HONEY TESTING

Saraswatie Sankar[1], Vernetta Lewis[2], Riaz Hosein[2], Faisal Mohammed[2], Terry Mohammed[2], Azad Mohammed[1], Hayden Sinanan[3]

[1] Department of Life Sciences, Faculty of Science and Technology, The University of the West Indies St. Augustine, Trinidad and Tobago
[2] Department of Chemistry, Faculty of Science and Technology, The University of the West Indies St. Augustine, Trinidad and Tobago
[3] Ministry of Agriculture, Land and Fisheries, Trinidad and Tobago

Defining Honey

Honey is the natural sweet substance produced by honeybees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects. [1]

Blossom honey is produced from the nectar of plants and includes multifloral or unifloral types.

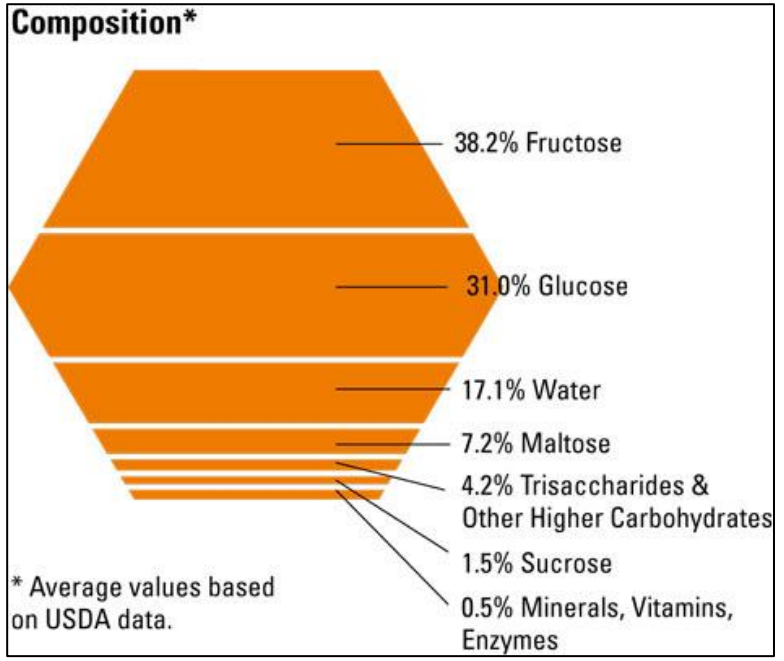
Honeydew honey is produced from other excretions from plants, caused by excretions of plant-sucking insects.

Source: bugguide.net

Source: beekeepinginsider.com

Honey content and properties

- different sugars, predominantly fructose and glucose
- water
- carbohydrates
- organic acids
- minerals, vitamins, enzymes
- solid particles derived from honey collection



Source: Howland Blackston

- Nearly colorless to dark brown colours
- Fluid, viscous or partly to entirely crystallized consistencies
- Varying flavor and aroma derived from plant origin

Source: kenyonbee.com

Dearth of research on honey in Trinidad and Tobago

- “No scientific research done to examine the specific qualities of and composition of honey and its relation to the foraging environment in this region.”[2]

- “….the lack of quality and standard control. To date, there is neither an Apiaries Unit nor a testing honey laboratory available to beekeepers in Trinidad and Tobago.” [3]

Therefore, on-going research can assist with :

- classifying types of honey based on specific properties
- creating local standards and assessing quality
- contributing to marketing and branding of honey

Objectives

- **To determine pollen content of honey samples from local apiaries throughout Trinidad and Tobago.**

- **To determine the following :**

Physicochemical properties	Antioxidant properties
Optical Density	Total Phenolic Content (TPC)
Moisture	Total Flavonoid Content (TFC)
pH	Total Antioxidant Content (TAC)
Specific Rotation	DPPH free radical scavenging activity
Electrical Conductivity	Ferric Reducing/Antioxidant Power (FRAP) assay

- **To compare the aforementioned properties with international standards to establish acceptable levels for consumer use.**

