

Determining Total Phenolic and Anthocyanin Content in Varieties of *Capsicum annuum* (Hot Pepper) in Guam

Nathan Grant Sala, Stanford University

Mentor: Mari Marutani PhD, University of Guam

Coordinating Center: University of Hawaii at Manoa

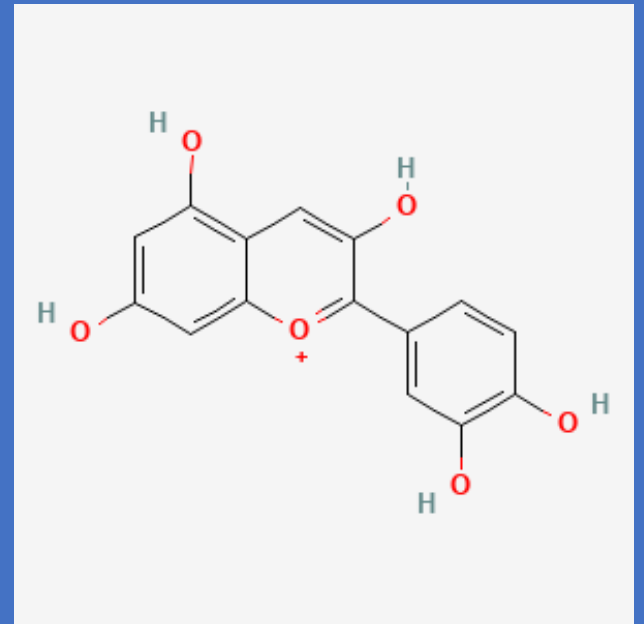


What are Phenolics?

- A type of phytochemical ("plant chemical") that are commonly found in fruits and vegetables.
- Commonly associated with the antioxidant activity of plants.

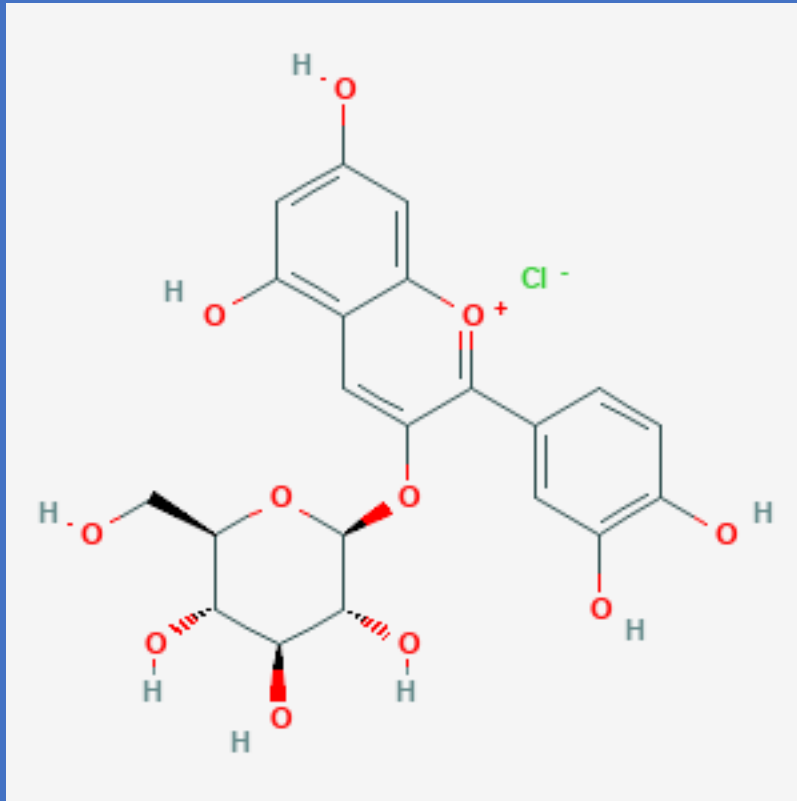
What are Anthocyanins?

- A type of flavonoid (subgroup of phenolics) that commonly present as orange, red, or purple color in plants.
- Possible use as natural food colorant.
- Also associated with antioxidant activity.

