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Effects of Temperature and Feeding Practices on Viscosity and Physicochemical Properties of Honey

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Abstract – Honey's viscosity is a crucial factor affecting its processing, storage, and overall quality. This study examines the relationship between temperature and viscosity across different honey samples, demonstrating an inverse correlation—higher temperatures reduce viscosity, enhancing honey's fluidity. Viscosity measurements, conducted using a digital viscometer at temperatures ranging from 25 °C to 60 °C, confirmed that natural honey exhibited distinct molecular characteristics compared to sugar-fed honey, as revealed by Fourier transform infrared spectroscopy (FTIR). Additionally, microscopic imaging showed a greater diversity of pollen grains in natural honey, emphasizing the impact of botanical origin on composition. The research also found that artificial feeding methods, such as sugar syrup supplementation, resulted in lower viscosity, altered acidity, and reduced bioactive components. The mixture sample (honey + fruit + sugar) displayed the highest viscosity at 25 °C, while sugar-fed honey had the lowest viscosity at 60 °C. These findings highlight the significance of temperature regulation and feeding practices in maintaining honey's physicochemical properties, which are essential for quality control and applications in the food industry. Promoting natural feeding practices among beekeepers can enhance honey's nutritional and medicinal properties, contributing to improved product quality and sustainability.

Keywords – Viscosity, Temperature, Physicochemical Properties, Honey, Fruit-Based Feeding, Honeybee Nutrition.

I. INTRODUCTION

Honey, the main bee product, is the energy source of bees. Honey bees gather their honey from two sources: nectar and honeydew [1]. Codex Alimentarius Commission defined honey as 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 on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in wax honeycombs to ripen and mature. The EU definition states that honey is only honey according to the definition when it is produced by Apis mellifera honeybees [2, 3].

Honey contains several constituents of small amounts, such as minerals, free amino acids, proteins, vitamins, enzymes, organic acids, flavonoids, phenolic acids, and other organic acids in addition to other phytochemicals compounds [4, 5]. A key physicochemical attribute of honey is its viscosity, which affects its flow behavior, processing, and crystallization tendencies [6, 7].

Viscosity of honey depends on several factors: such as temperature, moisture and crystal structure, it is important to evaluate these factors before using honey as active substance or excipient in pharmaceutical products. Viscosity, one of the most important rheological parameters of honey, largely affects product quality and processing conditions related to the design of honeyprocessing equipment [8].

Freshly extracted honey is a viscous liquid food, and its viscosity depends on the various honey constituents. Hence, the viscosity is greatly influenced by the composition of honey, mainly its water content [9]. In contact with the air, honey absorbs water, a phenomenon known as hygroscopicity. The water content absorbed depends on the relative humidity of the air. The hygroscopicity of honey is conditioned by the large amount of sugar. This process can increase the amount of water in the surface layer of the honey that can affect its quality during storage [10].

Honey will absorb moisture from air at a relative humidity of about 60 %. Another factor affecting the physical appearance of honey is surface tension, which is influenced by the colloidal substances in the honey, a reflection of the honey’s botanical origin. The surface tension and high viscosity of honey cause the foaming appearance of honey [9].Temperature plays a crucial role in affecting the viscosity of honey. At lower temperatures, honey becomes thicker and more resistant to flow, whereas at higher temperatures, it becomes more fluid due to reduced intermolecular forces [7, 11, 12].

This relationship is essential in beekeeping, food processing, and pharmaceutical industries, where honey is often stored, transported, and processed under different temperature conditions [13, 14]. In general, the quality of honey and its chemical composition are related to many factors, such as its geographic, botanical or plant origin, climate and seasonality; other factors could be external, such as environmental factors, processing methods of honey by beekeepers, storage conditions and deliberate adulteration by producers [15].

The storage conditions will influence honey quality; refrigeration temperatures lead to a darkening of the color, whereas storage at room temperatures and higher (28 °C) results in modification of the acidity and an increase in HMF and the microbiota in honeys, together with a small modification of the composition in monosaccharides [16]. Samples stored at -18 °C showed no difference in the physico-chemical parameters from the fresh ones; it may affect honey viscosity [17].

Dehydration of honeys at 40 °C goes through several steps: at the two first steps, water loss occurs, the third step is characterized by volatiles’ loss, whereas, at the last dehydration step both volatiles and water are removed from samples [18]. The authors studied the dependence of shear stress on shear rate, and the dependence of dynamic viscosity on temperature. Although a higher content in water generally corresponds to a lower honey viscosity [19]. Analyzing honey's physicochemical properties using instruments like pH meters, refractometers, viscometers, FTIR spectroscopy, density measurements, and microscopic imaging is essential for assessing its quality, authenticity, and origin [20, 21].

