



The Antibacterial and Antioxidant Properties of Northern Californian Honey Samples

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Abstract

The escalating global threat of antimicrobial resistance (AMR) is projected to become the leading cause of mortality worldwide by 2050, largely driven by hospital-acquired infections caused by ESKAPE pathogens. Honey, historically celebrated for its wound-healing properties, has re-emerged as a promising natural therapy against resistant pathogens. Medical-grade honey, characterized by high bioactivity, offers potent antimicrobial, antioxidant, and anti-inflammatory benefits.

In this study, we conducted the **first comprehensive bioactivity analysis of Northern Californian honey samples**, evaluating their potential for medical-grade applications. Over 80 honey samples, sourced from a diverse range of Northern Californian cities, were assessed for antibacterial activity using zone of inhibition (ZOI) assays and for antioxidant capacity through DPPH radical scavenging assays. In addition, key physiochemical properties—color, pH, and moisture content—were measured to better understand factors contributing to bioactivity.

Our findings reveal that certain Northern CA honeys exhibit strong antibacterial and antioxidant activities, comparable to medical-grade honeys. These results suggest that **Northern CA honeys may serve as valuable candidates for wound care and infection prevention on wounds and medical devices**, opening new avenues for local honey producers and advancing natural, sustainable therapies in clinical settings.

Introduction

Honey is a natural substance produced by honeybees through the enzymatic processing of nectar collected from various plants. It contains over 200 biologically active compounds, including sugars, amino acids, vitamins, minerals, enzymes, flavonoids, phenolic acids, and antioxidants.

The antimicrobial potential of honey has gained renewed importance due to the escalating global threat of AMR, which jeopardizes the effective treatment of infectious diseases. Particularly concerning are the **ESKAPE pathogens**—a group of bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species)—known for their ability to "escape" the effects of conventional antibiotics. These organisms are responsible for a significant proportion of hospital-acquired infections and are adept at forming biofilms, further enhancing their resistance.

Honey's antimicrobial, antioxidant, and anti-inflammatory properties offer a promising natural alternative for combating antibiotic-resistant infections.