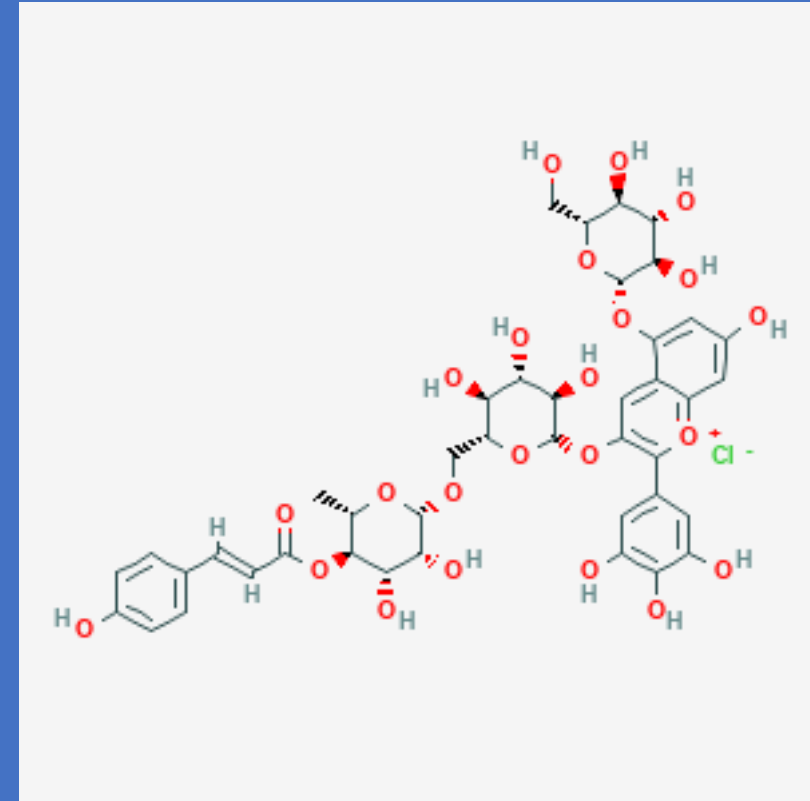


Basic Anthocyanin
Structure

Anthocyanins in *Capsicum annuum*



Chrysanthemins
(Cyanidin-3-glucoside)
Magenta, Orange, Crimson



Nasunins
(Delphinidin-3-p-coumaroylrutinoside-5-glucoside)
Purple

Varieties of Hot Peppers in Guam



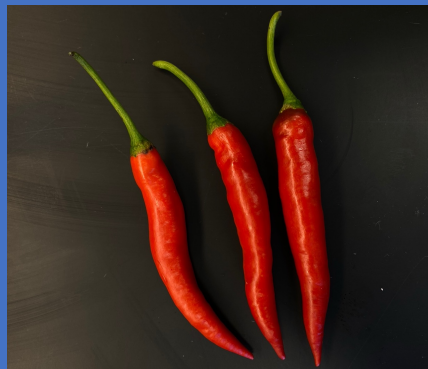
Guafi



Toves



Saipan



Barcinas



Hachon

Research Questions

- How can we numerically characterize differently colored peppers?
- Do different colored peppers have different amounts of phenolic compounds and anthocyanins?

Hypothesis

- **All varieties** of pepper have **comparable** CIELAB L^* , a^* , b^* color space (Lightness, Red/Green, Yellow/Blue) values.
- **All varieties** of pepper have **comparable** concentrations of total phenolics.
- **All varieties** of pepper have **comparable** concentrations of anthocyanins.

Objectives

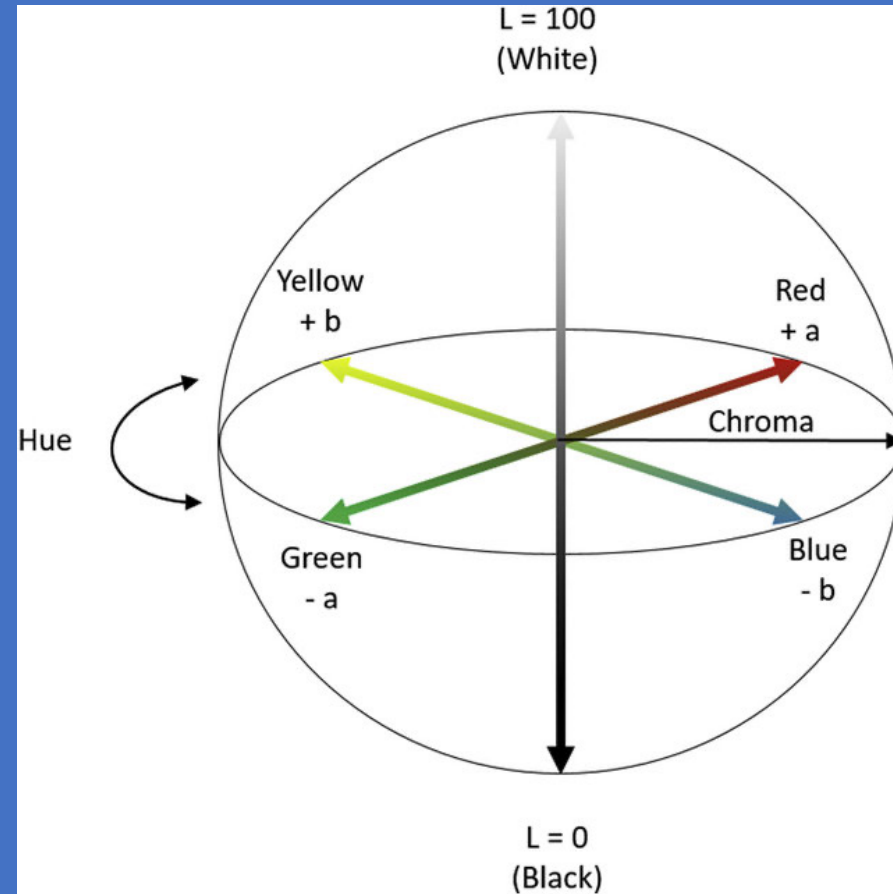
1. To determine **CIELAB Color Space L^* , a^* , b^*** values of locally grown hot peppers from Guam.
2. To determine the concentration of **total phenolics** in locally grown hot peppers from Guam.
3. To identify and quantify the **concentration** of **anthocyanins** present in locally grown hot peppers from Guam.

Methods

Objective 1: Determination of CIELAB Values



Colorimeter



CIELAB Color Space

Methods

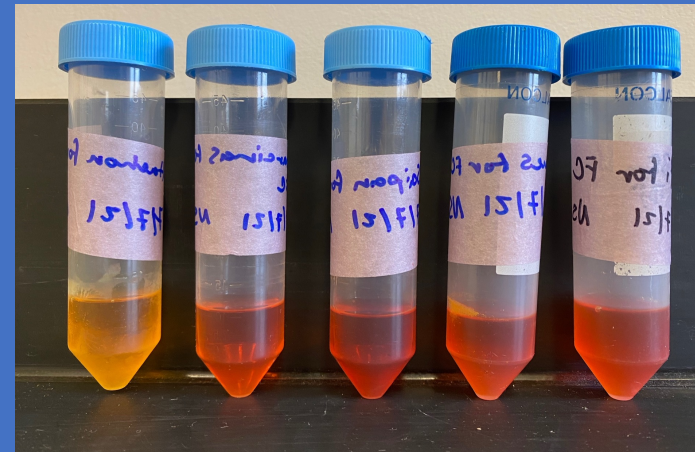
Objectives 2&3: Preparation of Hot Pepper Samples

- Fresh pepper fruits were harvested from the UOG Yigo Research Center.
- Pepper fruits were cleaned, cut into small pieces, and freeze-dried.
- Freeze-dried pepper was ground into powder and stored in the freezer for later use.

