to 10.990 mg/g. North Carolina had a much broader range, which could indicate that the metal concentration depends significantly on the North Carolina region that honey is derived from. The zinc range for North Carolina ended up being anywhere between 4.303 to 4.485 mg/g while the iron concentration varied from 4.113 to 8.152 mg/g.

Out of all the mentioned honey brands, Uncle Jims Southern Honey, sourced from South Carolina, contained the highest zinc concentration. In contrast, the honey with the highest iron concentration not limited by geographic range was Kirkland, 11.349 mg/g. These results would make sense since it is not limited to a singular geographic range. Sourcing honey from multiple

regions will increase the likelihood that contaminated areas will be incorporated into the final honey product. This would be in opposition to a single-sourcing method that could ensure that honey is pulled from an area low in pollutants. However, when sticking to the geographic range of interest, the highest concentration of iron was in Georgia. The Georgia honey source with the highest iron application was Bear Hug Honey. Bear Hug Honey had an average iron concentration of 10.990 mg/g. High concentrations discovered in both iron and zinc could be linked to minerals found in soils, sediments, and even runoff into water bodies. These factors could contribute to the observed concentration average of the geographical regions under consideration.

Discussion:

As previously mentioned, concentrations of both zinc and iron are common minerals found within environmental areas. Both zinc and iron have been denoted as essential trace elements that are vital to the health of individuals (Jeejeebhoy, 2009). These trace elements help increase the functionality of tissues while decreasing the likelihood that abnormalities within the human body arise (Jeejeebhoy, 2009). However, when the body receives an overload of these elements it can lead to negative health consequences that can interfere with the body's ability to function properly (Zhuang et al., 2012). This means that it is important to receive these trace minerals within reasonable limits to ensure that these minerals are contributing to the health of an individual rather than diminishing it.

In previous studies, the most abundant mineral found in honey products was iron due to its ability to be present in the atmosphere, high emission rate from steel complexes, and transferability into soils and nearby vegetation (Bartha et al.,2020). According to Katy Layman and others, iron is determined to be the most abundant mineral in natural soils due to the components of ferrous and ferric iron. These two components are composed of positively charged atoms that can stick to negatively charged soil particles (Layman, 2018). These soil particles can then be picked up by bees during the pollination process and added to their overall honey content. Since iron is a plentiful mineral discovered in soil, it can support the experimental results provided by table 1. As a reminder, this table emphasize that the average concentration of iron in various honey sources is considerably higher than zinc. Bartha mentions that the provided iron concentrations may even be considerably lower than those present within geographical regions. These values may seem lower than the actual amount of iron present within these regions because bees can sift out heavy metals that are present in their environment when consuming their food options (Bartha et al., 2020).

Although not prevalent in notably high amounts, the concentration of zinc can still exist in liquified honey samples. Traces of zinc have been discovered on both a local and regional scale in many geographical areas. Zinc has been noted to be prevalent in both surface and groundwater. Since zinc can enter the environment through drainage, runoff, and increasing urban development (Christos, Miltiadis, & Karyotis, 2018). However, because iron is the most abundant mineral in soil components, it can validate why iron concentrations are higher than zinc when analyzing the geographical distribution of local honey products.

To confirm that the discovered concentrations were accurately depicted, these ranges were compared to the honey concentrations derived from West Mediterranean regions found throughout Turkey. When comparing these values, there was not an extreme difference in the literature levels of both zinc and iron. In this study, the range for zinc varied from 0.32 to 6.00 mg/g (Tutun et al., 2019). When comparing this range to the honey concentration discovered in sampled honey sources from Coastal Carolina University, zinc fell within the middle of this

broad range. After comparing the literature values of zinc to the observed zinc values, iron was the next mineral under investigation. The average iron concentration for West Mediterranean regions varied between 0.20 to 12.60 mg/g (Tutun et al., 2019). When comparing this to the average honey concentrations described in table 1, the range for iron tended to be on the higher end of the described literature values. Overall, the values in the study provided by Tutun and others displayed similar literature values to the average honey concentrations conveyed in this study. Additionally, the honey concentrations discovered when the analysis of West Mediterranean regions in Turkey were determined to be within acceptable limits and considered safe for human consumption (Tutun et al., 2019). Since the literature values were close to the observed values, this confirms that the honey samples provided by Coastal Carolina University are also safe for human consumption, given that they fall within the acceptable limits provided by the West Mediterranean regions.

