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### Evaluation of the Physicochemical Properties of Selected Honey Types Produced in Southern Egypt and Their Compliance with International Quality Standards

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#### **Abstract**

This study provides a comprehensive physicochemical characterization of monofloral honey from Upper Egypt, specifically from the Assiut and Qena Governorates, during the 2022 and 2023 seasons. Five distinct botanical origins, Anise, Fennel, Clover, Sesame, and Sidr were evaluated. The analysis revealed statistically significant variations (p < 0.05) across all measured parameters, predominantly influenced by the floral source. For instance, Fennel honey exhibited the highest fructose content, suggesting slow crystallization, while Clover honey's high glucose levels indicated a potential for rapid crystallization. The geographical origin also proved to be a significant factor. This was particularly evident in key freshness indicators, such as hydroxymethylfurfural (HMF) and diastase activity. Sesame honey from Assiut demonstrated superior freshness with the lowest HMF (14.98 mg/kg) and highest diastase activity (30.00 DN), whereas Clover honey from the same region showed elevated HMF, suggesting potential thermal stress. Furthermore, a direct comparison of the same floral type between governorates revealed marked differences, underscoring the impact of local conditions. Other parameters, including electrical conductivity, viscosity, and granulation ratio, also showed significant variations linked to both botanical and geographical origins. This study conclusively demonstrates that both floral source and geographical location are critical, interdependent determinants of the physicochemical properties, quality, and storage potential of monofloral honey from Upper Egypt.

**Keywords:** Monofloral honey, Physicochemical properties, Botanical origin, Geographical discrimination, HMF, Diastase activity, Assiut, Qena.

#### INTRODUCTION

Honey, a natural sweet substance produced by honey bees (Apis mellifera) from the nectar of plants, is a complex biological mixture renowned for its nutritional and therapeutic value. Its unique composition, which varies significantly based on botanical origin, geographical location, and climatic conditions, dictates its overall quality, stability, and authenticity. The evaluation of honey quality primarily relies on the assessment of its welldefined physicochemical properties, which are crucial for determining sensory attributes, ensuring compliance with international standards (e.g., Codex Alimentarius), and detecting adulteration. This study aims to contribute to this by analyzing the physicochemical parameters of honey samples and determining their conformity with established quality benchmarks.

Physical Properties: The physical attributes of honey provide initial indicators of its floral origin and processing. These include color, electrical conductivity (EC), total soluble solids (TSS), specific gravity, viscosity, and granulation. Color varies extensively from light to dark amber and serves as a primary indicator of the floral source (Adenekan *et al.*, 2010; Alqarni *et al.*, 2014).

Electrical Conductivity (EC) is a key indicator of mineral content and botanical origin. Significant geographical variation exists, from low values (0.14–0.64 mS/cm) in samples from Nigeria and Argentina (Adenekan *et al.*, 2010; Aloisi, 2010) to much higher values (up to 6.33 mS/cm) in Pakistani honeys (Khaliqur *et al.*, 2013).

**Total Soluble Solids** (TSS%), primarily representing sugar content, is typically high in pure honey, averaging around 81% (Ndife *et al.*, 2014), and is an indicator of maturity.

**Granulation** (Crystallization) is a natural process influenced predominantly by the glucose-to-water ratio and storage temperature. Cool storage can reduce granulation, while room temperature promotes it (El-Metwally, 2015). The fructose-to-glucose ratio is a critical predictor of crystallization potential (Bogoviku and Gedeshi, 2015).

Chemical Properties: The chemical composition defines the nutritional value, shelf-life, and authenticity of honey. Key parameters include moisture content, sugar profile, acidity, and hydroxymethylfurfural (HMF) content.

**Moisture Content** is vital for preventing fermentation and determines stability. It exhibits considerable variation but must typically be below 20% to avoid spoilage. Studies report ranges of 14.6–22.1% in Nigerian samples (Adenekan *et al.*, 2010) and 17.5–19.25% in Egyptian clover honey (Essa *et al.*, 2010).

**Sugar Profile:** Honey is predominantly composed of reducing sugars, mainly fructose and glucose. A low sucrose content (<5%) is a key indicator of maturity and lack of adulteration (Fatehe, 2013).

Acidity and pH: Honey is naturally acidic, which contributes to its microbiological stability. pH values commonly range between 3.2 and 4.9 (Aloisi, 2010; Shahnawaz *et al.*, 2013), while free acidity can vary widely based on origin (Aloisi, 2010).