Methods

Objective 2: Preparation of Pepper Extract for Phenolics Analysis

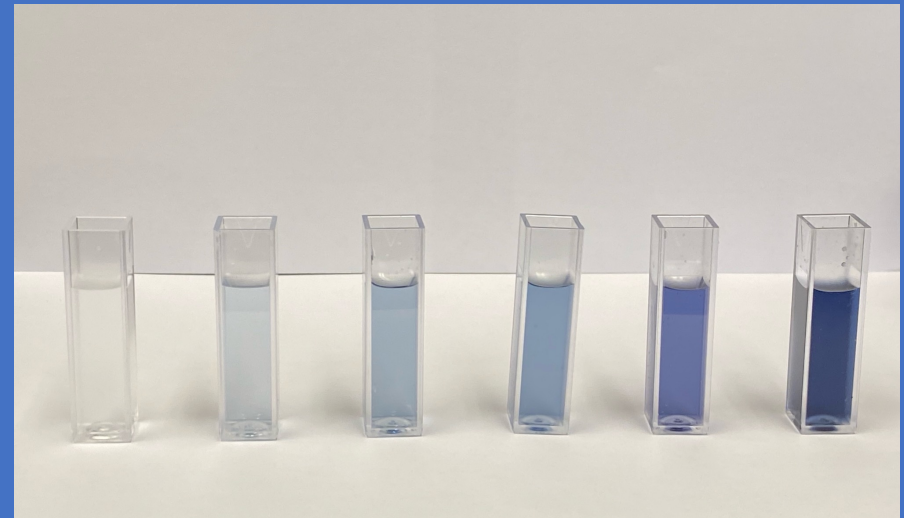
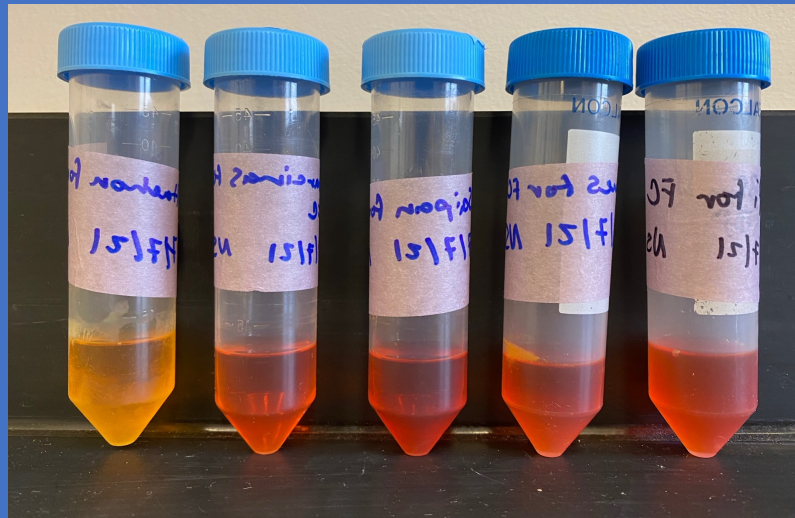
- Pepper powder (2g) was mixed with acidified methanol (20 mL 0.005% HCl) and phenolics extracted by magnetic stirring over 15 minutes.
- Crude extracts were filtered using filter paper and a 0.2 μ m syringe filter.



Methods

Objective 2: Analysis of Total Phenolics

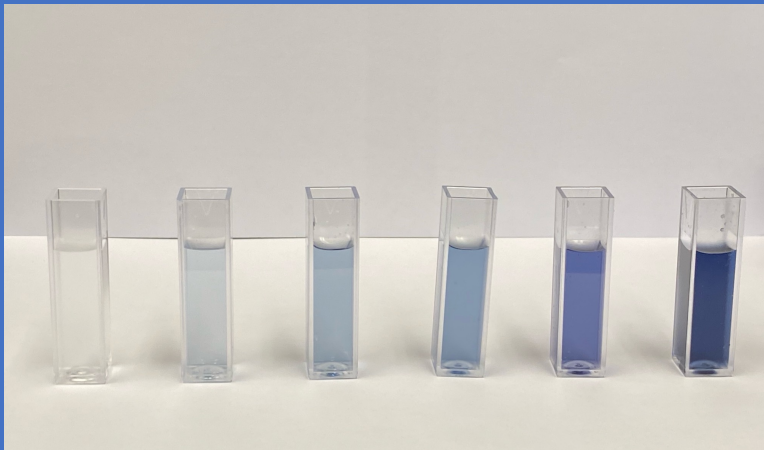
- For each extract prepared from peppers, samples were prepared for Folin-Ciocalteu analysis as described in literature (Arnnok, 2012).



Methods

Objective 2: Analysis of Total Phenolics

- Absorbance of each sample at 745 nm was recorded using UV-Visible spectroscopy.
- Absorbance values of samples were compared to absorbance values of gallic acid standards of known concentration.



Methods

Objective 3: Preparation of Hot Pepper Samples for Anthocyanin Analysis

- Pepper powder (10g) was mixed with acidified methanol (50 mL 2% HCl) and anthocyanins extracted using magnetic stir plate and bar for 15 minutes.
- Crude extracts were filtered using filter paper and a 0.2 μm syringe filter.