Conclusion:

The average concentration of two prevalent honey metals, zinc, and iron can be helpful in trace amounts, but become toxic in large amounts. Large quantities of these minerals can lead to unforeseen health consequences, making the consumption of honey unsafe for humans. Iron is a mineral commonly found in soil and can even be picked up by bees during the pollination process. Furthermore, zinc is a common mineral introduced into the surface of groundwaters due to nearby runoff. The levels of these metals can vary depending on the geographic region that these sources are extracted from. South Carolina honey sources were determined to be the highest in average zinc concentrations, while Georgia honey sources contained the highest iron concentration. Although these honey sources were on the higher end when compared to past literature values, they were within reasonable limits. Since these honey brands fell within the

acceptable limits, they are safe for human consumption. In future studies, the geographic region of honey sources under consideration could be surveyed. This technique will allow researchers to better understand the areas physical features and components that may contribute to high metal concentrations found in honey products. Table 1: Adjusted Calculated Average Concentration by Honey Brand. The table above shows the adjusted average honey concentration by brand and metal. This was calculated by taking the average concentration provided by AA, multiplying it by liquid amount contained within the volumetric flask (25 mL) and then dividing it by the mass of honey measured in each crucible (around 5 g). The honey brands highlighted in yellow are from South Carolina, light blue brands are sourced from the North Carolina area, and the brilliant green highlights brands from Georgia.

Honey Brand	Zinc Concentration Average	Iron Concentration Average
Yellow Sweet Clover	4.333	5.200
Blue Ridge Honey Company	4.583	7.759
Simply Nature	4.489	9.370
Aunt Sues Raw and Unfiltered	4.329	6.503
Between the Rivers	4.407	6.507
Local Honey Hive	4.373	6.669
Beach Road Honey	4.303	4.113
Hubert Lowe	4.458	<mark>4.978</mark>
Virgina Brand	4.523	12.443
Silver Spoon Alpines	4.411	4.245
Uncle Jims Southern Honey	<mark>4.580</mark>	<mark>9.510</mark>
Savannah Honey	4.432	7.464
Silver Blossom	4.430	<mark>6.480</mark>
Orange Blossom	4.405	8.152
South Carolina Honey	<mark>4.496</mark>	<mark>8.780</mark>
Paradise Honey	4.359	5.131
Ole Smoley Candy Kitchen	4.462	7.232
Green Swamp Apiary	4.384	8.472
Bear Hug Honey	4.446	10.990
Heartland Honey	4.393	2.809
Kirkland	4.543	11.349

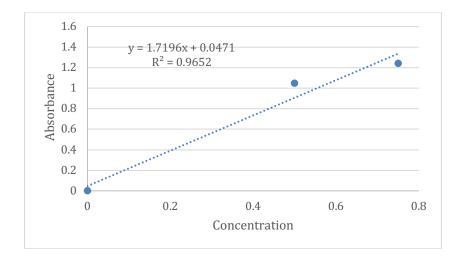


Figure 1: The above figure displays the calibration curve of zinc. The exact zinc concentration values that qualified as stock solutions were 0.5 and 0.75. The R^2 value was close to 1 but not exact, so this could help to explain the nonlinearity of the calibration curve.

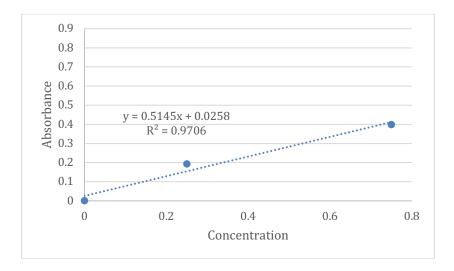


Figure 2: The above figure displays the calibration curve of iron. The exact iron concentration values that qualified as stock solutions were 0.25 and 0.75. The R^2 value was close to 1 but not exact, so this could help to explain the nonlinearity of the calibration curve.

References:

- Bartha, Szilárd, et al. "Heavy Metal Content in Polyfloral honey and Potential Health Risk. A Case Study of Copşa Mică, Romania." *International Journal of Environmental Research and Public Health*, vol. 17, no. 5, 2020, p. 1507., https://doi.org/10.3390/ijerph17051507.
- Chafik, Bouden, and Belabed Adnène. "Determination of Heavy Metals in Honey Samples from Different Regions of the Northeast of Algeria: According to an Urban Gradient." *Pollution*, vol. 8, no. 3, 2022, pp. 820–829., https://doi.org/DOI: 10.22059/POLL.2022.334317.1259.
- Christos, Miltiadis, and Theodore Karyotis. "Zinc in soils, water, and food crops." *Journal of Trace Elements in Medicine and Biology*, vol. 49, 2018, pp. 252-260., https://doi.org/10.1016/j.jtemb.2018.02.009.
- Frost, Jim. "How to Interpret R-Squared in Regression Analysis." *Statistics By Jim*, 16 Oct. 2023, statisticsbyjim.com/regression/interpret-r-squared-regression/.
- Jeejeebhoy, Khursheed, et al. "Zinc: An Essential Trace Element for Parenteral Nutrition." *Gastroenterology*, W.B. Saunders, 27 Oct. 2009,

https://www.sciencedirect.com/science/article/abs/pii/S0016508509014528.