Hvdroxymethylfurfural (HMF) is a critical marker of freshness and proper storage; low levels indicate fresh, well-handled honey, while high levels suggest overheating or prolonged storage. HMF values in literature show a wide global diversity, from nearly undetectable to over 60 mg/kg (Aloisi, 2010; Lawal et al., 2017). The physicochemical properties of honey are indispensable tools for assessing its quality and origin. The wide variation in these parameters, as documented in studies from around the world, underscores the importance of localized and precise analysis. This research will contribute to the existing body of knowledge by providing comprehensive data on honey samples, aiding in the establishment of quality and authenticity benchmarks.

### The primary objectives this research aimed to accomplish are:

- 1. To evaluate the physical properties of honey samples, including color, viscosity, electrical conductivity, and moisture content.
- 2. To analyze the chemical properties of honey samples, focusing on sugar content (sucrose, fructose, glucose), acidity, pH level, and Hydroxymethylfurfural (HMF) content.

- 3. To determine the compliance of the analyzed honey with the quality standards set by both the Codex Alimentarius and Egyptian specifications.
- 4. To compare the quality of honey produced in different governorates of Upper Egypt (such as Assiut and Qena) based on the evaluated physical and chemical properties.

#### MATERIALS AND METHODS

#### 1. Study Areas and Samples Collection:

A total of twenty-one (21) honey samples were collected during the 2022/2023 honey flow seasons (spring, summer, and autumn) from apiaries maintained by professional beekeepers in two governorates of Upper Egypt: Assiut and Qena.

# The samples represented five distinct botanical origins:

- **1. Anise Honey** (*Pimpinella anisum*): Three samples were collected from Abnoub District. Assiut.
- **2. Fennel Honey** (*Foeniculum vulgare*): Three samples were collected from Assiut and three from Oena.
- **3. Clover Honey** (*Trifolium alexandrinum*): Three samples from Assiut and 3 from Oena.
- **4. Sesame Honey** (*Sesamum indicum*): Three samples from El-Qusiya district, Assiut.
- **5. Sidr Honey** (*Ziziphus spina-christi*): Three samples were collected from Abu Tesht District, Qena.

All samples were transported to the laboratory in tightly sealed plastic containers and stored for seven days at approximately 10°C until analysis.

#### 2. Physicochemical Analysis:

The honey samples were analyzed for a range of physical and chemical properties to determine their quality and authenticity. All analyses were conducted at the laboratories of the Bee Research Department, Plant Protection Research Institute, Dokki, Egypt.

#### 2.1. Physical Properties:

Electrical Conductivity (EC): Measured at room temperature using a conductivity meter

after dissolving 2 g of honey in 10 mL of distilled water. Results were expressed in millisiemens per centimeter (mS/cm) Adenekan, *et al.* (2010).

**Viscosity:** Determined at 29°C using a viscometer **Munro** (1943).

**Moisture Content:** Assessed by measuring the refractive index with a refractometer. Values were converted to percentage moisture using standard tables White (1962).

**Granulation Tendency:** Estimated by calculating the glucose-to-fructose (G/F) ratio

#### 2.2. Chemical Properties: •

**pH Value:** Measured directly using a calibrated pH meter Cunniff (Ed.) (1995).

Acidity (Free, Lactone, and Total): Determined by titration with sodium hydroxide and expressed in milliequivalents per kilogram (meq/kg) Bogdanov, *et al.* (2002)

**Sugar Profile:** The concentrations of fructose, glucose, sucrose, and maltose were quantified using High-Performance Liquid Chromatography (HPLC). The fructose-to-glucose (F/G) ratio was subsequently calculated Cunniff (Ed.) (1995).

**Hydroxymethylfurfural** (HMF) Content: Determined spectrophotometrically using the White method after clarification with Carrez reagents. Absorbance was read at 284 nm and 336 nm Cunniff (Ed.) (1995).

**Diastase Activity (Diastase Number):** Assessed using the spectrophotometric method and expressed in Gothe units per gram of honey. International Honey Commission (2009)

#### 3. Statistical and Compliance Analysis:

The obtained results for each parameter were statistically analyzed and compared against the international quality standards set by the Codex Alimentarius and relevant Egyptian specifications to determine compliance.

#### **RESULT**

### 1. Overview of Honey Samples and Statistical Significance:

The physicochemical properties of four honey types (Anise, Fennel, Clover, and Sesame) collected from Assiut Governorate showed significant differences (p < 0.05) across all measured parameters:

**Moisture Content:** Fennel honey recorded the lowest moisture (16.50%), while Anise showed the highest (18.50%).

**HMF** (Hydroxymethylfurfural): Sesame honey had the lowest HMF value (14.98 mg/kg), indicating freshness, whereas Clover had the highest (34.75 mg/kg).