Investigating the Pollen Composition of Trinidad and Tobago Honey

Presenter: SARASWATIE SANKAR

M. Phil candidate Environmental Biology

Pollen use and storage

- Pollen is a main source of protein which also provides fats, lipids, minerals, and vitamins

- Pollen is collected by worker bees, combined with nectar and stored in honeycomb cells as bee bread

- Pollen in the nectar collected by the honeybee is regurgitated together and deposited into the same honeycomb cell [4]

Source: thepopulistfarmer. com

Source: Beekeepersgram

Pollen is combined into honey through various ways

- Pollen can fall into the open cells when a honeybee grooms herself

- Airborne pollen from plants not visited by honeybees can enter the hive on air currents and fall into the open cells

- Pollen can fall onto the honeycomb frames when being removed by the beekeeper [5]

Source: honeybeesuite.com

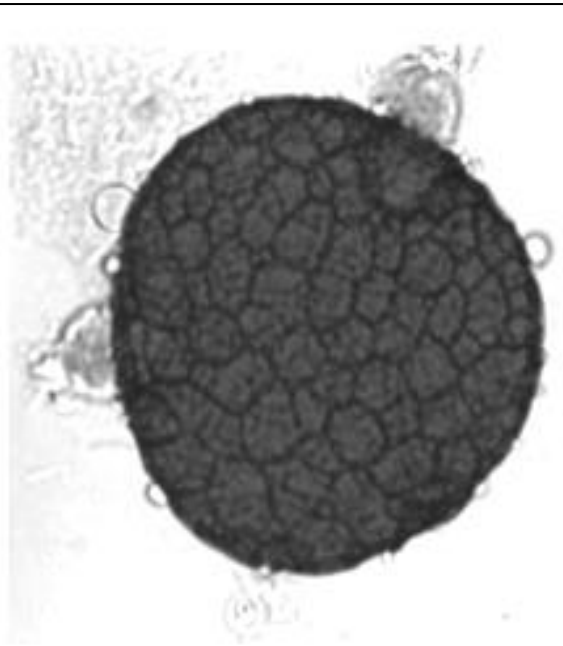
Source: Aliexpress.com

Pollen analysis determines the Botanical and Geographical origin of honey

- Melissopalynology is the analysis of the pollen contents of honey and pollen loads of an area
- The method proposed by the International Commission for Bee Botany (ICBB) is a well-established method in most European and American laboratories involved in routine honey analyses to determine nectar and pollen sources [6]
- Pollen research helps to establish the nature and quality of the hone
- It will also support the creation of a local pollen atlas for assisting beekeepers with identifying bee forage plants.

Characteristics of pollen used for identification

- Dispersal unit
- Aperture number and type
- Ornamentation
- Tectum
- Shape



Dispersal unit	Monad	Polyad
Aperture number	4	0
Aperture type	Multi-aperturate	Inaperturate
Ornamentation	Verrucate	Psilate
Tectum	Semi-tectate	Tectate
Shape	Spheroidal	Sub-prolate

