# Analyzing Antioxidant Properties of Twenty-Two Texan Honey Samples


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## Abstract


Twenty-two Texan honey samples were analyzed to assess their antioxidant capacity for potential medical use. To measure antioxidant capacity in honey a simple method, such as DPPH Radical Scavenging Activity (RSA%), was used and results were compared to Total Phenolic Content (TPC), Total Flavonoid Content (TPC), pH Levels, and the pFund Color Scale. Results showed a strong correlation of DPPH between TPC and TFC. Notable antioxidant activity overall, when DPPH was compared to TPC and TFC, was observed in honey samples 23H-74 (100%, TPC:179, and TFC:100.889) and 83 (99%, TPC:177, and TFC:133.267), suggesting potential therapeutic benefits against oxidative stress-related diseases and making them of dietary importance due to their high values in DPPH and TPC/TFC. Conversely, pH Levels is a significant contributor to the antimicrobial properties of honey but not antioxidant properties. Based on the results, a correlation between DPPH and pH levels in honey was not found. For color analysis vs DPPH, the pFund Color Scale was used and aligned closely with the USDA's classification system, which delineates honey into seven color categories, such as Water White, Extra White, White, Extra Light Amber, Light Amber, Amber, and Dark Amber. Lighter hues are expected to be less antioxidant and with sweeter taste, than darker hues expected to have higher antioxidant activity. However, based on the results this was not the case as honey samples 23H-74 and 83, that exhibit a high RSA percentage, have lighter hue colors such as Extra Light Amber, leaving a correlation in question.


## Introduction

Analyzing the antioxidant properties of honey is important to understand its composition and benefits for medicinal purposes. Honey is often known for its natural sweetness, but within this lies a multitude of compounds, including phenolic acids, flavonoids, vitamins, and enzymes, each contributing to its remarkable antioxidant properties. The role of these antioxidants is pivotal in counteracting oxidative stress, a key factor contributing to an array of chronic diseases such as cancer, coronary diseases, and neurological degeneration. To delve into the antioxidant potential of honey, various parameters are employed, including the DPPH radical-scavenging assay, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), pH, and Color Pfund analysis. The DPPH assay stands as a cornerstone in evaluating honey's antioxidant activity, measuring its ability to neutralize the DPPH radical, thereby reflecting its overall antioxidant capacity. This method, known for its simplicity and efficacy, has become a standard in the assessment of honey's antioxidative activity. Analyzing Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) offers crucial insights into honey's antioxidant potential. TPC, assessed via the Folin-Ciocalteu method, reveals the diversity of phenolic compounds, while TFC highlights the abundance of flavonoids like rutin, luteolin, and catechin. Additionally, analyzing honey's color and pH provides further indicators of its quality and antioxidant activity. Ultimately, the combination of these methods provides a thorough grasp of honey's antioxidant characteristics, highlighting its potential health benefits and solidifying its position as a natural medical source promoting health and vitality.

## Objective

This study aims to analyze twenty-two local Texan honey samples for their antioxidant activity using the DPPH Radical Scavenging Activity Assay and investigating its correlation between Total Phenolic Content (TPC), Total Flavonoid Content (TFC), pH Levels, and the pFund Color Scale. Antioxidant activity (RSA%) is measured to identify the honey samples with the highest antioxidant activity most suitable for medical purposes.

## Materials and Methods

**Folin-Ciocalteu (FC) Reagent Assay Method:** This method is designed to determine the Total Phenolic Content (TPC) in honey. The assay is performed by mixing FC reagent and sodium carbonate with the test sample, measuring the absorbance at 765 nm after a specified incubation period, and plotting a graph to find the TPC of unknown samples.

**Colorimetric Assay Method:** This method is designed to determine Total Flavonoid Content (TFC) in honey, such as rutin, luteolin, and catechin. This procedure is based on aluminum complex formation but in alkaline medium regarding the absorbance at 510 nm and using catechin as a reference compound.

**DPPH Radical Scavenging Power Assay Methods for DPPH:** This simple, cost-effective method looks at each honey sample to determine whether or not they can combat the DPPH (difenil-1-picril-hidrazil) radical and then measures the evident (or lack of) antioxidant activity.

**Color Intensity Methods of Beretta et al.:** This method is designed to measure the color intensity of honey. This procedure is done by using the pFund classifier and comparing results to the United States Department of Agriculture approved color standards.