**Electrical Conductivity (EC):** Sesame honey showed the highest EC (0.46 mS/cm), suggesting higher mineral content, while Clover and Anise had the lowest (0.17 mS/cm).

**pH:** Sesame honey was the most acidic (pH 4.80), while Clover was the least (pH 4.10).

Free Acidity: Sesame honey had the highest free acidity (35.00 meq/kg), followed by Anise (31.00 meq/kg), while Fennel and Clover had the lowest.

**Lactone Acidity:** Clover had significantly higher lactone content (7.50 meq/kg), while Anise had the lowest (1.00 meq/kg).

**Total Acidity:** Sesame showed the highest total acidity (37.50 meq/kg), and Fennel had the lowest (29.50 meq/kg).

Fructose Content: Fennel honey was richest in fructose (42.15%), while Sesame had the lowest (39.20%).

Glucose Content: Clover had the highest glucose level (30.12%), while Anise had the lowest (25.71%).

**Sucrose Content:** Sucrose levels were low in all samples, with Clover and Anise recording the highest (0.13%), and Fennel the lowest (0.09%), all within acceptable limits.

**Maltose Content:** Sesame honey showed the highest 0maltose (9.76%), while Anise had the lowest (7.94%).

**Diastase Activity:** Sesame honey exhibited the highest enzymatic activity (30.00), indicating freshness and high quality, while Fennel showed the lowest (10.00).

# 2. Geographical and Botanical Variations in Physicochemical Properties

#### 2.1. Honey from Assiut Governorate:

Data presented in Table (1) for Sesame, Clover, Fennel, and Anise honeys collected from Assiut revealed clear differences in their physicochemical properties, reflecting their botanical origin:

#### **Quality and Freshness Indicators:**

Anise honey recorded the highest moisture content (18.50% c), which may affect its stability and shelf life. In contrast, Fennel honey had the lowest moisture content (16.50% a), indicating better storage potential. Regarding HMF values, Clover honey exhibited the highest level (34.75 mg/kg d), suggesting potential heat exposure or prolonged storage. Meanwhile, Sesame honey showed the lowest HMF value (14.98 mg/kg a), indicating better freshness and minimal processing impact. This variation in moisture and HMF highlights the influence of floral source and post-harvest handling on honey quality.

#### **Mineral Content and Acidity:**

Clover honey had the highest electrical conductivity (EC: 0.51 mS/cm a), indicating a richer mineral content. Fennel honey displayed the most pronounced acidic character, with the highest free acidity (28.50 meq/kg c) and total acidity (46.00 meq/kg a). Based on Table 1, the results for honey samples from Assiut Governorate regarding sugar composition and enzymatic activity are summarized as follows:

## **Sugar Composition and Crystallization Tendency:**

**Fennel honey** exhibited the highest fructose content (42.15% a), indicating a lower tendency to crystallize and suggesting good quality. In contrast, Clover honey had the highest glucose level (30.12% a), which predicts a faster crystallization rate.

**Sucrose values** ranged from 0.09% to 0.13%, all within the acceptable limit (<5%), confirming the authenticity and purity of the honey samples. Enzymatic Activity (Diastase Number):

**Sesame honey** showed the highest diastase activity (30.00 DN a), reflecting high enzymatic integrity and minimal exposure to heat.

Fennel honey recorded the lowest diastase number (10.00 DN c), possibly due to thermal degradation or extended storage. These findings highlight clear botanical and geographical variations among the honey types from Assiut, directly influencing their physicochemical characteristics and quality indicators.

Table (1): Statistical comparison of physicochemical properties of different honey types from Assiut Governorate

Parameter							
Parameter	Type of honey						
	Anise	Anise Fennel Clover Sesame					
Moisture	18.50c	16.50 a	18.00b	18.00b			
H.M.F	21.31b	24.58 c	34.75 d	14.98 a			
E.C	0.17c	0.18 b	0.17c	0.46a			
PH	4.50 b	4.50 b	4.10 a	4.80c			
Free	31.00b	27.00c	27.50c	35.00a			
Lactone	1.00c	2.50b	7.50a	2.50b			
Total acidity	32.00b	29.50a	35.00c	37.50d			
Fructose	40.66ab	42.15a	39.30b	39.20b			
Glucose	25.71c	25.78c	30.12a	26.88b			
Sucrose	0.13a	0.09b	0.13a	0.10b			
Maltose	7.94c	9.63b	8.13c	9.76a			
Diastase	12.00 b	10.00 c	12.00 b	30.00 a			

Means followed by the same letter per row do not differ by the Duncan's test (P = 0.05).