Understanding the relationship between temperature and viscosity, along with the effects of different feeding methods, is essential for optimizing honey processing, storage, and quality control. Temperature variations significantly influence honey’s flow properties, which in turn affect its usability in the food industry and other applications. Moreover, the botanical and nutritional composition of honey is directly impacted by the bees' diet, with natural feeding resulting in superior quality compared to artificial sugar-based diets. This study aims to investigate the impact of temperature on honey viscosity while also analyzing how different feeding methods alter its physicochemical properties. The findings will provide valuable insights for beekeepers, food industries, and researchers to enhance honey quality and sustainability.

II. MATERIAL AND METHOD

Collection of samples honey

Four liquid honey samples were collected from *Apis mellifera* or *Apis florea* bees' colonies during July and August. After harvesting the honey or removing it from the hive boxes, the bees were fed fruit like date (*Phoenix dactylifera L.*), mixture (honey+fruit+sugar), and Sugar syrup, bees were fed 1 kg per week for one month to replicate the (unnatural honey) production process, sourced from beehives in Akre, while (natural honey) from Rania located in the northern region of Iraq. Local beekeepers prepared the honey samples using traditional methods and stored them in clean, sealed plastic bags in a dark environment at 20 °C until analysis [22]. Following this period, physicochemical analyses were performed on the honey. The study also involves collecting honey from bee boxes, as depicted in Figure1.

Figure 1. Bee colonies were fed dates (Phoenix dactylifera L.), with honey samples collected to assess the effects on composition

Physicochemical properties of honey

The physicochemical properties of honey samples were analyzed using various techniques, including FTIR spectroscopy, refractometer, viscometer, microscopic imaging, electrical conductivity, pH, and density measurements.

FTIR analysis

Fourier transform infrared spectroscopy (FTIR) was performed using the Shimadzu IR Spirit QATR-S instrument to analyze the molecular composition of honey samples. A small aliquot of honey was placed directly onto the Attenuated Total Reflectance (ATR) crystal, and spectra were recorded in the 4000–500 cm^{-1} range with a resolution of 4 cm^{-1}, and 32 scans were averaged per sample to ensure accurate results. The instrument was calibrated using a background scan before each measurement to eliminate interference from the atmosphere. Each analysis was conducted in triplicate for reproducibility. The spectra were analyzed using Shimadzu Lab Solutions IR software to identify functional groups and detect potential adulterants. This method is highly effective in characterizing honey's sugars, organic acids, and phenolic compounds [23]. Structured FTIR peak identify in table 1, for the honey samples based on common FTIR absorption bands..

Refractive index (%Brix)

The refractive index of samples honey was measured using a handheld refractometer (Atago PAL-1, Japan) to determine the moisture content and sugar concentration. A drop of honey was placed on the prism, and the Brix value was recorded at (20 °C), and humidity (36 %). The refractometer was calibrated with distilled water prior to use. This method provides a rapid and non-destructive assessment of honey quality, as the refractive index is directly correlated with the total soluble solids content [24].

Viscosity measurements

Measurements were made on Lamy RM 100 Plus rotary viscometer. A digital viscometer was used to assess viscosity at temperatures ranging from 25 °C to 60 °C. The measurement was conducted using the spindle 28 at a constant humidity level (36 %) and a rotational speed of 20 rpm [25]. The instrument was calibrated before each measurement using a standard viscosity reference fluid. Viscosity readings were recorded in centipoise (mPa·s), and each measurement was performed in triplicate to ensure accuracy and reproducibility. For a Lamy RM 100 Plus rotary viscometer, viscosity is the measured resistance to flow encountered by a spring when a spindle rotates in liquid. The most common application of this type of measurement is the quantification of a fluid's viscosity when compared to either a target performance or when compared against another fluid. For example, similar Brookfield instrumentation was used in developing target viscosities for the standardization of nectar-thick and honey-thick barium products that are used during videofluoroscopic evaluations of swallowing [26].