Methods

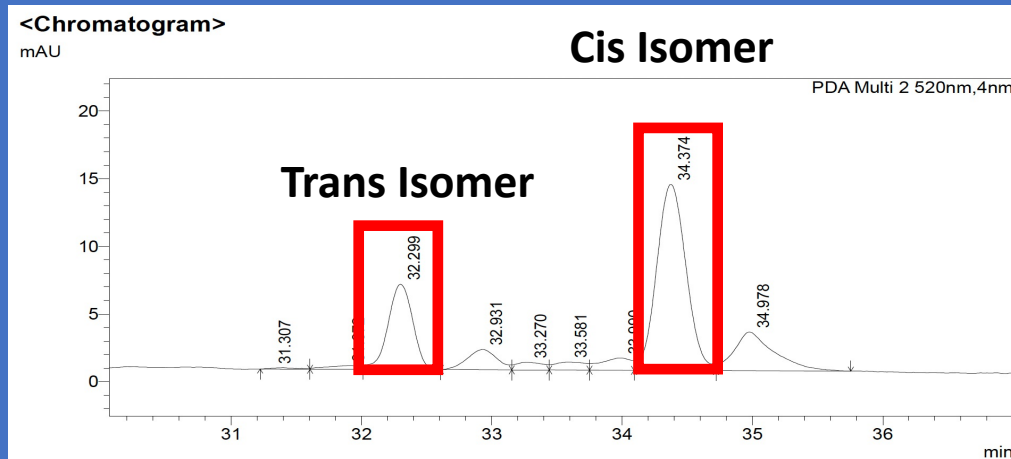
Objective 3: Analysis of Anthocyanins by HPLC

- Extracts were run using High-Performance Liquid Chromatography.
 - Wavelength: 520 nm
 - **Chrysanthemins: Commercial Standard**
 - **Nasunin: Comparison**

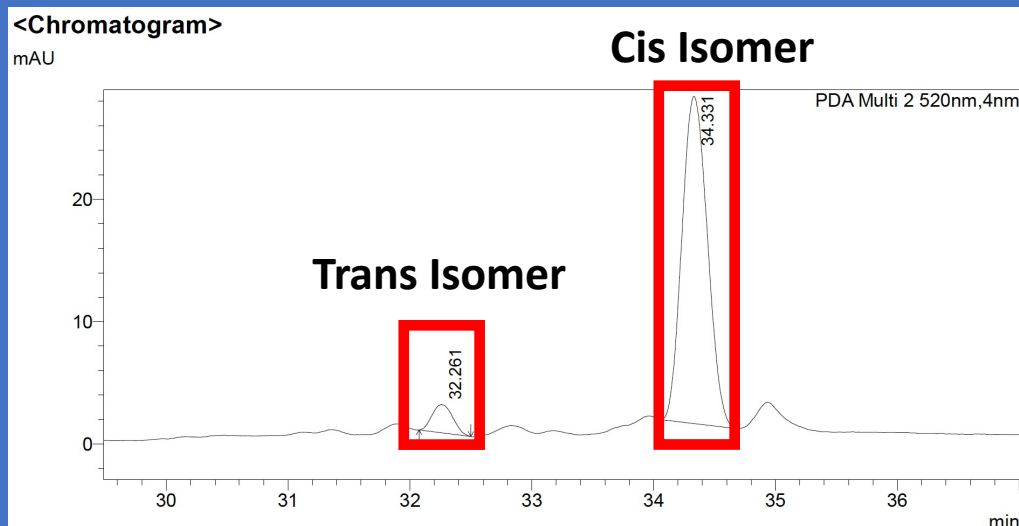


Methods

Objective 4: Identification of Anthocyanins



Presumptive Nasunin
Peaks
(Guam Hot Peppers)



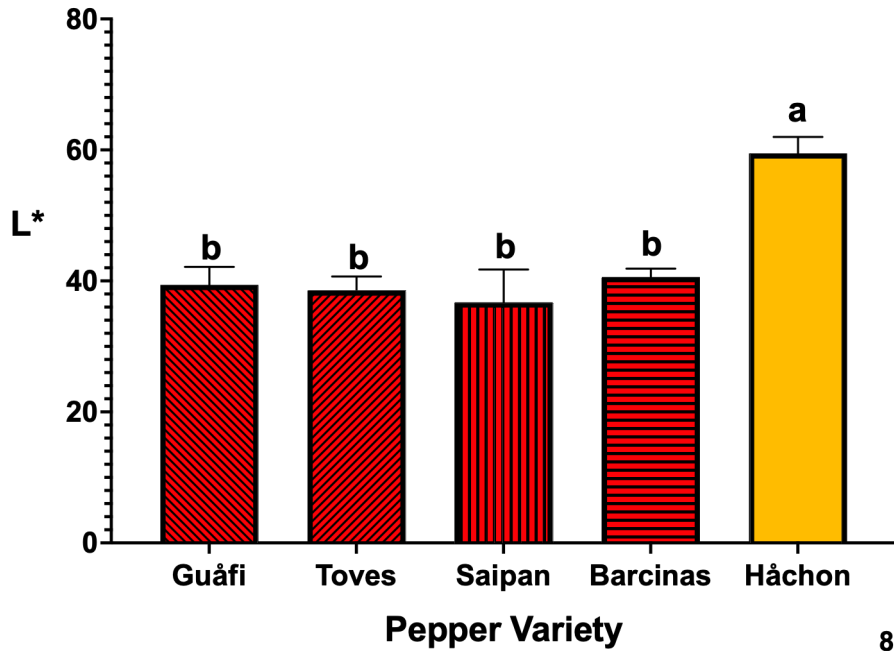
Confirmed Nasunin
Peaks in Arbol Pepper
(Aza-Gonzalez, 2012)