#### 2.2. Honey from Qena Governorate:

Results based on Table (2): Physicochemical properties of honey types from Qena Governorate:

**Moisture Content:** Clover and Sidr honeys recorded the highest moisture content (18.00% a), while Fennel honey showed the lowest value (17.00% b).

Hydroxymethylfurfural (H.M.F): Sidr honey had the highest HMF (8.64 a), followed by Clover (6.13 b), and the lowest value was in Fennel honey (4.60 c), indicating greater freshness.

**Electrical Conductivity (EC):** Sidr honey showed the highest EC (0.51 a), while Fennel had a moderate value (0.24 b), and Clover recorded the lowest (0.16 c).

**pH:** The highest pH was found in Sidr honey (5.40 a), followed by Fennel (4.70 b), and the lowest in Clover (3.90 c).

Free Acidity: Clover honey had the highest free acidity (28.50 a), Fennel was intermediate (21.50 b), and Sidr the lowest (18.17 c).

**Lactone Content:** Clover showed significantly higher lactone levels (17.50 a), Fennel (3.00 b), and the lowest was in Sidr (1.00 c).

**Total Acidity:** Clover honey recorded the highest total acidity (46.00 a), followed by Fennel (24.50 b), and the lowest in Sidr (19.50 c).

Fructose Content: Clover and Fennel honeys had the highest fructose values (44.31 a and 42.81 a), while Sidr showed significantly lower content (37.89 b).

Glucose Content: Clover honey had the highest glucose level (36.82 a), followed by Fennel (31.35 b), and Sidr the lowest (26.49 c).

**Sucrose Content:** Fennel honey recorded the highest sucrose (0.60 a), Clover moderate (0.36 b), and Sidr the lowest (0.12 c), all within acceptable limits.

Maltose Content: Sidr honey had the highest maltose (11.89 a), Fennel intermediate (8.92 b), and Clover the lowest (7.31 c).

**Diastase Activity:** Clover honey exhibited the highest diastase number (60.00 a), followed by Sidr (30.00 b), and Fennel with the lowest

(20.00 c). These findings confirm significant botanical influence on honey composition across types.

#### **Distinctive Mineral and Acidic Profiles:**

Clover honey exhibited the highest free acidity (28.50 meq/kg a) and lactone content (17.50 a), indicating a pronounced acidic profile. In contrast, Sidr honey showed the highest electrical conductivity (0.51 mS/cm a), suggesting a richer mineral composition. Fennel honey displayed moderate values for both acidity and conductivity, reflecting a more balanced chemical profile.

Table (2): Statistical analysis of physicochemical properties of different honey types from Qena Governorate

Damamatan	Qena Governorate				
Parameter		Type of honey			
	Fennel	Clover	Sidr		
Moisture	17.00 <sup>b</sup>	18.00a	18.00 <sup>a</sup>		
H.M.F	4.60°	6.13 <sup>b</sup>	8.64ª		
E.C	0.24 <sup>b</sup>	0.16 <sup>C</sup>	0.51 <sup>a</sup>		
PH	4.70 <sup>b</sup>	3.90 °	5.40 <sup>a</sup>		
Free	21.50 <sup>b</sup>	28.50 <sup>a</sup>	18.17°		
Lactone	$3.00^{b}$	17.50 <sup>a</sup>	1.00°		
Total acidity	24.50 <sup>b</sup>	46.00a	19.50°		
Fructose	42.81a	44.31a	37.89 <sup>b</sup>		
Glucose	31.35 <sup>b</sup>	36.82a	26.49°		
Sucrose	$0.60^{a}$	0.36 <sup>b</sup>	0.12°		
Maltose	8.92 <sup>b</sup>	7.31°	11.89 <sup>a</sup>		
Diastase	20.00°	60.00 <sup>a</sup>	30.00 <sup>b</sup>		

Means followed by the same letter per row do not differ by the Duncan's test (P = 0.05).

**2.3.** Comparative Analysis: Assiut and Qena honeys, the direct comparison (Table3) underscored the impact of geography. Significant variations were observed between honey samples from Assiut and Qena ( $p \le 0.05$  or  $p \le 0.0001$ ). Clover honey from Assiut had the highest HMF (34.75 mg/kg), while Qena honeys showed much lower levels, indicating better freshness. Sider honey from Qena recorded the highest EC (0.51 mS/cm),

reflecting higher mineral content. Clover from Qena also showed the highest total acidity (46.00 meq/kg), while Sider had the lowest. In sugar content, Qena clover honey had the highest fructose and glucose, while fennel from Qena had the most sucrose. Diastase activity peaked in Qena clover (60.00 DN), suggesting higher enzymatic freshness. These differences highlight the impact of geography, floral origin, and handling on honey quality.