Microscopic images

Microscopic images were conducted to identify pollen grains, crystals, and other particulate matter in honey samples. A small amount of honey was diluted with distilled water, and a drop of the mixture was placed on a glass slide. Images of honey samples were examined with a Dcorn HDMI LCD Digital Microscope (7 Inc IPS Screen – 16 MP). The morphology of pollen grains was analyzed to determine the botanical origin of the honey, while the presence of foreign particles indicated potential adulteration [27].

pH test

The pH, conductivity, and total dissolved solids (TDS) of honey samples were measured using an AZ 86505 multi parameter meter. These parameters are critical for assessing the stability and quality of honey. This method provides critical information about the acidity, mineral content, and stability of honey. For instance, pH influences microbial growth, while conductivity reflects the mineral content [28]. The pH and electrical properties of the honey sample were measured using a digital pH meter (model 86505) at a controlled temperature of 25 °C and a relative humidity of 36 %. A volume of 10 mL of honey was carefully placed into a clean beaker, and the pH probe was immersed in the sample, ensuring full electrode contact. The instrument was calibrated using standard buffer solutions (pH 4.0, 7.0, and 10.0) before measurement. Additionally, millivolt (mV), electrical conductivity (μS/cm), and total dissolved solids (ppm) readings were recorded. Each measurement was performed in triplicate to ensure accuracy and reproducibility.

Determination of density

The pycnometry method was used to determine the density of the honey sample using the expression Eq. 1. The mass of the SAMPLE with honey was recorded using an analytical balance (accuracy: ±0.0001 g).

$$\text{Density (g/mL)} = \frac{\text{W2}-\text{W1 (g)}}{\text{V (mL)}} \qquad \text{(Eq. 1)}$$

Where W1: mass of the empty pycnometer, and W2: mass of the filled pycnometer. All honey samples were heated at (50 °C) for 30 min to become less viscous and easy to pour into the pycnometer [29].

III. RESULTS AND DISCUSSIONS

The physicochemical properties of honey samples were analyzed using various techniques, including FTIR spectroscopy, Refractometer, Viscometer, Microscopic Imaging, Electrical Conductivity, pH, and Density measurements.

Table 1 shows the FTIR peak analysis for honey samples. FTIR spectroscopy revealed significant differences in honey samples based on feeding methods.

Table 1. FTIR spectra analysis for honey samples

Position of bands [cm^{-1}]	Functional Group	Honey Component	Ref.
3300–3400	O–H stretching vibration	Water, Sugers (Glucose, Fructose)	[30, 31]
2925–2850	C–H stretching vibrations	Carbohydrates (Glucose, Fructose)	[32, 33]
1740–1710	C=O stretching vibration,	organic acids (e.g., gluconic acid) and esters	[34, 35]
1650–1600	C=C stretching vibration	Phenolic Compounds, Flavonoides	[36, 37]
1550–1500	N–H bending vibrations	proteins and amino acids	[38, 39]
1450–1350	C–H bending vibrations	sugars and organic acids	[30, 40]
1200–1000	C–O stretching vibrations	glucose,fructose,polysaccharides	[41, 42]
1100–900	C–O–C stretching vibrations	glycosidic bonds in oligosaccharides and polysaccharides	[30, 43]
900–650	C–H out-of-plane bending vibrations	aromatic, phenolic compounds and trace organic impurities	[36]

FTIR spectra of different honey types are shown in Figure 2, Figure 3, Figure 4, and Figure 5. FTIR analysis identified key functional groups in honey, including, O-H stretching (3300–3400 cm^{-1}) related to water and sugars, C=O stretching (1740–1710 cm^{-1}) associated with organic acids, C-H bending (1450–1350 cm^{-1}) linked to carbohydrates. The results suggest that feeding methods impact the molecular composition of honey, particularly in terms of sugar content and organic compounds.

