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# Research Article Physiochemical Parameters and Rheological Properties of Citrus, Clover and Marjoram Egyptian Bee Honeys

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## Abstract

**Background and Objective:** The quality of bee honey depend on many factors which affecting honey contents, moisture, monosaccharide's (glucose and fructose), pH, optical density and electrical conductivity. Beside that the rheological properties as a new method to judge the quality of honey was used. Thus, the main aim of the present study was to measure the physicochemical and rheological properties of three most valuable Egyptian bee honeys. **Materials and Methods:** Physiochemical properties of the most widespread Egyptian bee honeys (Citrus, Clover and Marjoram bee honeys) were detected (moisture, glucose, fructose, electrical conductivity, pH and optical density) which were consistent with the international regulation and the rheological parameters were analyzed under temperature 25 °C to analyze (viscosity (cP), Shear stress (D/cm<sup>2</sup>) and Torque (%). **Results:** Results obtained showed about the physiochemical properties, the moisture of citrus honey was the highest one while marjoram honey recorded the lowest value. Besides that, the pH of bee honey samples recorded low value in citrus and the high value noted with marjoram honey. The sugar content (glucose and fructose) was measured, the maximum of glucose and fructose were recorded in clover honey. Whereas the optical density (O.D.) was recorded the highest value with marjoram honey, while it's recorded the lowest value of rheological parameter while, citrus honey scored the lowest values for viscosity, shear stress and torque, respectively. **Conclusion:** The results of this study indicated that, the marjoram bee honey was considered a superior in terms of physiological and rheological properties compared to other bee honeys under this study.

Key words: Bee honey, Marjoram honey, physiochemical properties, citrus honey, rheological properties, clover honey

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

The sweet substance which produced from flowers and other living parts of secretion plants by honeybee called honey<sup>1</sup>. The major component of bee honey is carbohydrate materials of which the monosaccharide glucose and fructose and the water in which the sugars are dissolved<sup>2,3</sup> 17-20% and contains many other potentially biologically active components such as antioxidant, enzymes, vitamins, minerals and proteins<sup>4</sup>. Bee honey and its water mixture are commonly used ingredients in the food industry for its sweetness, texture and preservative properties. It has been reported that carbohydrate components play a major role in influencing Tg in food systems<sup>5</sup>.

Natural honey has been used as an effective medicine around the world since ancient time. The ancient Egyptians, Assyrians, Chinese, Greeks and Romans used honeys for wounded and diseases of the gut<sup>6</sup>. Health benefits of honey have been investigated from a variety of conditions including microbial infections, glucose tolerance, inflammation, wound healing and analgesia<sup>7</sup>.

Rheological measurements of substances are very important and applicable in many fields of human activity. Determination of rheological behavior of substances is particularly important for designing of equipments, for transport, pumping and storage of substances<sup>5</sup>. Also play an important role in food industry, where rheology relates with quality control or sensory properties of foods including honey<sup>5</sup>. Viscosity is one of the most important and significant characteristics of honey which affects its quality. Beside that it's depend on several factors, temperature, moisture content and chemical constitution<sup>8</sup>. The rheological properties of bee honey from different countries have been briefly analyzed: Australia<sup>9</sup>, China<sup>10</sup>, Korea<sup>11</sup>, Greece<sup>8</sup>, India<sup>12,13</sup>, Spain<sup>14</sup>, Jordan<sup>15</sup> and Poland<sup>16</sup>.

However, research describing rheological properties of honeys from different areas are sporadic. Therefore, the objective of this study was to extend the investigation in this field and deal with the rheological behavior of selected honeys in Egypt because of studies on rheological behaviors of honey are important for applications related to handling, storage, processing, pumping, transport, quality control and sensory analysis of foods.

#### **MATERIALS AND METHODS**

**Collecting and preparation of honey samples:** Three honey samples were collected from different governorates of Egypt,

Citrus honey was collected from Giza governorate, while Clover and Marjoram honeys were collected from Al-Fayoum during the active honey bee months, April, May, June and July months in 2018 at different apiaries of different Egyptian governorate (three samples for each) and stored at 4°C until analysis. The weight of each sample was approximately 1 kg.