Table (3): Comparativ	e analysis of physicochemical para	meters of monofloral noney	s irom Assiu	it and
Qena Governorates.				
	Type of he	oney		
·			1 - 1	

	Type of honey							
Parameter	Assiut			Qena			Pr	
	Anise	Fennel	Clover	Sesame	Fennel	Clover	Sider	
Moisture	18.50 a	18.50 a	18.00 b	18.00 b	17.00 c	18.00 b	18.00 b	0.0355 *
H.M.F	21.30 c	24.58 b	34.75 a	14.98 d	4.61 g	6.13 f	8.64 e	0.0001 **
E.C	0.17 d	0.18 c	0.17 d	0.46 a	0.24 b	0.16 d	0.51 a	0.0001 **
PH	4.50 b	4.50 b	4.10 c	4.80 a	4.70 b	3.90 d	5.40 a	0.0021 *
Free	31.00 a	27.00 b	27.50 b	35.00 a	21.50 c	28.50 b	18.50 d	0.0001 **
Lactone	1.00 d	2.50 c	7.50 b	2.50 c	3.00 c	17.50 a	1.00 d	0.0001 **
Total acidity	32.00 f	29.50 f	35.00 c	37.50 b	24.50 d	46.00 a	19.50 e	0.0001 **
Fructose	40.66 c	42.15 b	39.50 c	39.20 с	42.81 b	44.31 a	37.89 d	0.0001 **
Glucose	25.71 e	25.78 e	30.45 c	26.88 d	31.35 b	36.82 a	26.49 e	0.0001 **
Sucrose	0.13 c	0.09 e	0.13 c	0.10 d	0.60 a	0.36 b	0.12 d	0.000 **
Maltose	7.94 d	9.93 a	8.13 c	9.76 a	8.92 b	7.31 d	11.89 a	0.0001 **
Diastase	12.00 d	10.00 e	12.00 d	30 b	20 c	60 a	30 b	0.0001 **

Means followed by the same letter per row do not differ by the Duncan's test (P = 0.05).

### 3. Key Physical Properties Influencing Honey Quality:

Results of Table (4) demonstrate significant variations (p < 0.05 to p < 0.001) in the physical properties of different monofloral honey types collected from Assiut and Qena.

- Electrical Conductivity (EC): EC values varied significantly (p = 0.0001), ranging from 0.16 ± 0.005 mS/cm in Clover honey from Qena to 0.51 ± 0.011 mS/cm in Sider honey from Qena. The highest EC was observed in Sider and Sesame honeys, indicating a higher mineral or non-nectar origin, while Anise, Clover, and Fennel honeys from Assiut recorded the lowest values (0.17–0.18 mS/cm).
- Granulation (F/G ratio): The Fructose/Glucose (F/G) ratio showed significant differences (p = 0.04). The highest value was found in Fennel honey from Assiut (1.634  $\pm$  0.176), indicating slower crystallization, while the lowest was recorded in Clover honey from Qena (1.203  $\pm$  0.275), suggesting a higher tendency to granulate.

- Viscosity: Viscosity values ranged between  $55.0 \pm 5.0$  and  $70.0 \pm 5.0$  poise, with significant variation (p = 0.01). \*Sesame and Fennel honeys from both governorates showed the highest viscosities ( $70.0 \pm 5.0$ ), whereas Clover from Qena had the lowest ( $55.0 \pm 5.0$ ). Viscosity is closely linked to moisture and sugar content.
- Moisture Content: Moisture content varied significantly (p = 0.0355), but all samples were within the acceptable limit (<20%). The lowest value was in Fennel honey from Qena (17.00 ± 0.577%), indicating better stability and lower fermentation risk. The highest was found in Anise and Fennel honeys from Assiut (18.50 ± 0.264–0.208%).

However, the observed variations in electrical conductivity, fructose/glucose ratio, viscosity, and moisture content highlight the influence of both botanical origin and geographical location on the physical properties of bee honey. These parameters serve as important indicators for assessing honey quality, crystallization behavior, storage potential, and commercial classification.

		EC	Granulation	Viscosity	Moisture (%)
Gov.	Type of honey	mS/cm	F/G ratio	Poise	g/100g
			(Mean	±SD)	
	Anise	$0.17 \pm 0.028$	1.581±0.229	$58.0 \pm 3.0$	$18.50 \pm 0.264$
A agint	Fennel	$0.18 \pm 0.011$	1.634±0.176	$65.0 \pm 5.0$	$18.50 \pm 0.208$
Assiut	Clover	$0.17 \pm 0.011$	1.297±0.174	59.0±5.0	$18.00 \pm 0.057$
	Sesame	$0.46 \pm 0.034$	1.458±0.327	70.0±5.0	$18.00 \pm 0.461$
	Fennel	$0.24 \pm 0.011$	1.345±0.150	70.0±5.0	$17.00 \pm 0.577$
Qena	Clover	$0.16 \pm 0.005$	1.203±0.275	55.0±5.0	$18.00 \pm 0.288$
	Sider	$0.51 \pm 0.011$	1.430±0.342	60.0±5.0	$18.00 \pm 0.577$
	p-value	0.0001***	0.04*	0.01*	0.0355*

Table (4):Physical properties of bee honey obtained from Assiut and Qena governorates.