Methods

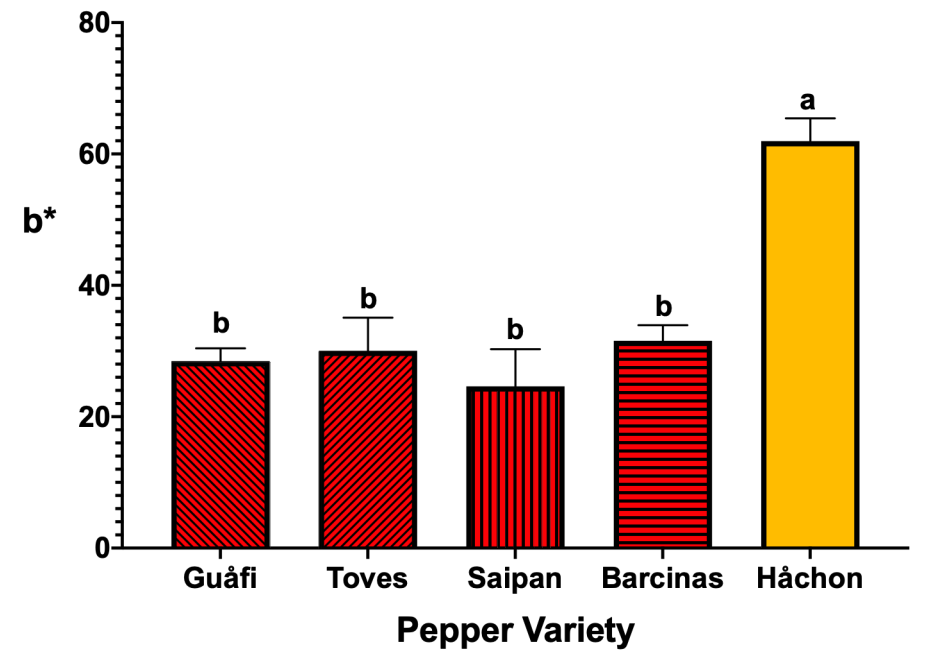
Data Analysis

- CIELab (L*a*b*): Five (5) Readings per pepper
- Phenolics and Anthocyanins: Three (3) Trials per pepper
- One-Way Analysis of Variance (ANOVA) and Tukey's Multiple Comparison tests performed on data collected.
- Graphs constructed using GraphPad Prism.
- 3-D Scatterplot constructed using Python (Matplotlib)