#### Physiochemical properties of some Egyptian bee honey

**samples:** Physiochemical analysis was conducted in "Elements Laboratory, Campus of Research Laboratories, FARP", Faculty of Agriculture, Cairo University Research Park. Determination of water content of bee honey was carried out by measurements of its refractive index value (ABBE WAY-IS) refractometer<sup>17</sup> at 20°C. The quantity of sugars (glucose and fructose) was performed by HPLC according to the method of Bogdanov *et al.*<sup>18</sup>. The electrical conductivity was determined by the method of Bogdanov<sup>19</sup>, using EC meter model EN50081-1 at room temperature (2 g of honey sample was dissolved in 10 mL of distilled water and the results were expressed as ppm). The optical density and color of the honey samples were measured by using the relation between optical density and USDA standard as indicated by White<sup>20</sup>.

The bee honey samples were classified according to their botanical source based on the method of Von Der Ohe<sup>21</sup>. Pollen classification as: Mostly pollen (more than 45%), important minor pollen (10-20%) and minority pollen (less than 3%) by Andrade *et al.*<sup>22</sup>.

**Rheological properties of some Egyptian bee honey samples:** Rheological measurements of bee honey samples (viscosity, shear stress and torque) were carried out in triplicates over temperature of 25°C using a concentric cylinder Brookfield Programmable viscometer (Model DV-II, Brookfield Engineering Laboratories, USA) with UL adaptor and using spindle 7 at several RPM (5-100) in the shear rate range<sup>23</sup> from 6.1-122 s<sup>-1</sup>. Win Gather version 1.1, (Brookfield Engineering Laboratories, Inc.) software was used to collect, store and plot the data on a personal computer connected to the viscometer.

**Statistical analysis:** The two-way statistical analysis of variance (ANOVA), mean separation and correlation required subprogram of MSTAT microcomputer statistical program<sup>24</sup> was applied to evaluate physiochemical and rheological parameters of some Egyptian honey samples. Simple and multiple linear regression analysis were applied and the student t-test was used to test mean at p<0.05.

#### RESULTS

#### Physiochemical properties of some Egyptian bee honey

**samples:** The physiochemical properties of three different types of Egyptian bee honey "citrus (*citrus* spp.), clover (*Trifolium alexandrenum*) and marjoram (*Majorana hortensis*) were determined. As shown in Table 1 there were clear significant differences in all tested parameters which was conducted on tested Egyptian honey types. The water content (moisture %) of bee honey samples ranged from 18.10% in marjoram honey to 19.74% in citrus honey. Whereas the electrical conductivity (EC) of citrus honey was recorded 0.007% while in clover and marjoram honeys were recorded 0.008 and 0.011%, respectively.

On the other hand pH values of the studied Egyptian honey types ranged between 3.56 in citrus honey and 3.84 in marjoram honey. The obtained data showed that, the glucose content was ranged from 28.00% (marjoram honey) to 30.95% (clover honey) and the fructose content was recorded 39.12% in clover honey, while in marjoram and citrus honeys were detected 37.50 and 37.17%, respectively. The optical density (O.D.) of bee honey samples were measured in Table 1. The O.D. was ranged from 0.25 in marjoram honey to 0.14 in citrus honey.

**Rheological properties of some Egyptian bee honey samples:** Rheological (viscosity, shear stress and torque) properties were periodically determined at 25°C in cP. Citrus honey scored the lowest values for viscosity 11400 cP, shear stress 1390.8 D\cm<sup>2</sup> and torque 875.1%, while marjoram honey showed the maximum values 22750 cP, 2775.5 D\cm<sup>2</sup> and 921.1%, respectively.

In ascending series, citrus honey, clover honey and marjoram honey were represented their values in Fig. 1. Shear stress expressed the force exerted on a specific sample area (1 cm<sup>2</sup>) followed the same trend as viscosity. The maximum shear stress and torque values were measure in marjoram, clover and citrus bee honeys, respectively as shown in Fig. 1.

Analysis of variance showed significant differences (p<0.001) in viscosity, shear stress and torque as influenced by type of honey and rpm (round per minutes). Statistical analysis of variance, range and regression analysis indicated that type of honey significantly (p<0.001) affected viscosity, shear stress and torque as shown in Table 2. Moreover, LSD confirmed significant differences within honeys Table 2 at  $\alpha = 0.05$  level also, high R<sup>2</sup> values assured corrected data.

A proportional relationship was found between shear stress torque and the rpm values. Steep rise in shear stress and torque occurred and found that significant differences were found between the honey bee types as a function of rpm. Different shear-dependent fluids behave differently: For some, their viscosity decreased when the shear rate increased as shown in Fig. 2a, b and c.