**Volumetric Method for pH levels:** This method uses a pH meter to measure the pH of diluted honey samples.

## Results

| Honey Sample | Sample Zipcode | DPPH | TPC | TFC | pH | pFund | USDA Color |
|---|---|---|---|---|---|---|---|
| 23H-74 | 78070 | 100% | 179.0 | 100.889 | 6.93 | 0.4373 | Extra Light Amber |
| 23H-83 | 78623 | 99% | 177.0 | 133.267 | 6.92 | 0.4663 | Extra Light Amber |
| 23H-76 | 78163 | 90% | 137.0 | 77.26 | 6.93 | 0.361 | White |
| 23H-112 | 78676 | 86% | 153.1 | 59.796 | 5.2867 | 1.281 | Light Amber |
| 23H-31 | 78023 | 85.60% | 203.3 | 133.813 | 6.32 | > 85 and ≤ 114 | Amber |
| 23H-49 | 78006 | 84% | 276.2 | 126.266 | 6.34 | 1.6212 | Amber |
| 23H-114 | 78737 | 84% | 155.1 | 98.075 | 4.6767 | 1.9803 | Amber |
| 23H-13 | 78006 | 83.79% | 275.0 | 112.737 | 6.33 | > 85 and ≤ 114 | Amber |
| 23H-29 | 78023 | 83.10% | 229.3 | 171.282 | 6.18 | > 85 and ≤ 114 | Amber |
| 23H-30 | 78063 | 81.80% | 171.4 | 80 | 6.8 | > 85 and ≤ 114 | Amber |
| 23H-40 | 78063 | 81% | 93.5 | 33.008 | 6.12 | > 85 and ≤ 114 | Amber |
| 23H-101 | 78735 | 81% | 229.7 | 137.301 | 6 | >85 and ≤ 114 | amber |
| 23H-26 | 78132 | 79.80% | 178.6 | 127.753 | 5.99 | > 85 and ≤ 114 | Amber |
| 23H-91 | 78606 | 77% | 187.3 | 58.812 | 6.7 | 1.64 | amber |
| 23H-113 | 78736 | 77% | 125.1 | 55.829 | 4.77 | 1.27575 | Light Amber |
| 23H-116 | 76092 | 77% | 147.0 | 79.14 | 4.91 | 1.9257 | Amber |
| 23H-33 | 78006 | 75.20% | 173.0 | 125.992 | 6.34 | > 85 and ≤ 114 | Amber |
| 23H-50 | 78132 | 74% | 294.4 | 130.22 | 6.25 | 1.9246 | Amber |
| 23H-77 | 77354 | 74% | 147.0 | 65.576 | 6.95 | 0.446 | Extra Light Amber |
| 23H-109 | 77340 | 74% | 118.3 | 66.396 | 4.4267 | 1.4931 | Amber |
| 23H-41 | 78006 | 71% | 68.7 | 35.085 | 6.04 | > 85 and ≤ 114 | Amber |
| 23H-75 | 78606 | 71% | 117.0 | 55.954 | 6.94 | 0.252 | White |