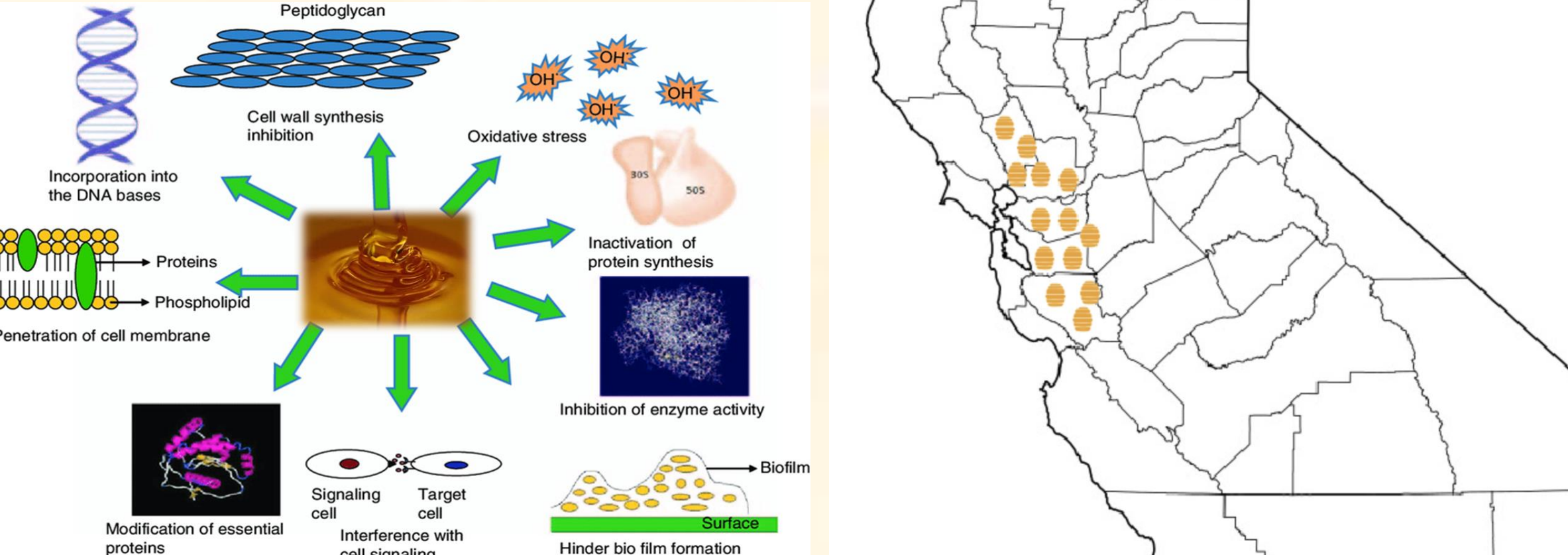


Figure 1: A. Mechanisms of antibacterial activity of honey - B. Zip Code Locations of honey samples collected from CA.

Objective

This study aimed to evaluate the antimicrobial and antioxidant properties of Northern Californian honey samples, with a focus on their activity against ESKAPE pathogens. By characterizing their bioactivity, we seek to identify honeys with potential for development into medical-grade treatments for hospital-acquired infections.

Materials and Methods

A total of **84 honey samples** were collected from local beekeepers in Alameda and Santa Clara counties (Northern CA) in **January 2025**. For each sample, data on **botanical origin**, **nectar source**, **beekeeper contact**, and **pest treatment history** were recorded.

The physical characteristics of the samples were evaluated as follows:

pH Measurement: Honey samples were measured without any dilution using a calibrated pH meter.

Moisture Content: A thin layer of each sample was applied to a refractometer, and moisture percentage was recorded.

Color Analysis: Samples were diluted to 50% concentration, transferred in triplicate to a 96-well plate, and the absorbance was measured at 560 nm using a spectrophotometer. Absorbance values were multiplied by 3.15, and corresponding color categories were assigned based on USDA standard color reference tables.



To assess **antimicrobial activity**, the agar well-diffusion method was used against *Staphylococcus aureus*. Mueller Hinton Agar (MHA) plates were inoculated with bacteria standardized to 10⁸ CFU/mL, and 8 mm wells were filled with 100 µg of each honey sample. Plates were incubated at 37°C for 18 hours, and zones of inhibition were measured. Honey samples producing inhibition zones greater than 16 mm were classified as having high antimicrobial activity based on CLSI guidelines.

To determine **antioxidant activity**, a DPPH radical-scavenging assay was performed. Honey samples (10% w/v) were prepared and added to a 96-well plate along with DPPH solution (17 mM). After 30 minutes of incubation in the dark, absorbance was measured at 517 nm. The radical scavenging activity (RSA) was calculated using the formula %RSA = [(AB - AA)/AB] × 100, where AB is the absorbance of the blank and AA is the absorbance after honey treatment.