## 4. Detailed Acidity Profile as a Fingerprint for Botanical Origin:

Results of Table (5): Acidity Profile of Fresh Bee Honey Samples from Assiut and Qena Governorates

**pH:** The pH values varied significantly among honey types and locations. Sider honey from Qena recorded the highest pH  $(5.40 \pm 0.04)$ , while Clover honey from Qena showed the lowest pH  $(3.90 \pm 0.04)$ . The pH differences were statistically significant (p = 0.0021\*\*).

Free Acidity (meq/kg): Assiut Sesame honey had the highest free acidity  $(35.0 \pm 2.00 \text{ meq/kg})$ , while Qena Sider honey had the lowest  $(18.17 \pm 0.47 \text{ meq/kg})$ . Free acidity differences were highly significant (p = 0.0001\*).

**Total Acidity (meq/kg):** Clover honey from Qena exhibited the highest total acidity  $(46.0 \pm 2.00 \text{ meq/kg})$ , followed by Sesame honey from Assiut  $(37.5 \pm 0.50)$ . Sider honey from Qena had the lowest total acidity  $(19.5 \pm 2.00)$ . Total acidity also showed highly significant differences  $(p = 0.0001^*)$ .

**Lactone Content:** The highest lactone content was found in Clover honey from Qena (17.50  $\pm$  0.50), while the lowest was in Sider honey from Qena (1.00  $\pm$  0.20) and Anise honey from Assiut

 $(1.00 \pm 0.20)$ . Lactone differences were highly significant (p = 0.0001\*). The detailed acidity analysis (Table 5) revealed highly significant differences (p<0.001). Fennel honey from Assiut consistently had the highest values for free acidity, total acidity, and lactones, defining its strong, characteristic flavor. This profile serves as a chemical fingerprint for its botanical origin.

**pH:** The pH values range from  $3.90 \pm 0.04$  (Qena Clover) to  $5.40 \pm 0.04$  (Qena Sider). There is a statistically significant difference in pH between honey types and regions (p = 0.0021\*\*).

Free Acidity (meq/kg): Free acidity varies significantly, with Assiut Sesame honey showing the highest value (35.0  $\pm$  2.00) and Qena Sider honey the lowest (18.17  $\pm$  0.47). Differences are highly significant (p < 0.0001\*).

**Total Acidity (meq/kg):** Total acidity is highest in Qena Clover honey  $(46.0 \pm 2.00)$  and lowest in Qena Sider honey  $(19.5 \pm 2.00)$ , showing significant variation  $(p < 0.0001^*)$ .

**Lactone (meq/kg):** Lactone content shows significant differences with the highest value in Qena Clover honey  $(17.50 \pm 0.50)$  and lowest in Assiut Anise and Qena Sider honeys  $(1.00 \pm 0.20)$  (p < 0.0001\*).

Gov.	Type of honey	PH	Free acidity Meg/kg	Total acidity Meq/Kg	Lacton
			(Mean±S	SD)	
	Anise	$4.5 \pm 0.20$	$31.0 \pm 1.50$	$32.0 \pm 1.00$	$1.00 \pm 0.20$
A ~~:4	Fennel	$4.5 \pm 0.30$	$27.0 \pm 2.00$	$29.5 \pm 0.50$	$2.50 \pm 0.50$
Assiut	Clover	$4.1 \pm 0.10$	$27.5 \pm 0.50$	$35.0 \pm 2.00$	$7.50 \pm 0.60$
	Sesame	$4.8 \pm 0.80$	$35.0\pm2.00$	$37.5 \pm 0.50$	$2.50 \pm 0.50$
	Fennel	$4.70 \pm 0.05$	$21.5 \pm 0.50$	$24.5 \pm 1.00$	$3.00 \pm 1.00$
Qena	Clover	$3.90\pm0.04$	$28.5 \pm 1.00$	$46.0 \pm 2.00$	$17.50 \pm 0.50$
	Sider	$5.40 \pm 0.04$	$18.17 \pm 0.47$	$19.5 \pm 2.00$	$1.00 \pm 0.20$
	p-value	0.0021**	0.0001***	0.0001***	0.0001***

Table (5): Acidity profile in fresh bee honey samples obtained from Assiut and Qena governorates.

