Antioxidant Properties of 20 San Antonio Honey Samples in Relation to their Color

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Abstract

Honey is a natural healing agent composed of many compounds, such as minerals, vitamins, proteins, and carbohydrates. Honey has multiple activities including antioxidant, antimicrobial, antiviral, etc., and known to protect the cardiovascular, nervous, respiratory, and gastrointestinal systems. Antioxidant properties can be affected depending on the honey's nectar source, temperature, and physical properties. In this research, we examined the antioxidant properties using TPC, TFC, and DPPH assays, along with the color detection assay to examine a correlation between honey color and antioxidants.
A total of 20 different San Antonio honey samples were used. The color detection of honey was determined using the absorption values and the Pfund scale. The absorbance of TFC, TPC, and the DPPH experiments were read where a standard curve was created to obtain individual sample results and determine how well they correlate. Our results showed correlation between the color of the honey and the antioxidant activity. There were three dark amber honey samples that exhibited a general high total phenolic content, total flavonoid content, and radical scavenging activity. Our results demonstrated that the dark amber honey samples had higher antioxidant properties.

Introduction (Background)

We chose to study antioxidant properties of honey because honey has been shown to reduce free radicals in the human body, therefore it can be used for medical practice [1]. Many countries around the world have researched antioxidant properties with their own honey samples, however, there is no research done in the United States.

Honey has been used since 2100 B.C. by ancient Egyptians, Greek, and Romans primarily for wound care [2], [3]. Honey contains over 200 compounds being broadly comprised of sugars, amino acids, vitamins, minerals, enzymes, flavonoids, phenolic acids, and antioxidants. Honey comprises different activities such as antioxidant, antimicrobial, antiviral, etc., but this research focuses on antioxidant properties. Honey protects the cardiovascular, nervous, and respiratory systems.

For this research, antioxidant activity is the primary focus. Honey consists of different antioxidants such as polyphenols and flavonoids, which were obtained by using total phenolic content (TPC) and total flavonoid content (TFC). The DPPH radical scavenging assay blocks the DPPH radical, which then measures the antioxidant activity in honey. Lastly, every honey sample has a different color. Research shows that generally the darker the honey, the higher the antioxidant properties it possesses [4].

Purpose

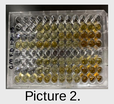
The purpose of this research was to collect data that informs about the antioxidant properties of 20 San Antonio honey samples. First, we determined the color for each honey sample using a UV-spectrophotometer and calculating using the Pfund scale [5]. After, we performed the TPC, TFC, and DPPH assays for each honey sample. The goal was to determine if there was any correlation between the color of the honey with its antioxidant properties.

Hypothesis: If the honey sample exhibits a dark color, then the more antioxidant properties it will have because the color of honey can depict the content of pigments with antioxidant properties (polyphenols, catenoids, flavonoids).

Methods

Experimental Methods to Determine Antioxidant Properties		
Total Phenolic Content (TPC)	Total Flavonoid Content (TFC)	DPPH Radical Scavenging
The antioxidant activity of various honey samples was determined by measuring their TPC absorbance as they reacted with the Folin-Ciocalteu (FC) reagent. To express the result as an equivalent of gallic acid (GAE) per kg of honey, an equation was used. Equation: C= C1 • V/m C = TPC in mg/g C1 = concentration of gallic acid V = the volume of honey solution m =the weight of the honey	The total flavonoid content (TFC) method is based on aluminum complex production in an alkaline medium, detecting the absorbance at 510 nm, and using catechin as a reference component to identify the three most prevalent flavonoids in honey, rutin, luteolin, and catechin. A calibration curve was created using a standard solution of catechin (20, 40, 60, 80, and 100 μg/mL; r 2 = 0.998).	The DPPH radical-scavenging assay is based on the ability of antioxidants to block the 2,2- diphenyl-1-picril-hidrazil (DPPH) radical and is used to measure the antioxidant activity in the honey samples. The absorbance of the solution at 517 nm was used to determine the reduction of the DPPH radical. Equation: % RSA = [(AB-AA)/AB] x 100 AB = absorbance of blank AA= absorbance of honey solution

Picture 1.
ABS SpectroMax



Picture 2.
96 Well Plate

Color Method

The average of the honey triplicate values were obtained. The average was then multiplied by 3.15 to obtain the formula and then compared to the sample result range in the Pfund scale. This number would indicate which honey color it was.