Results

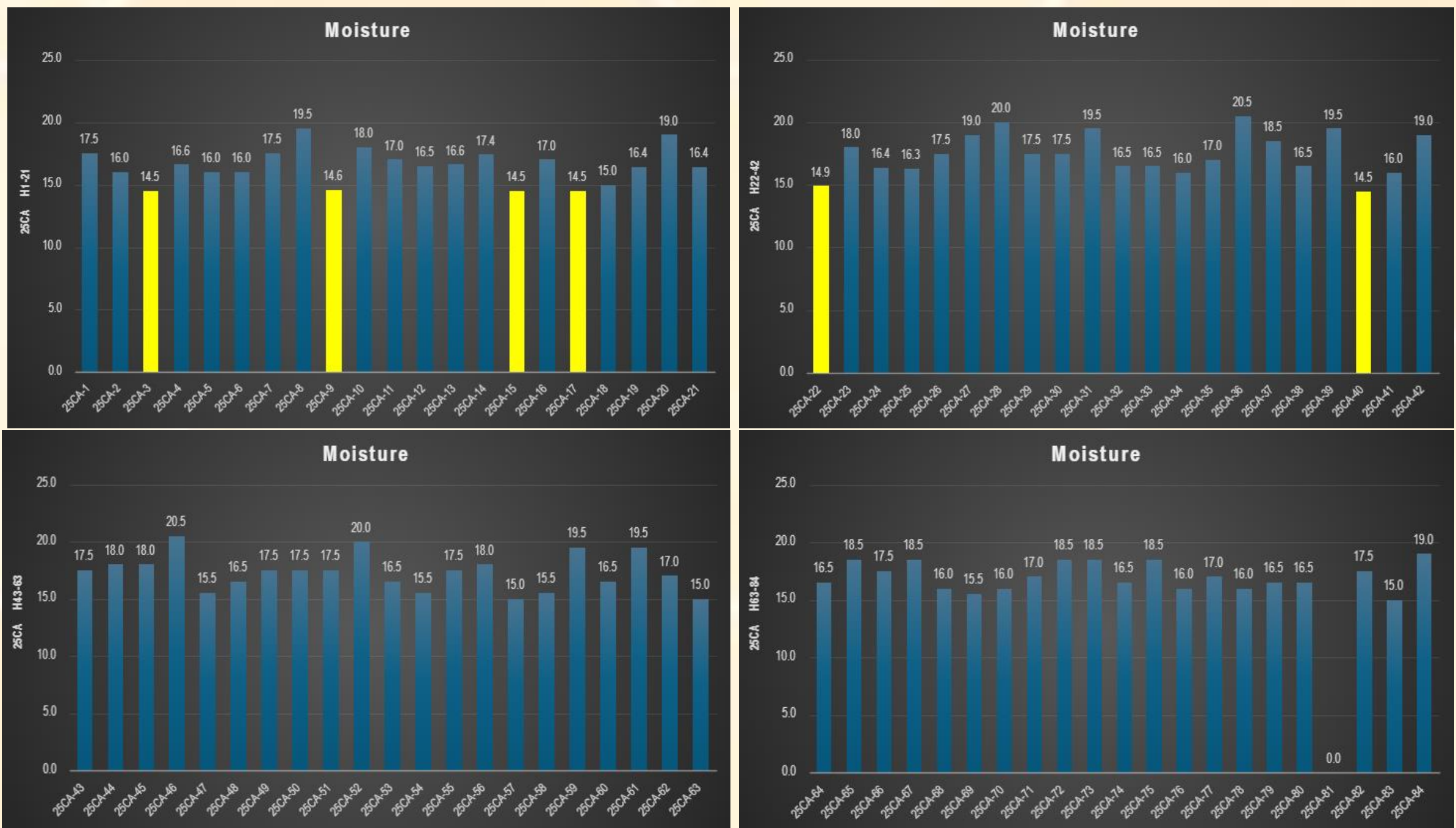


Figure 2. Moisture Content of Northern CA Honey Samples
Moisture content was measured using a refractometer to assess honey quality and stability. Samples with lower moisture levels are more resistant to fermentation and microbial growth.