Forage plant sample collection and processing

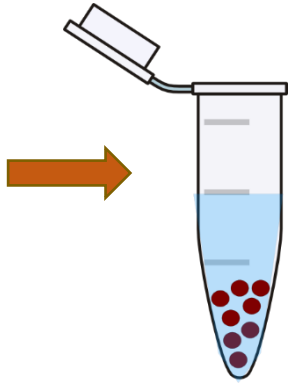
Collection of plant specimens by RBS

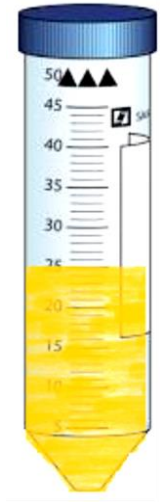
Pressing and Identification

Preparing voucher specimens

Source: University of Florida Herbarium

Unacetolysed pollen extraction from flowers and honey



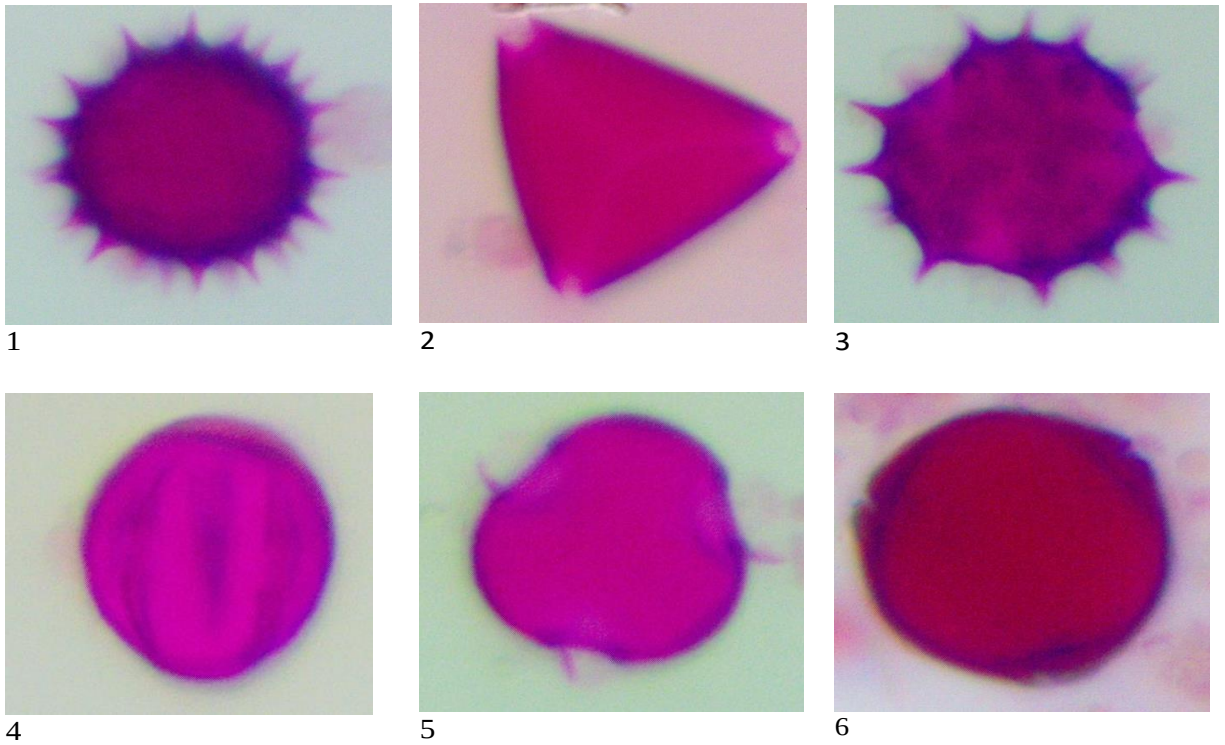


Centrifuge
3000/8000 rpm
for 5 minutes
Wash (x2)
distilled water

Discard
supernatant
Retain Pellet for
Slide preparation

Pollen identification

- 5 pollen grains from the forage plants were photographed and characteristics for identification were recorded
- 300 pollen grains from the honey sample were counted, numbered and photographed per slide at suitable magnification (x400 to x1000)
- pollen characteristics were listed in excel for grouping into pollen types which were identified using the forage plant pollen and international pollen atlases and databases such as pollenatlas.net and paldat.org



Photographs of selected pollen grains recovered from the honey samples.

1. Asteraceae type 15 µm
2. Unidentified 19 µm
3. Asteraceae type 18 µm
4. Combretaceae type 12 µm
5. Oxalidaceae type 14 µm
6. Rubiaceae type 17 µm

Pollen frequency and results

- $\boldsymbol{Relative\ frequency}$
- $= \frac{\boldsymbol{P\ (pollen\ type)}}{\boldsymbol{TP(total\ number\ pollen\ grains\ per\ slide)}} \times \mathbf{100}$

- Pollen predominantly from a given botanical origin produces unifloral honey if the relative frequency of the pollen of one *taxon* exceeds 45%.

