High-Throughput Quantification of Fructose and Glucose in Texan Honeys Using a Microplate-Based Assay

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Abstract

Honey is a natural substance renowned for its nutritional and medicinal properties, largely due to its complex composition of sugars and bioactive compounds. This study aims to quantify the sugar profiles specifically fructose and glucose of 46 honey samples collected from various regions across Texas and to investigate how these sugar concentrations influence honey's bioactivity levels. Sugar content was determined using the Megazyme Sucrose/D-Fructose/D-Glucose Assay Kit, adapted to a 96-well plate format. Bioactivity was assessed through antioxidant activity (via DPPH radical scavenging), antimicrobial effects (Zone of Inhibition), and physicochemical properties including pH, moisture content, and color. Statistical analyses such as Ratio, Pearson correlation, and regression analysis were used to explore relationships between sugar composition and bioactivity indicators. Preliminary results revealed that approximately 56% of the honey samples had fructose to glucose below 1.3, indicating a lower relative sweetness. Additionally, 72% of the honeys showed no signs of granulation, suggesting minimal crystallization. Contrary to literature suggesting a positive association between glucose and antimicrobial properties, this study found no significant correlation between glucose levels and antimicrobial or antioxidant activity. Likewise, no correlation was observed between fructose levels and bioactivity indicators.

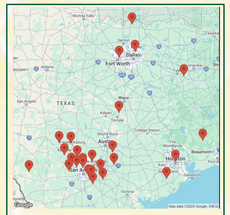
Introduction

- Honey contains sugars, polyphenols, flavonoids, and proteins.
- There 13 major types of sugars in honey: fructose, glucose, sucrose, maltose, turanose, isomaltose, kojibiose, nigerose, erlose, melezitose, raffinose, panose, and maltotriose.
- Fructose and glucose are the most abundant sugars.
- These properties contribute to the antimicrobial, antioxidant, anti-inflammatory, glycemic index, and wound-healing effects of honey.
- Honey composition is influenced by floral source, geographic location, season, and environmental conditions, which affect both nutritional and therapeutic properties.
- Sugar content plays a key role in determining bioactivity levels of honey, with the fructose and glucose concentrations influence antioxidant and antimicrobial effects.
- Higher fructose content contributes to antioxidant activity.
- Higher glucose contents are linked to stronger antimicrobial activity
- Gas chromatography (GC) is used for sugar quantification
- There are no clear or consistent relationship established between sugar profiles and overall bioactivity
- Definition and standardization of medical-grade honey is needed.
- High throughput quantification of sugars is not available

Objective

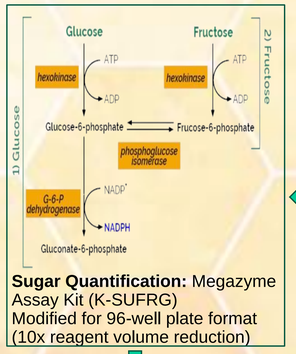
To quantify the concentrations of fructose and glucose sugars in Texan honeys using a microplate assay and explore how sugar profiles influence honey's antioxidant and antimicrobial activities.

Materials and Methods

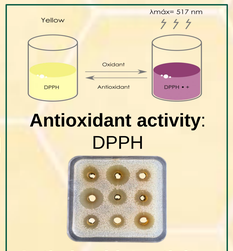


Sampling: 46 Honey Samples in Texas

Physiochemical Properties: pH, moisture and color



Sugar Quantification: Megazyme Assay Kit (K-SUFRG) Modified for 96-well plate format (10x reagent volume reduction)



Antioxidant activity: DPPH

Antimicrobial activity: Zone of Inhibition (ZOI)

Ratio: Fructose to Glucose Glucose to Water

Correlation: Pearson for sugar-bioactivity relationships

Regression Analysis: To predict bioactivity from sugar types

Result Analysis

Results

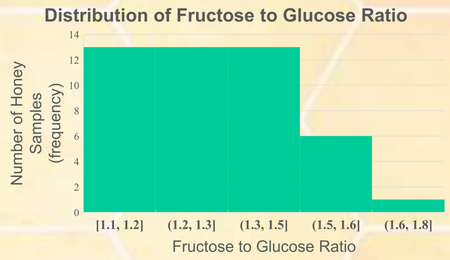


Figure 1. Histogram illustrating the distribution of honey samples according to their **fructose-to-glucose ratios**.