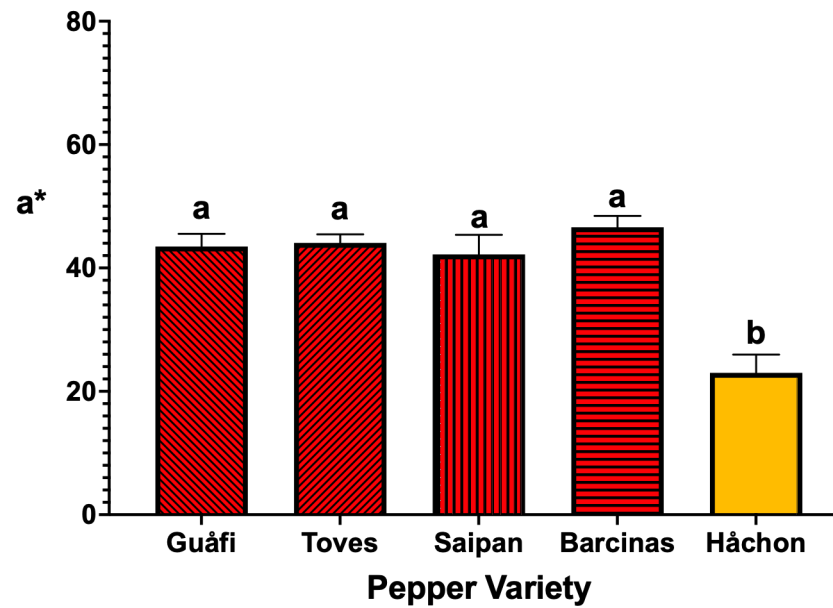
L*
Lightness



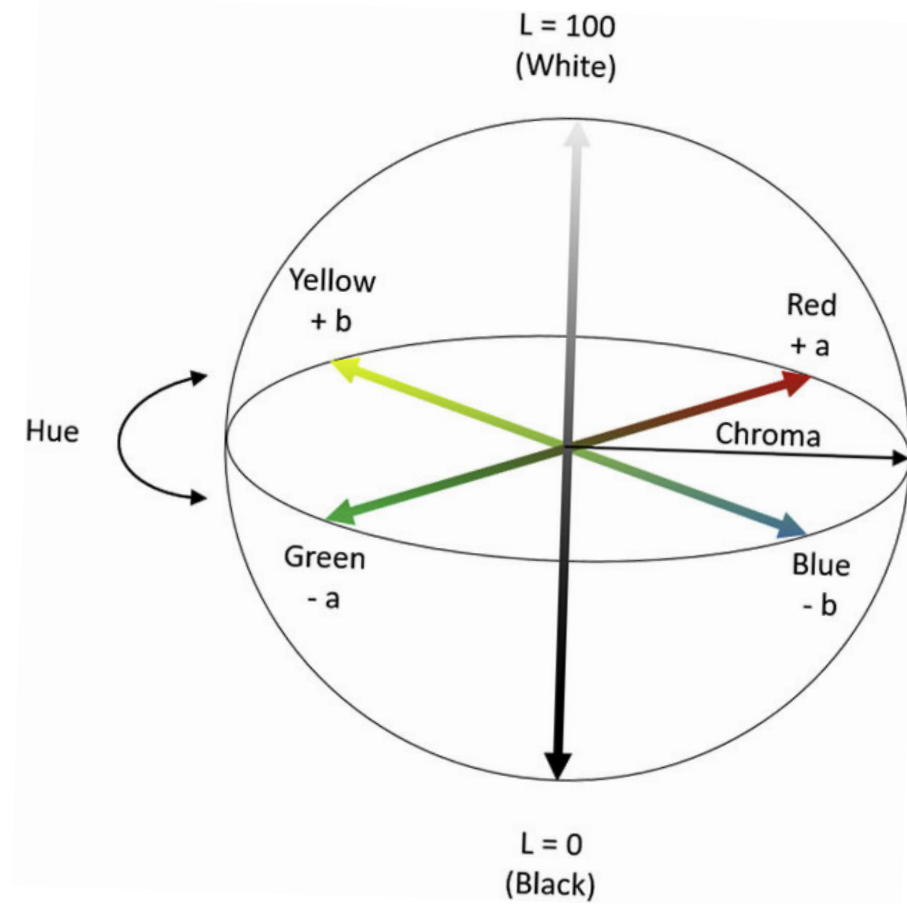
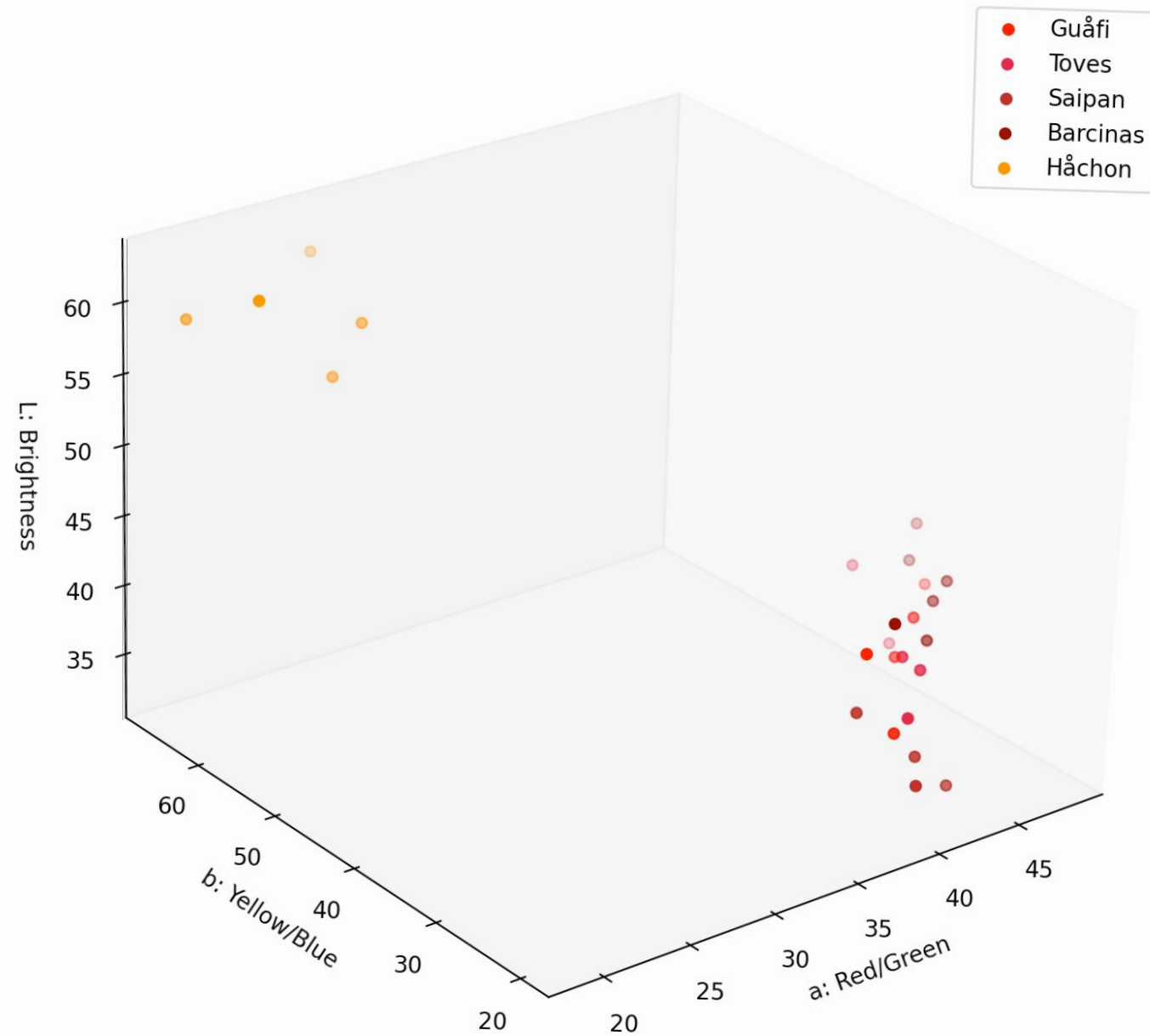
b*
Yellow/Blue