- Layman, Katy, et al. "Identifying and Correcting Iron Deficiencies in Ornamentals Oklahoma State University." *Identifying and Correcting Iron Deficiencies in Ornamentals Oklahoma State University*, 1 Nov. 2018, extension.okstate.edu/fact-sheets/identifying-andcorrecting-iron-deficiencies-in-ornamentals.html.
- Lefton, Jennifer. "Iron: Benefits, Side Effects, Dosage, and Interactions." *Verywell Health*,11 Oct. 2022, https://www.verywellhealth.com/iron-supplements-benefts-4718814#tocwhat-happen s-if-i-take-too-much-iron.
- Levine, Mindy. "ICP-Oes ICP Chemistry, ICP-Oes Analysis, Strengths and Limitations." *Analysis & Separations from Technology Networks*, 17 Mar. 2021, https://www.technologynetworks.com/analysis/articles/icp-oes-icp-chemistry-icp-oesanalysis-strengths-and-limitations-342265#:~:text=Inductively%20coupled%20plasmaoptical%20emission%20spectroscopy%20%28ICPOES%29%20is%20an,each%20elem ent%20within%20the%20complexity%20of%20a%20compound.
- Kubala, Jillian. "Zinc: Benefits, Deficiency, Food Sources and Side Effects." *Healthline*, Healthline Media, 28 Nov. 2022, https://www.healthline.com/nutrition/zinc#bottom_

line.

Meixner, Makayla. "7 Signs and Symptoms of Zinc Overdose." *Healthline*, Healthline Media, 14 Mar. 2023, https://www.healthline.com/nutrition/zinc-overdose -symptoms#treatments.

- Nail, Altunay, et al. "Monitoring of Some Trace Metals in Honeys by Flame Atomic Absorption Spectrometry after Ultrasound Assisted-Dispersive Liquid Microextraction Using Natural Deep Eutectic Solvent." *Microchemical Journal*, Elsevier, 2 Mar. 2019, www.sciencedirect.com/science/article/pii/S0026265X19302589#s0105.
- Pita-Calvo, Consuelo, et al. "Analytical Methods Used in the Quality Control of Honey." Journal of Agricultural and Food Chemistry, vol. 65, no. 4, 2017, pp. 690–703., https://doi.org/10.1021/acs.jafc.6b04776.
- Terrab, et al. "Contribution to the Study of Avocado Honeys by Their Mineral Contents Using Inductively Coupled Plasma Optical Emission Spectrometry." *Food Chemistry*, Elsevier, 22 Oct. 2004, reader.elsevier.com/reader/sd/pii/S0308814604006965?token=86B92105 E0F4684087A2AE8D407E9B57120845DB26FB5D14630BA03E267D4ABDE458C5026 7DB2EFC33119B8876CFE3EA&originRegion=us-east-1&origin=20230502010 434.
- Tibebe, Dessie, et al. "Assessment of Selected Heavy Metals in Honey Samples Using Flame Atomic Absorption Spectroscopy (FAAS), Ethiopia." *BMC Chemistry*, vol. 16, no. 1, 2022, https://doi.org/10.1186/s13065-022-00878-y.
- Tutun, Kahraman, et al. "Investigation of some metals in honey samples from West Mediterranean region of Turkey." *Vet Res Forum*, vol. 10, no. 3, 2019, pp. 181-186. 10.30466/vrf.2019.96726.2312
- Visser, Deon. "Atomic Absorption Spectroscopy, Principles and Applications." *Analysis & Separations from Technology Networks*,16 Dec.2021,https://www.technologynetworls.com/analysis/articles/atomic-absorption-spectroscopy-principles-and-applications-356829.
- Zhuang, P., et al. "Risk Assessment of Heavy Metals in Honey Consumed in Zhejiang Province, Southeastern China." *Food and Chemical Toxicology*, Pergamon, 19 Dec. 2012, www.sciencedirect.com/science/article/pii/S0278691512008885?via%3Dihub.