**Table 1. Twenty-Two Texan Honey Sample Values**

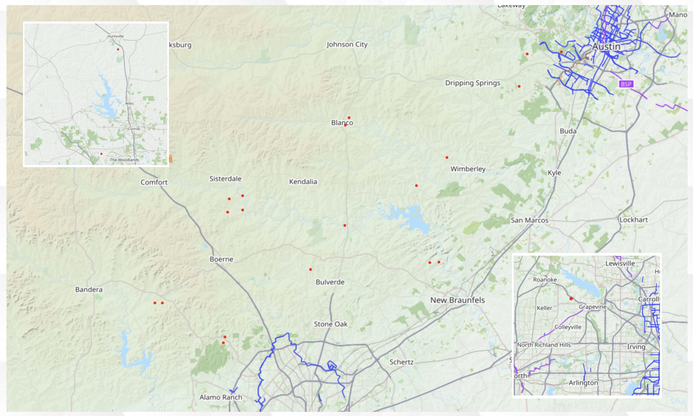


**Figure 1. 22 Texan Honey Samples Zip Code Location**

## Results (Cont.)

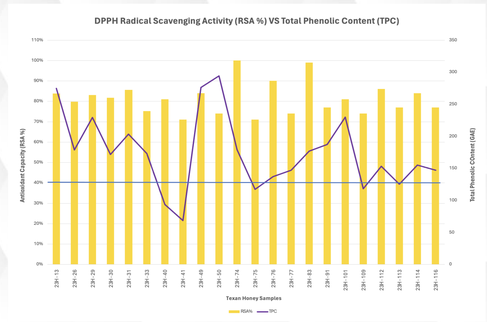


**Figure 2. DPPH Radical Scavenging Activity (RSA%) vs Total Phenolic Content (TPC)**
Amongst all honey samples, Total Phenolic Content (TPC) and DPPH Radical Scavenging Activity (RSA%) was the highest in honey samples 23H-74 (RSA of 100% and TPC of 179), and 23H-83 (RSA of 99% and TPC of 177), whereas honey samples 23H-40 (RSA of 81% and TPC of 93.5), and 23H-41 (RSA of 71% and TPC of 68.7) exhibited lower values, lower antioxidant activity. Honey samples 23H-74 and 23H-83 would be best suited for therapeutic potential against diseases linked to oxidative stress, such as cancer, coronary, and neurological degeneration, due to their high antioxidant activity.

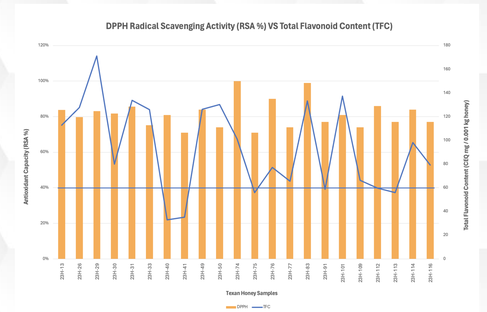


**Figure 3. DPPH Radical Scavenging Activity (RSA%) vs Total Flavonoid Content (TFC)**
Greater DPPH Radical Scavenging Activity (RSA%) and Total Flavonoid Content (TFC) was exhibited in honey samples 23H-74 (RSA of 100% and TFC of 100.889) and 23H-83 (RSA of 99% and TFC of 133.267) have a strong correlation between DPPH and TFC, indicating high antioxidant activity. On the other hand, honey samples 23H- 40 (RSA of 81% and TFC of 33.008) and 23H-41 (RSA of 71% and TFC of 35.085) exhibited the lowest RSA percentage and TFC content. Honey samples 23H-74 and 23H-83 can offer greater beneficial properties such as antibacterial, anti-inflammatory, and anti-thrombotic effects.

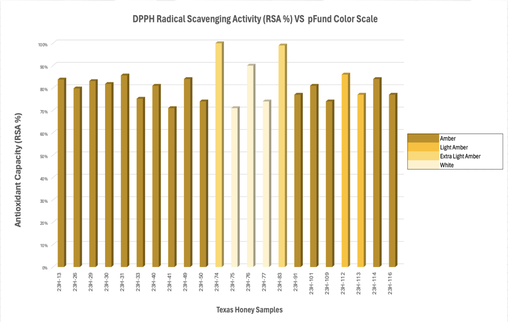


**Figure 4. DPPH Radical Scavenging Activity (RSA%) vs pFund Color Scale**
A correlation between DPPH Radical Scavenging Activity (RSA%) and color pFund was found to be strong, more research is needed to understand this correlation. Honey samples 23H-74 (100%) and 23H-83 (99%), demonstrated to be white and extra light amber. Lightest honey samples had the highest antioxidant activity. Darker hues, such as honey samples 23H-41 (71% / amber) and 23H-50 (74% / amber), exhibited lower antioxidant activity. Based on these results, lighter honey samples in this cohort have higher antioxidant activity.

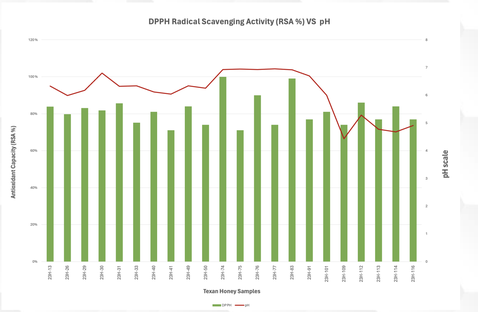


**Figure 5. DPPH Radical Scavenging Activity (RSA%) vs pH**
No correlation was exhibited between acidity (pH levels) and DPPH Radical Scavenging Activity (RSA%).

## Conclusions

- Honey samples with the highest DPPH Radical Scavenging Activity (RSA%) exhibited the highest Total Phenolic Content (TPC) and Total Flavonoid Content (TFC).
- Honey samples 23H-74 and 23H-83, overall had the highest values having the highest antioxidant activity and capacity to be promising for medical purposes in human health and vitality.
- No correlation was found between pH and antioxidant activity.
- A correlation was present between color and antioxidants in honey. However, it was contradicting as usually darker color honey is expected to have higher antioxidant activity. In this study, honey samples with the highest antioxidant activity had lighter hues such as white and extra light amber.

## References


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