a*
Red/Green

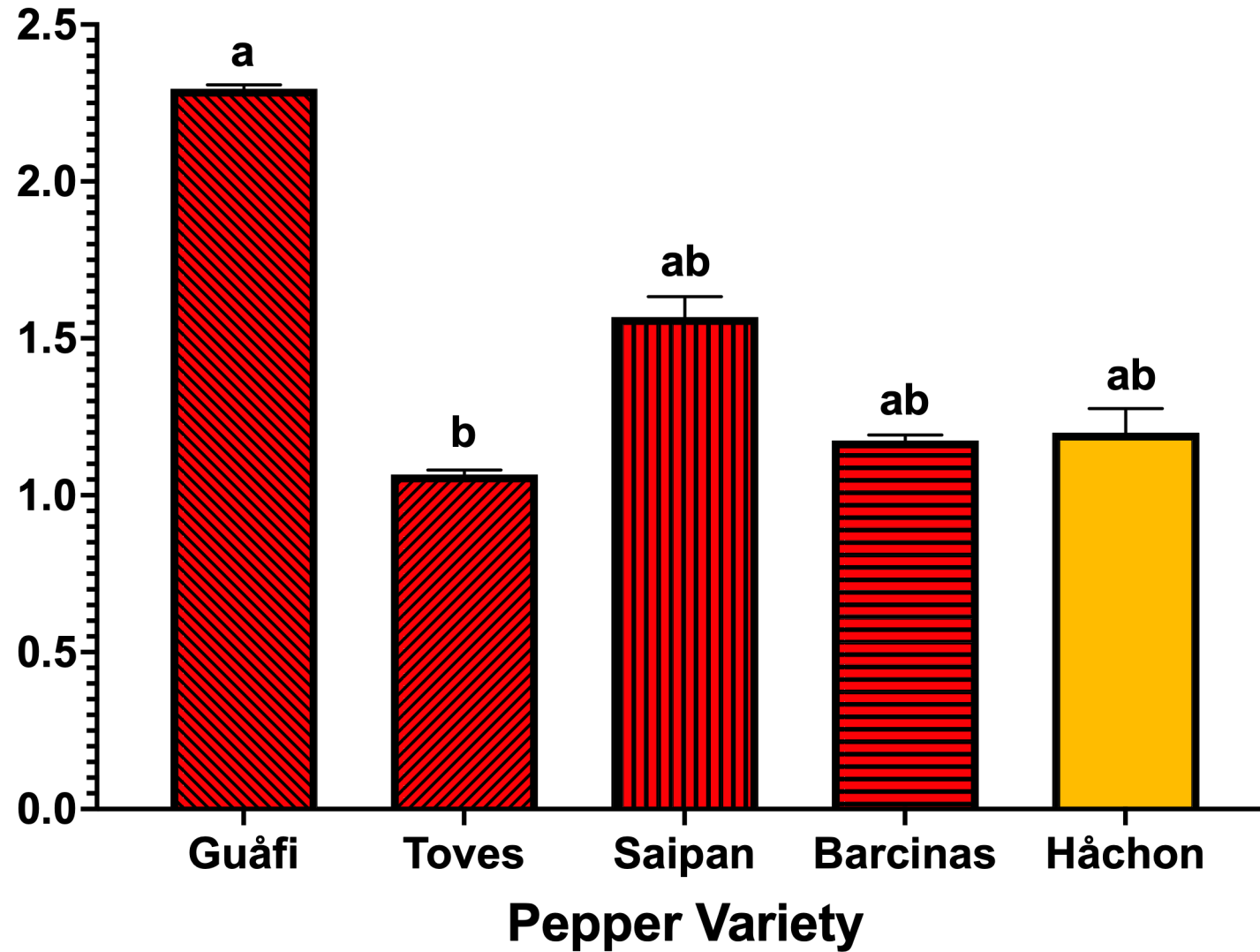


Guam Hot Peppers

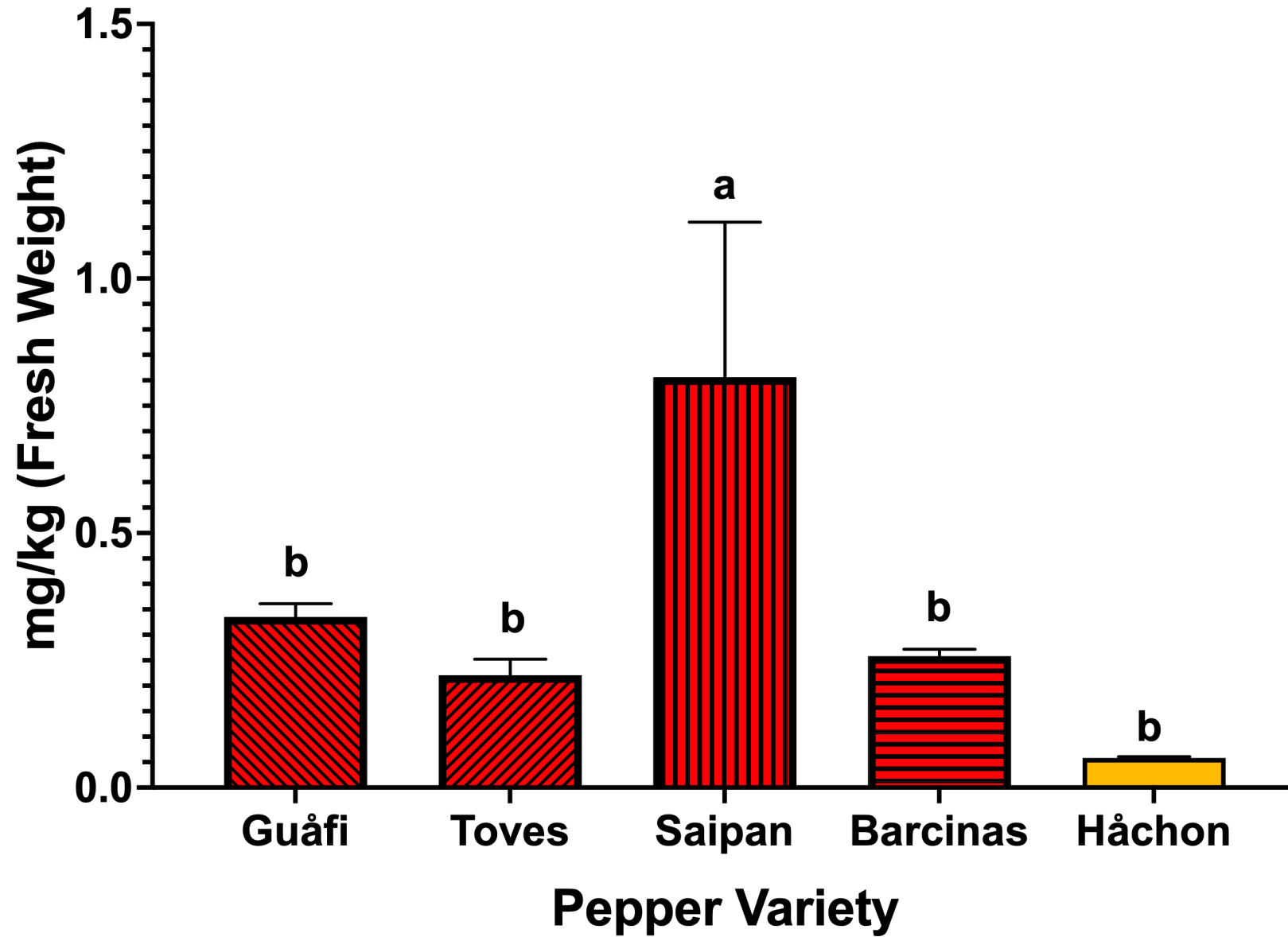


mg Gallic Acid Equivalents/g (Fresh Weight)