- If no one species makes up 45% pollen, the honey is considered to be multifloral.[28]

- Both samples were classified as multi-floral as the relative frequency of none of the taxa exceeded 45%.

Plant family	Plant common names	Honey sample no.	
		H1 (North)	H2 (South)
Anacardiaceae	Mango, Hogplum, Cashew	14.33	20.33
Arecaceae	Coconut, Alexander and Royal Palms	2.67	12.67
Asteraceae	Daisy, Marigold, Zebapique	14.33	4.67
Calophyllaceae	Mammey apple	0	2.67
Combretaceae	Almond, Oliviere	5.00	8.33
Cucurbitaceae	Jingay, Cucumbers, Watermelon	9.00	4.00
Fabaceae	Immortelle, Mora, Robble, Peas	12.00	7.67
Lauraceae	Avocado, Bay, Cinnamon	6.33	11.67
Oxalidaceae	Fivefinger, Bilimbi	4.00	14.00
Rubiaceae	Coffee, Juniper	10.67	4.67
Unknown	-	21.67	9.33

Investigating the Antioxidant Properties of Trinidad and Tobago Honey

Presenter: VERNETTA LEWIS
M. Phil candidate Analytical Chemistry

Defining Antioxidants

- A substance that inhibits oxidation or reactions promoted by oxygen, peroxides or free radicals.[7]
- Elevated free radicals and reactive oxygen species (ROS) can cause damage to lipids, proteins, and nucleic acids leading to carcinogenesis, mutagenesis, aging and atherosclerosis.[8]
- Antioxidants intercept these free radicals before they can cause damage.[8]
  - Phenolic acids
  - Flavonoids
- The antioxidant properties of honey is greatly influenced by its geographical and botanical origin.[9]

Description of methods

Total Phenolic Content

- **Secondary metabolite in plants[10]**
- **Responsible for sensorial properties[10]**
- **Lowers the incidence of cancer, diabetes, Alzheimer's disease[11]**
- **Subdivided into 16 major classes[10]**

Total Flavonoid Content

- **Most abundant phenolic compounds in nature[10]**
- **Protection , coloration of flowers[12]**
- **Reduces risk of diabetes, cancer and cardiovascular diseases[13]**
- **Subdivided into 6 classes[14]**

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity

- **Tests the ability of the compounds to act as free radical scavengers or hydrogen donors[15]**
- **High DPPH free radical scavenging activity confers superiority antioxidant activity of the sample**

Ferric reducing/antioxidant power assay (FRAP)

- **A simple and direct test to determine antioxidant activity**
- **Reduces ferric (Fe^{III}) to ferrous (Fe^{II})[16]**

Sample collection, identification, storage

1) Sample Collection

- Collect pure honey samples directly from hive

2) Sample Identification:

- Date of extraction
- Apiary number
- Geographic Location/Address

3) Sample Storage

- Store at room temperature in dry area away from direct sunlight, until analysis

Methods and materials used for Antioxidant testing

ANTIOXIDANT TEST	SUMMARY OF METHOD
	❖ Honey sample dissolved in Methanol
Total Phenolic Content [17]	• Addition of Folin's reagent and Na_2CO_3 to filtered sample • Sample incubated at room temperature for 2 hours • Absorbance recorded at 765 nm
Total Flavonoid Content [9]	• Addition of 2% w/v $AlCl_3$ in Methanol • Sample incubated at room temperature for 10 minutes • Absorbance recorded at 415 nm
Total Antioxidant Capacity [17]	• Addition of de-ionized water and reagent solution (sulphuric acid, ammonium molybdate, sodium phosphate) • Sample incubated for 90 minutes at 95^{o}C • Absorbance recorded at 695 nm.
DPPH Free radical-scavenging activity [9]	• Addition of Tris-HCl and DPPH • Sample incubated at room temperature for 2 hours in the dark • Absorbance recorded at 517 nm.
Ferric Reducing /Antioxidant Power Assay [16]	• Addition of FRAP reagent to honey solution sample • Sample incubated for 4 minutes in a water bath at 37^{o}C • Absorbance recorded at 593 nm.