Results

	Glucose	Fructose	ZOI	DPPH	Biological Activity Score
Glucose	1				
Fructose	0.79344961	1			
ZOI	-0.0793986	-0.2545206	1		
DPPH	-0.3675599	-0.4847223	0.56273245	1	
Biological Activity Score	-0.2727566	-0.4331953	0.8510264	0.91298093	1

Figure 2. Correlation table showing the pattern between fructose and glucose to antioxidant, antimicrobial and bioactivity score.

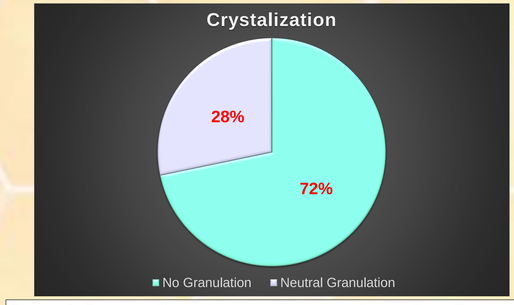


Figure 3. Pie chart illustrating the percentage of crystallized Texan honey samples based on their **glucose-to-water ratios.**

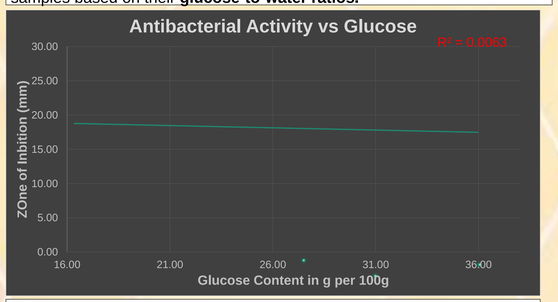


Figure 4. Linear Regression analysis examining the relationship between glucose concentration and antibacterial activity.

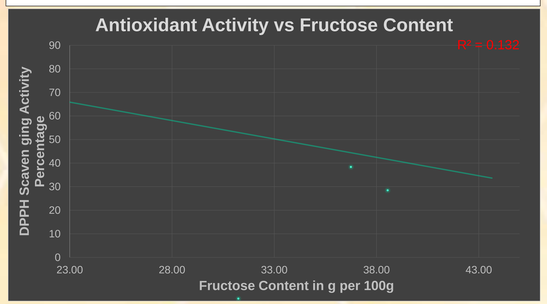


Figure 5. Linear regression analysis evaluating the relationship between fructose concentration and antioxidant activity.

Results

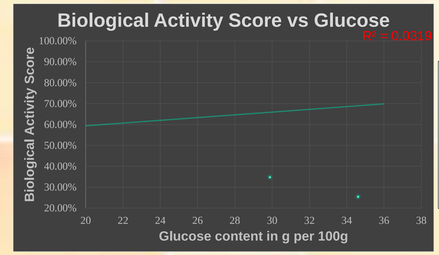
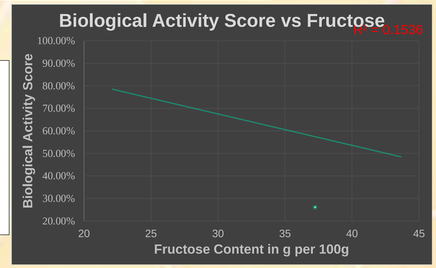


Figure 6. Linear Regression analysis assessing whether glucose content can predict the biological activity score.

Figure 7. Linear Regression assessing the predictive relationship between fructose content and biological activity score.



Conclusions

- Of 39 Texan honey samples, 72% were classified as sweet honeys based on fructose-to-glucose ratios and exhibited resistance to granulation (crystallization).
- No significant correlations were found between sugar content (glucose or fructose) and bioactivity, antimicrobial, or antioxidant properties.
- These findings help producers and consumers select smoother, sweeter Texan honeys and support the development of honey-based supplements and functional foods.
- Future studies will investigate the impact of geographic variation on fructose-to-glucose ratios and assess the glycemic index of Texan honeys.

References

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