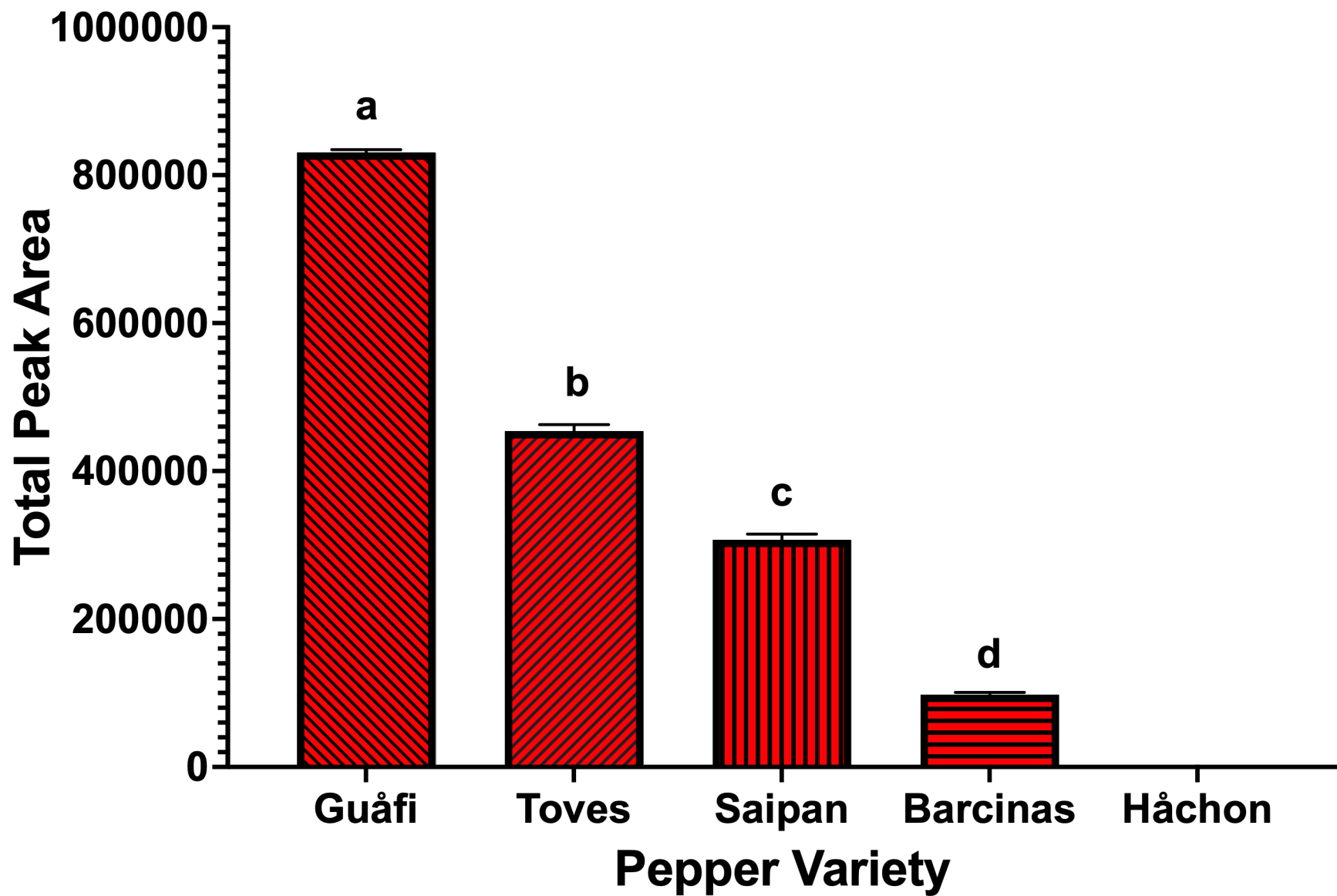
Total Phenolics



Anthocyanins: Chrysanthemin



Anthocyanins: Nasunin



Discussion – Objective 1

- L^* , a^* , b^* values were similar for red-colored varieties of peppers from Guam.
- L^* , a^* , b^* values of yellow-colored Håchon differed from red-colored varieties of peppers.

Discussion – Objective 2

- Total phenolics concentrations of hot peppers from Guam were within the range reported in literature (Arnnok, 2012).
 - **Literature:** 0.782– 4.516 g/kg (Fresh Weight)
 - **Guam Peppers:** 1.074 – 2.308 g/kg (Fresh Weight)

Discussion – Objective 3

- Chrysanthemin concentrations of hot peppers from Guam were overall lower than reported in literature (Arnnok, 2012).
 - **Literature:** 0.795 – 2.78 mg/kg (Fresh Weight)
 - **Guam Peppers:** 0.056 – 1.134 mg/kg (Fresh Weight)
- Nasunin identified in all red-colored varieties.
 - Not detected in Håchon.

Future Study

- Quantification of Nasunin concentration in local varieties of hot pepper.
- Determining the concentration of other phytochemicals in local varieties of hot pepper.
- Determining the antioxidant activity of local varieties of hot pepper by DPPH and TEAC assays.

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Questions?

Thank you!