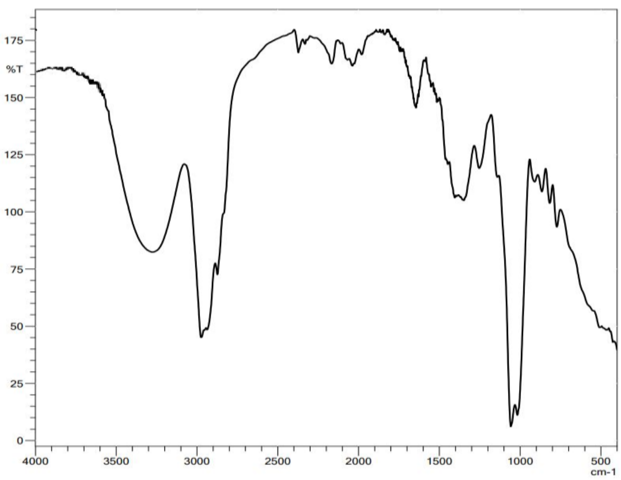

Figure 2. FTIR spectra of Rania natural sample honey

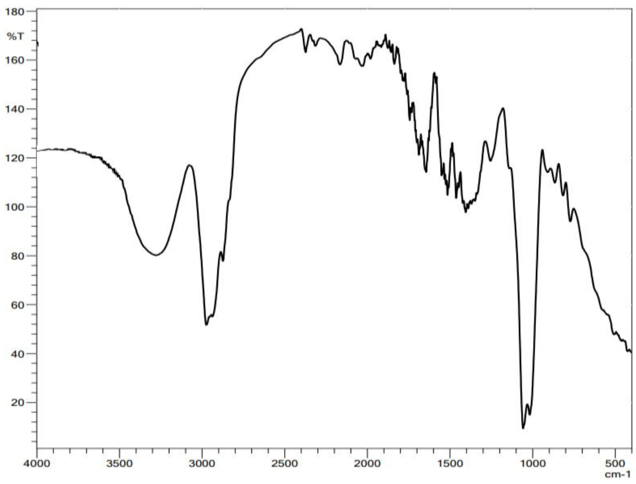

Figure 3. FTIR spectra of date sample honey

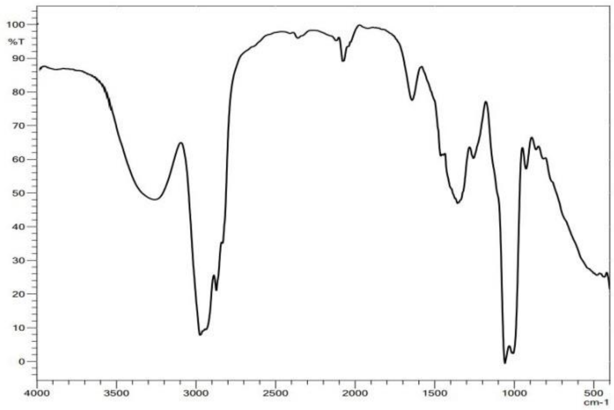

Figure 4. FTIR spectra of sugar sample honey

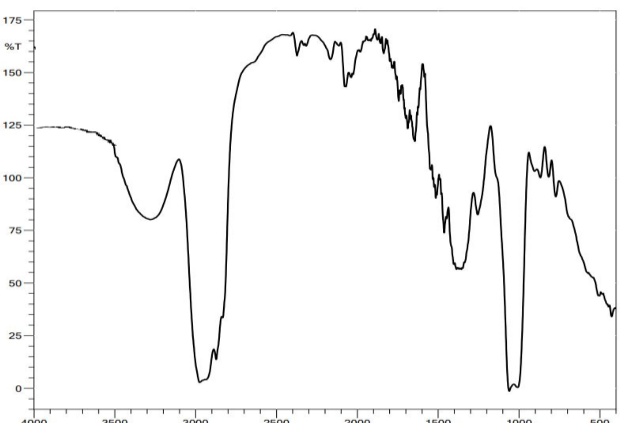

Figure 5. FTIR of mixture fed (honey+fruit+sugar) sample honey

Table 2 shows the refractometer (Brix) measurement results of honey samples. The Brix values varied among honey types, with Rania and mixture (honey+fruit+sugar) sample honey showing the highest values (83°Bx), and sugar honey the lowest (74 °Bx).

Table 2. Refractometer (Brix) of samples honey

Type of Honey	Brix3 (^{o}Bx)
Rania sample	83
Date sample	79
Sugar sample	74
Mixture sample (honey+fruit+sugar)	83

Viscosity values for the honey samples decreased significantly with increasing temperature. Mixture (honey+fruit+sugar) sample the highest viscosity values (28542 mPa·s) were observed at 25 °C, while sugar sample the lowest viscosity values were recorded at 60 °C. The results confirm that temperature influences the rheological properties of honey, reducing its resistance to flow at higher temperatures. Table 3 shows the viscosity results of honey samples at different temperatures (T), 36% humidity and 20 rpm shear rate.

Table 3. Viscosity results of honey samples at different temperatures

T (°C)	**Viscosity (mPa·s)**			
	Rania sample	**Date sample**	**Sugar sample**	**honey+ fruit+ sugar**
25	27208	6175	5775	28542
30	16725	3825	425	18750
35	9525	2125	300	11450
40	5825	1300	225	6925
45	3325	800	175	3825
50	2000	550	125	2325
55	1100	375	75	1425
60	800	300	50	950

Figure 6, Figure 7, Figure 8, and Figure 9, show microscope images of different honey samples. Microscopic examination revealed differences in pollen grains and crystal structures. Natural honey exhibited diverse pollen types, while sugar-fed honey contained fewer pollen particles.