Results

Honey Color	Honey Samples
Dark Amber	SA-4, SA-5, SA-15
Amber	SA-6, SA-7, SA-8, SA-9, SA-13, SA-16, SA-18, SA-20, SA-42
Light Amber	SA-10, SA-11, SA-12, SA-14, SA-17, SA-19, SA-41, SA-43

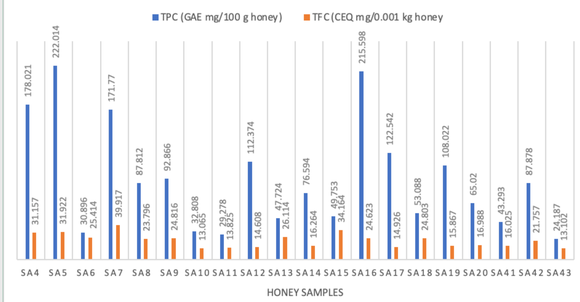


Figure 1. This graph compares TPC values with TFC values.

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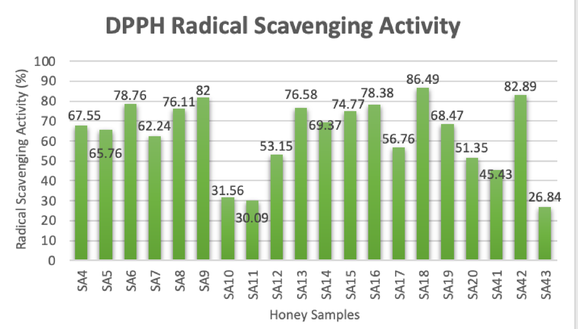

Figure 2. This chart shows the DPPH radical scavenging activity for each honey sample.

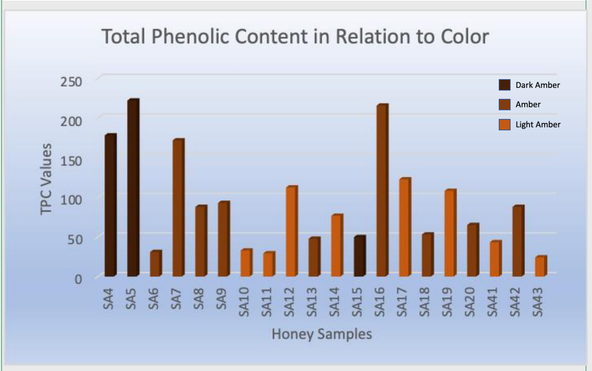

Figure 3. TPC Values in Relation to the Honey Color.

Summary of Results:
Total phenolic content determines the antioxidant capacity, whereas the total flavonoid content determines the antioxidant activity. SA-4 and SA-5 had a high total phenolic content which means that the FC reagent reacted strongly with the gallic acid, which is a phenolic compound. Both SA-4 and SA-5 had a dark amber color, which exhibits a relationship between the TPC and the color.
Total flavonoid content is based on aluminum complex formation. SA-4, SA-5, and SA-15 had a high TFC value, which indicates that the aluminum chloride reacts with the flavonoids to change color in the 96 well plate. Overall showing a strong total flavonoid content.
The DPPH radical assay allows antioxidants to block the DPPH radical (2,2-difenil-1-picril-hidrazil). Therefore, SA-18 was the highest antioxidant because it had the highest ability to block the DPPH radical, and it had an amber color, the second highest honey color.
Our hypothesis is generally correct because our results align with our hypothesis.

Conclusions

- SA-4, SA-5, and SA-15 had the darkest color, dark amber.
- SA-4 and SA-5 had the highest total phenolic content (TPC) values.
- SA-4, SA-5, SA-7, SA-15 had the highest total flavonoid content (TFC) values.
- For DPPH, SA-18 had the highest potential to block the DPPH radical. Following was SA-42, SA-16, and SA-8. The strongest DPPH radical blocking honey samples were amber colored.
- There was a relationship between the color of the honey and its antioxidants. All the dark amber colored honey samples showed the highest antioxidant activity.

References

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