Results

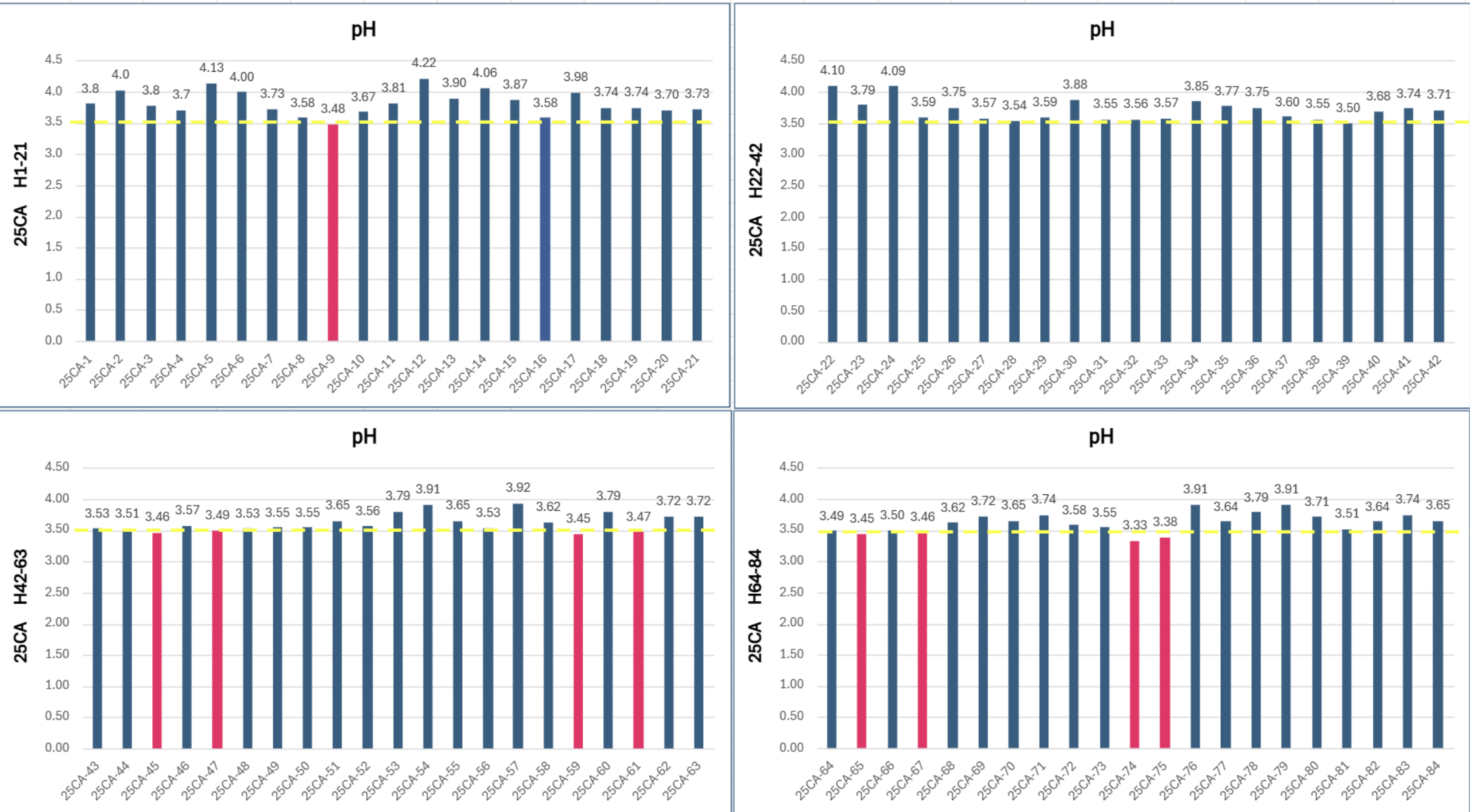


Figure 2. pH measurements of Northern CA Honey Samples
The average pH of honey samples was 3.7.