## 6. Sugar Composition: Purity and Botanical Signature:

Table (6): Sugars content (%) of bee honey samples from Assiut and Qena governorates:

Fructose (g/100g): Values range from 37.89  $\pm$  0.57 (Qena Sider) to 44.31  $\pm$  0.04 (Qena Clover). The difference between samples is highly significant (p < 0.0001\*).

Glucose (g/100g): Glucose content varies significantly, with the highest in Qena Clover

 $(36.82 \pm 1.93)$  and the lowest in Assiut Anise  $(25.71 \pm 1.01)$  (p < 0.0001\*).

**Sucrose (g/100g):** Sucrose is lowest in Assiut Fennel (0.09  $\pm$  0.01) and highest in Qena Fennel (0.60  $\pm$  0.20), with highly significant differences (p < 0.000\*).

**Maltose (g/100g):** Maltose content ranges from  $7.31 \pm 0.69$  (Qena Clover) to  $11.89 \pm 1.10$  (Qena Sider), with significant differences across samples (p < 0.0001\*).

Table (6): Sugars content (%) of bee honey samples obtained from Assiut and Qena governorates.

Gov.	Kind honey	Fructose (g/100g)	Glucose (g/100g)	Sucrose (g/100g)	Maltose (g/100g)
			(Mean	±SD)	
	Anise	$40.66 \pm 2.01$	$25.71 \pm 1.01$	$0.13 \pm 0.03$	$7.94 \pm 0.10$
Assiut	Fennel	$42.15 \pm 1.05$	$25.78 \pm 1.07$	$0.09 \pm 0.01$	$9.63 \pm 0.65$
Assiut	Clover	$39.50 \pm 0.70$	$30.45 \pm 0.29$	$0.13 \pm 0.03$	$8.13 \pm 0.87$
	Sesame	$39.20 \pm 0.40$	$26.88 \pm 0.12$	$0.10\pm0.02$	$9.76 \pm 0.33$
	Fennel	$42.81 \pm 2.04$	$31.35 \pm 0.77$	$0.60\pm0.20$	$8.92 \pm 0.37$
Qena	Clover	$44.31 \pm 0.04$	$36.82 \pm 1.93$	$0.36 \pm 0.04$	$7.31 \pm 0.69$
	Sider	$37.89 \pm 0.57$	$26.49 \pm 3.61$	$0.12\pm0.02$	$11.89 \pm 1.10$
	p-value	0.0001***	0.0001***	0.000***	0.0001***

# 7. Critical Quality Indicators: HMF and Diastase Activity:

Results of Table (7): Hydroxymethylfurfural (HMF) content and diastase enzyme activity of bee honey samples from Assiut and Qena governorates:

**HMF** (mg/kg): HMF content is significantly higher in Assiut samples, with values ranging

from 14.98  $\pm$  0.08 (Sesame) to 34.75  $\pm$  1.96 (Clover). Qena samples have much lower HMF values, between 4.60  $\pm$  0.02 (Fennel) and 8.64  $\pm$  0.04 (Sider). The difference is highly significant (p < 0.0001\*).

**Diastase activity (DN):** Diastase enzyme activity varies significantly, with Assiut honey showing lower activity  $(10.0 \pm 1.0 \text{ to } 30.0 \pm 1.0)$ 

compared to Qena honey, especially Clover from Qena with the highest activity at 60.0  $\pm$ 

10.0 DN. This difference is also highly significant (p < 0.0001\*).

Table (7): Hydroxymethylfurfural (HMF) content and diastase enzymes activity of bee honey samples

obtained from Assiut and Qena governorates.

Governorate	Kind of honey	HMF (mg/kg)	Diastase (DN)
Governorate	Kind of honey	(Mear	n±SD)
	Anise	$21.31 \pm 0.006$	$12.0 \pm 1.0$
Assiut	Fennel	$24.58 \pm 0.08$	$10.0 \pm 1.0$
Assiut	Clover	$34.75 \pm 1.96$	$12.0 \pm 2.0$
	Sesame	$14.98 \pm 0.08$	$30.0 \pm 1.0$
	Fennel	$4.60 \pm 0.02$	$20.0 \pm 5.0$
Qena	Clover	$6.13 \pm 0.01$	$60.0 \pm 10.0$
	Sider	$8.64 \pm 0.04$	$30.0 \pm 5.0$
p-v	alue	0.0001***	0.0001***

#### **DISCUSSION**

The present study revealed clear and statistically significant variations in the physicochemical properties of monofloral honey types collected from Assiut and Qena governorates. These findings strongly confirm that the qualitative characteristics of honey are heavily influenced by its botanical origin and the geographical and climatic conditions of the production area (Gul and Pehlivan, 2018; Aliferis et al., 2010). Overall, all samples complied with the basic requirements of the standard for honey (Codex international Alimentarius Commission, 2001), with the observed variations reflecting the distinct characteristics of each type and origin.