Figure 6. Rania natural sample honey under microscope images

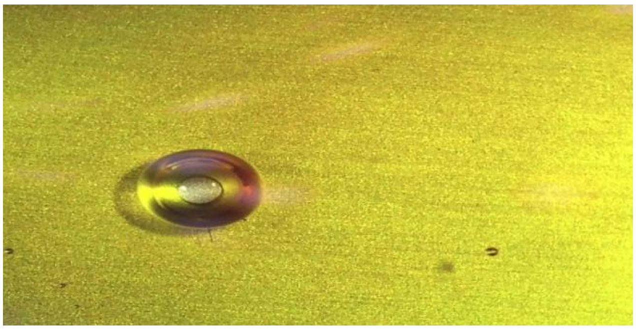

Figure 7. Date sample honey under microscope images

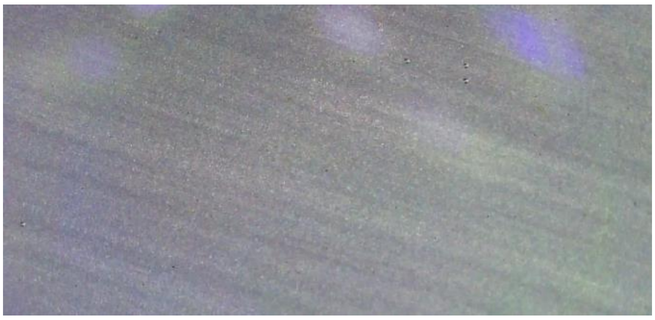

Figure 8. Sugar sample honey under microscope images

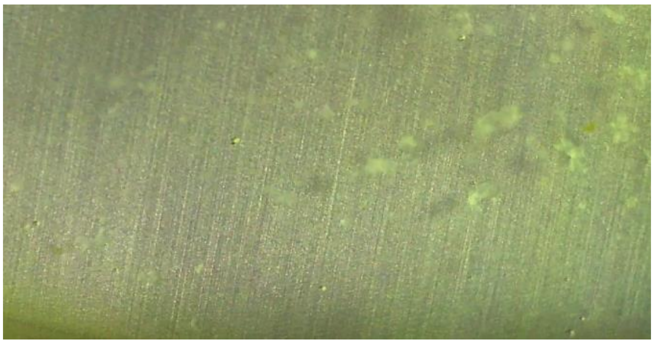

Figure 9. Mixture (honey+fruit+sugar) sample honey under microscope images

Table 4 shows the results of measuring honey samples with a pH meter at room temperature and 36% relative humidity. The pH analysis showed acidity variations, with sugar honey having the lowest pH (3.15) and Mixture (honey+fruit+sugar) sample the highest (4.24), influencing honey's stability and antimicrobial properties.

Table 4. Some properties of honey samples at 25 °C

Type of Honey	pH	mV	µS	ppm
Rania sample	3.79	191.4	0.05	0.03
Date sample	3.71	190.9	0.05	0.03
Sugar sample	3.15	220	0.05	0.03
Honey+fruit+sugar sample	4.24	156.5	0.05	0.03

Table 5 shows the density of honey samples at 25 °C. Density varied across samples, with sugar honey showing the highest density (2.855 g/cm^3), likely due to artificial sugar concentrations, whereas natural Rania sample exhibited the lowest density (1.468 g/cm^3), suggesting an optimal natural composition.

Table 5. Density of honey samples at 25 °C

Type of Honey	Density (g/cm^3)
Rania sample	1.468
Date sample	1.488
Sugar sample	2.855
Honey+fruit+sugar sample	1.576

IV. CONCLUSIONS

The study highlighted the significant impact of temperature and feeding methods on honey's physicochemical properties. FTIR analysis showed that honey composition varies based on the bees' feeding regimen, with natural honey (e.g., Rania sample) exhibiting distinct molecular characteristics compared to sugar-fed honey. Viscosity measurements confirmed an inverse relationship between temperature and viscosity, with honey becoming more fluid as temperature increased. The mixture (honey+fruit+sugar) sample exhibited the highest viscosity at 25 °C, while sugar honey had the lowest at 60 °C. Microscopic imaging revealed that natural honey contained more diverse pollen grains, while sugar-fed honey showed fewer, supporting the idea that botanical origin influences honey composition.

Overall, the study underscores how various factors, such as feeding methods and temperature, influence honey's chemical and physical properties, which are crucial for its quality control and applications in food processing. Artificial feeding methods, such as sugar syrup, lead to lower viscosity, altered acidity, and reduced bioactive components. Encouraging natural feeding practices among beekeepers can improve honey quality and sustainability.

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