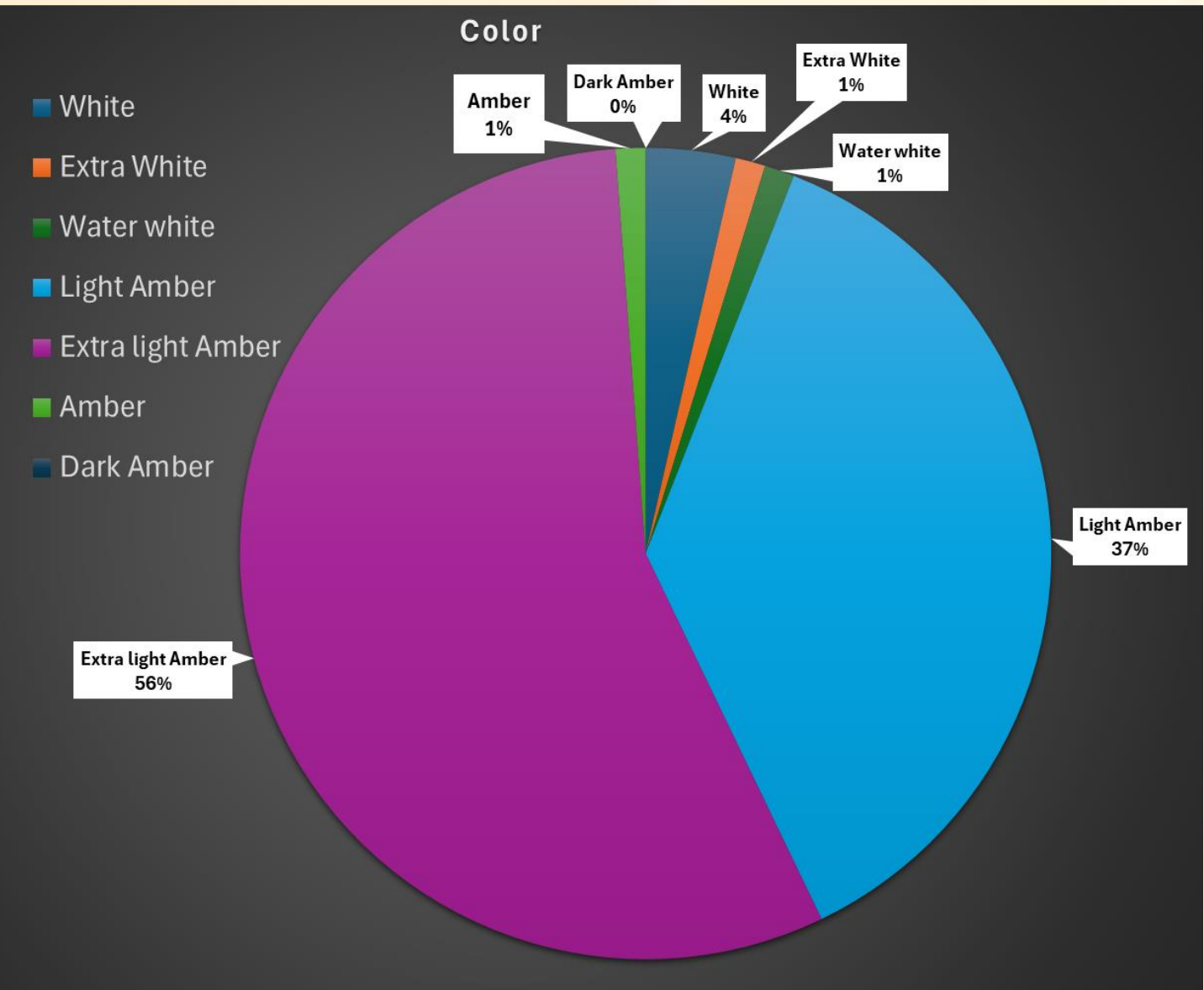


Figure 3. Color Analysis of Northern CA Honey Samples

93% of the honey samples had extra light and light amber colors, which correlates with low antioxidant activity (RSA %)