#### 1. Moisture Content and Stability:

Moisture values in the samples ranged from 16.50% to 18.50%, all within the permissible limit (≤20%) as per the Codex standard. The lowest moisture content was found in Fennel honey from Assiut (16.50%), indicating better storage potential and higher stability against fermentation. In contrast, Anise honey from Assiut recorded the highest moisture (18.50%), which may affect its shelf life. This variation between honey types and geographical regions has been observed in previous studies (Saxena, Gautam and Sharma, 2010; Gul and Pehlivan, 2018).

### 2. Hydroxymethylfurfural (HMF) and Diastase Activity: Critical Quality Indicators:

These indicators are the most sensitive for assessing honey freshness and the suitability of its storage conditions (White, 1994). A striking geographical variation was observed. Honeys from Qena governorate (Fennel, Clover, Sidr) showed remarkably low HMF values (4.60 to 8.64 mg/kg), indicating excellent freshness and minimal thermal processing. In contrast, Assiut honeys, particularly Clover honey, showed significantly higher HMF levels (up to 34.75 mg/kg), which clearly suggests improper storage or excessive heating, as HMF forms rapidly under thermal stress (Fallico, Zappalà, Arena and Verzera, 2004). These findings are strongly supported by the Diastase activity results. Qena Clover honey exhibited the highest enzymatic activity (60.00 DN), confirming optimal processing and storage. Conversely, the lower Diastase numbers in Assiut honeys, especially Fennel (10.00)DN), further corroborate the potential thermal degradation or extended storage implied by the higher HMF values.

### **3. Electrical Conductivity (EC) and Mineral Content:**

The electrical conductivity values showed significant variation, with Sidr honey from Qena and Sesame honey from Assiut recording the highest values (0.51 and 0.46 mS/cm, respectively). This reflects their higher mineral content. This finding is consistent with

literature indicating that certain blossom honeys (like Sesame) and honeydew-influenced honeys (like Sidr) typically exhibit higher electrical conductivity (Bogdanov, Ruoff and Persano Oddo, 2004; Escuredo et al., 2013). On the other hand, Clover and Anise honeys from Assiut showed the lowest EC values (0.17 mS/cm), highlighting the profound impact of botanical origin on this parameter.

#### 4. Acidity Profile: A Chemical Fingerprint:

The detailed acidity analysis revealed highly significant differences. The pH values ranged from very acidic in Qena Clover honey (3.90) to less acidic in Qena Sidr honey (5.40). Qena Clover honey exhibited a pronounced acidic profile, with the highest free acidity (28.50 meg/kg), lactone content (17.50 meg/kg), and consequently, the highest total acidity (46.00 meq/kg). This unique profile can serve as a chemical fingerprint for its botanical origin (Terrab, González, Díez and Heredia, 2003). In Assiut, Sesame honey showed the highest free acidity (35.00 meq/kg). The significant variation in lactone and total acidity values across all samples underscores the influence of both floral source and geographical factors on the organic acid composition of honey.

## 5. Sugar Composition and Crystallization Tendency:

The sugar profile provided clear insights into purity and crystallization behavior.All sucrose values were well below the 5% limit (Codex Alimentarius Commission, the honey's maturity confirming authenticity. Fennel honey from Assiut was the richest in fructose (42.15%), which grants it higher sweetness and a lower tendency to crystallize. In contrast, Clover honey from Qena had the highest glucose content (36.82%), predicting a faster crystallization (Ouchemoukh, Louaileche and Schweitzer, 2007). These observations are directly supported by the Fructose/Glucose (F/G) ratio, where the highest value in Fennel honey from Assiut (1.634) indicates slower crystallization, and the lowest in Qena Clover honey (1.203) suggests a higher tendency to granulate.

#### **CONCLUSION**

In summary, the results conclusively that the physicochemical demonstrate properties of honey serve as an effective fingerprint for determining its botanical and geographical origin. The clear differences in key quality indicators like HMF and Diastase activity between Assiut and Qena honeys not only reflect their floral sources but also highlight the critical impact of postharvest handling and local practices in each region on the final quality of the honey. The interplay of these factors—botanical origin, geography, and beekeeping practices ultimately defines the unique characteristics of each honey type.

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