Comparison of TPC, TFC and TAC of T&T honey samples with other regions

LOCATION	FLORAL CLASSIFICATION	TPC/(mg GAE/100 g)	TFC/(mg QE/100 g)	TAC/(mg AAE/g)
Trinidad	**Multifloral**	**55.30 ± 29.62**	**3.04 ± 2.02**	**24.56 ± 5.62**
Slovenia[19]	**Multifloral**	**15.73 ± 2.09**	_____	_____
Poland[23]	**Multifloral**	**19.03 ± 4.60**	_____	_____
Burkina Faso[9]	Multifloral	70.68 ± 16.76	2.79 ± 2.24	23.31 ± 7.96
Burkina Faso[9]	Unifloral	72.59 ± 21.62	1.96 ± 1.98	36.53 ± 24.57
Turkey[17]	Unifloral	48.63 ± 37.71	_____	33.55 ± 11.89
Malaysia[18]	Unifloral	83.96 ± 4.53	50.45 ± 1.83	53.06 ± 0.41
Algeria[20]	Unifloral	45.98 ± 0.19	5.42 ± 0.06	27.82 ± 0.43
Malaysia[21]	Unifloral	5.26 ± 0.12	3.46 ± 0.05	_____
Kenya[22]	Unifloral	66.72 ± 51.60	29.19 ±12.19	_____
Algeria[20]	Unifloral	45.98 ± 0.19	5.42 ± 0.06	27.82 ± 0.43

Profiling of the physicochemical properties of Trinidad and Tobago honey

Presenter: RIAZ HOSEIN

M. Phil candidate Analytical Chemistry

Importance of Physicochemical Properties

- Honey quality is mainly determined by its sensory, physicochemical and microbiological characteristics.
- Physicochemical testing is conducted internationally on honey to discern its quality and to identify the adulteration of honey.
- These properties include moisture, pH, electrical conductivity etc.

Physicochemical properties investigated

Moisture	→	Determines fermentation
pH	→	Delays growth of micro bacteria
Electrical Conductivity	→	Relates to the concentration of mineral salts
Specific Rotation	→	Differentiates between blossom and honeydew honey
Optical Density	→	Classifies honey colour

Summary Of Methods[26]

Property	Equipment	Description
		• Honey samples were diluted with distilled water
pH		• pH was recorded using a calibrated pH meter
EC		• Electrical Conductivity was recorded using a calibrated Conductivity meter
Specific Rotation		• Carrez I and II solutions were added • Subjected to angular rotation measurements
Optical Density		• Optical Density was recorded using a spectrophotometer at 560 nm
Moisture		• Homogenized sample was heated briefly in a water bath at 50^{o}C • Moisture content was recorded using a refractometer

Comparison of Physicochemical Properties Alongside Other Countries

Property	Trinidad	Palestine[5]	Italy[25]	Portugal[24]	Codex Standard
pH	3.77	3.92	4.0	3.67	3.4 – 6.1
EC/mS	0.20	0.40	N.A	0.37	< 0.8
Moisture/%	19.4	18.46	18.1	16.03	< 20
Specific Rotation	-19.13	N.A	N.A	-14.3	(-) value – blossom honey (+) value – honeydew honey
Optical Density	0.33	0.13	N.A	N.A	N.A

Colour Names and Optical Density *: Water white- 0.0945 ,* ***Extra white – 0.189*** *,* ***White – 0.378*** *, Extra Light Amber – 0.595 , Light Amber – 1.389 , Amber - 3.008*

Summary

- Pollen types belonging to 10 families were identified from 2 honey samples which were classified as multifloral.
- Antioxidant properties of T&T honey were comparable to honeys from other regions.
- Differences in antioxidant properties attributed to different botanical origins and variations in feeding patterns of the bees between T&T honey and others
- Physicochemical parameters observed for local samples were within range established in the Codex Standard for honey.

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