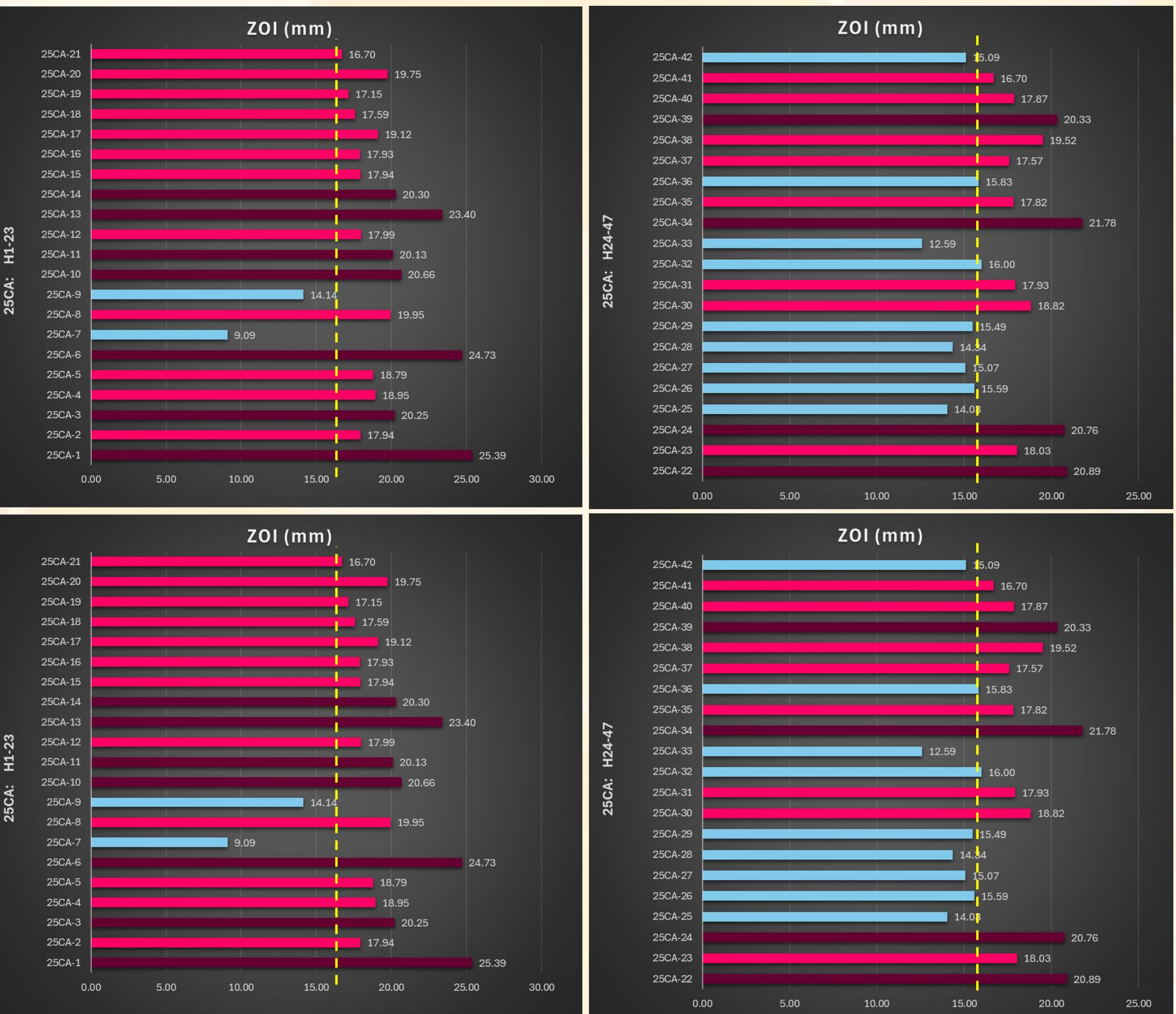


Figure 3. Zone of inhibition (ZOI) measurements for Northern Californian honey samples against *Staphylococcus aureus*. Samples are color-coded by antimicrobial activity: **Blue** = Low ZOI, **Pink** = Moderate ZOI, **Red** = High ZOI
Larger zones indicate stronger antibacterial effects. ZOI was measured in millimeters, including the well diameter.

Results

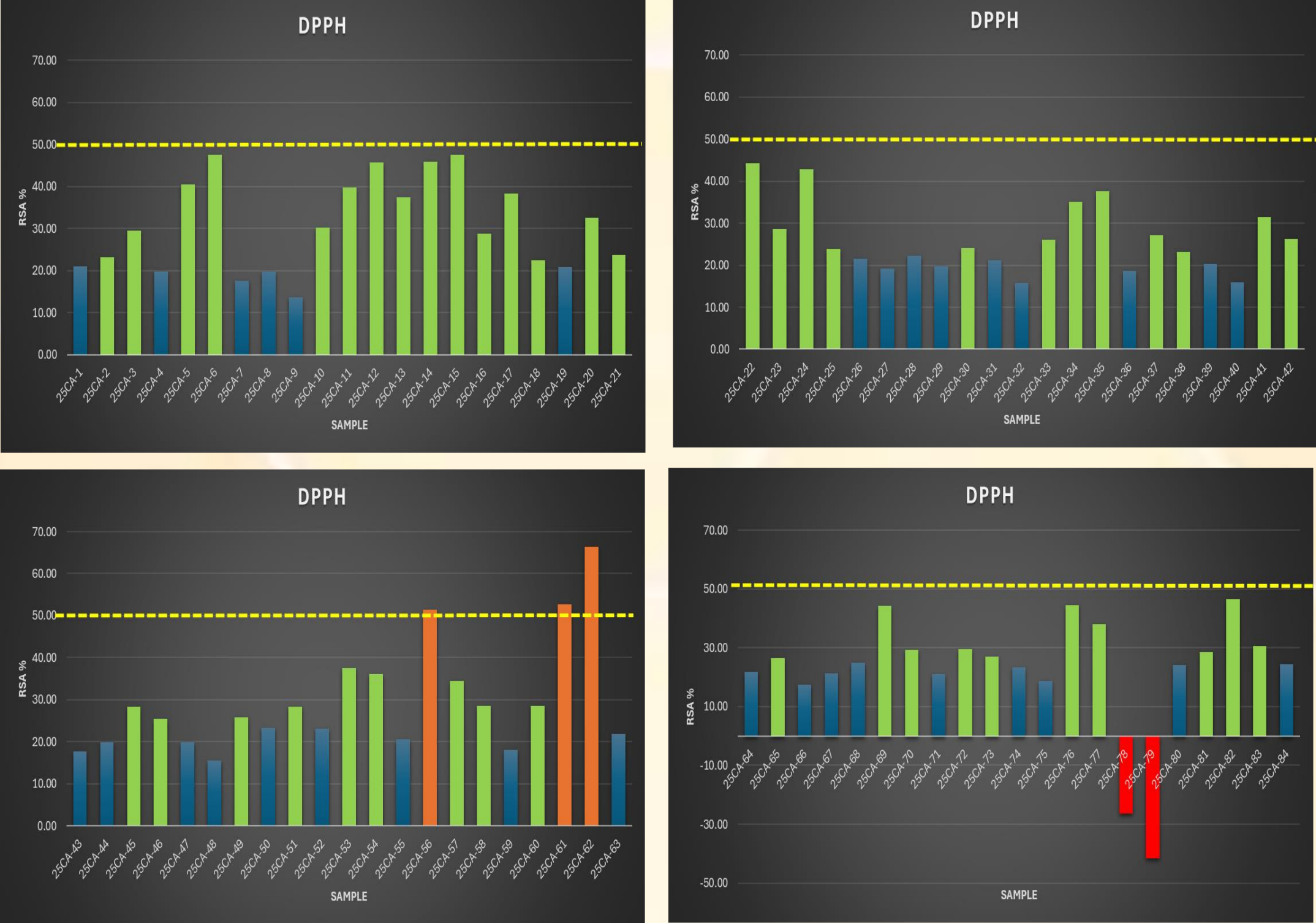


Figure 4. Antioxidant Activity of Northern CA Honey Samples (DPPH)
Free radical scavenging activity for all the honey samples was measured using DPPH assay. Samples are color-coded by antioxidant activity: **Blue** = Low, **Green** = Strong, **Red** = Not detectable
Higher DPPH values correspond to stronger antioxidant properties.

Conclusions

- Antibacterial Activity:** 68% of Northern Californian honey samples exhibited strong antibacterial effects against *Staphylococcus aureus*, a prominent ESKAPE pathogen.
- Antioxidant Capacity:** The majority of samples showed low antioxidant activity, which correlated with their lighter color, consistent with known relationships between color and phenolic content.
- pH and Moisture Content:** Honey samples had acidic pH values and moisture levels within the normal expected range for natural honey.
- Clinical Potential:** These results suggest that Northern CA honey may serve as a promising candidate for development as natural agents in wound care and infection control.

References

- Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Front Microbiol. 2019 Apr 1;10:539. doi: 10.3389/fmicb.2019.00539.
- The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0, 2018. <http://www.eucast.org>.

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