Fig. 1: Average viscosity (cP), shear stress (SS values\*10) and torque (T, values\*10) at 25 °C

Table 1: Physiochemical properties of the tested Egyptian bee honeys

Types of honey	Physiochemical properties						
	Moisture (%)	E.C. (%)	рН	Glucose (%)	Fructose (%)	0.D.	
Citrus honey	19.74±0.26	0.007±0.00	3.56±0.08	28.20±0.30	37.17±0.35	0.14±0.02	
Clover honey	19.42±0.22	0.008±0.01	3.63±0.09	30.95±0.25	39.12±0.26	0.16±0.02	
Marjoram honey	18.10±0.17	0.011±0.00	3.84±0.11	28.00±0.00	37.50±0.00	$0.25 \pm 0.00$	
OD: Optical density							

Table 2: Statistical analysis of variance, range and regression analysis of honeys

Statistical analysis	F-value	Probability	LSD	R <sup>2</sup>				
Rheological properties								
Viscosity	35683.5900	***	1.8910	0.940				
Shear stress	38506.8900	***	0.9706	0.920				
Torque	10.9251	***	0.2901	0.803				

\*\*\*p<0.001, \*\*p<0.01 \*p<0.05, NS: Not significant



Fig. 2(a-c): Viscosity, shear stress and torque of (a) Marjoram honey, (b) Clover honey and (c) Citrus honey at different shear rate values

As shown in Fig. 2, the viscosity decrease as a shear rate change by increasing the round per minute (rpm) of the plunger in tested honey as shown in Fig. 2a while torque and the shear stress values increased. The viscosity of each honey type had been measured over a range of shear rates from 6.12-122 sec<sup>-1</sup> the shear stress was proportional to the strain rate in the fluid Fig. 2a, b and c. A thixotropic fluid can be described as a shear thinning system where the viscosity decreases with increasing shear rate as shown in Fig. 2. Honeys were subjected to increasing shear rates. The viscosity of a shear thinning fluid decreases with increasing shear rate. This shear rate dependency of the viscosity can differ substantially between different honeys type depending on concentration of total solid and water contents. The reason for shear thinning flow behavior is that an increased shear rate deforms and rearranges particles, resulting in lower flow resistance and consequently lower viscosity while, shear stress values increased due to need more stress for deformation of particles.

#### DISCUSSION

Results of the physicochemical analysis of three types of Egyptian bee honey samples suggested that, the floral sources of honey are the most important factor in its variety. The data obtained are presented in Table 1 which were in the range of related standards and were similar to the values found by other researchers<sup>25</sup> and showed in general a good quality of the studied honey samples. Higher moisture contents could lead to undesirable honey fermentation during storage, caused by the action of osmotolerant yeasts, The moisture values which obtained in this study were similar to those found with Serem and Bester<sup>25</sup> which recorded from 15.3-21.7 g/100 g and with Saxena *et al.*<sup>26</sup> which recorded (from 14.6-21.8 g/100 g).

In general, colour and conductivity are parameters that are inter-correlated and also with the mineral content and the botanical and geographic origin of honey. The E.C. values were in the same range as those reported by other authors in bee honey<sup>27,28</sup>. The darker the honey higher was the mineral content and the conductivity<sup>29,30</sup>. On the other hand, the sugar content (glucose and fructose) of bee honey samples were similar to those found with Escriche et al.<sup>31</sup> which detected the levels of glucose (from 27.8-31.9 g/100 g) and fructose (38.3 and 42.7 g/100 g). Honeys with high fructose/glucose ratio would remain liquid for longer periods because of the modification of the saturated level of glucose by the presence of the larger amount of fructose. The pH values of three honey samples were measured and the obtained results confirmed that, all tested samples were acidic, pH values were agree with that obtained by Rateb<sup>32</sup>.

The high acidity of honey correlates with the fermentation of sugars present in the honey into organic acid, which is responsible for two important characteristics of honey: Flavor and stability against microbial spoilage<sup>33</sup>.

Knowing the viscosity of honey is important. Many of the steps involved in extracting and processing honey are difficult to do if the honey is too viscous. Bee honey's viscosity depends upon the amount of water and the type and amount of sugar it contains. If the concentration of water is increased, bee honey becomes less viscous<sup>28</sup>. Temperature also changes the viscosity of bee honey and heat is often used to make the honey easier to process.

There are no other studies in the literature regarding the prediction of Egyptian honey rheological parameters using a concentric cylinder Brookfield Programmable viscometer (Model DV-II, Brookfield Engineering Laboratories, USA).

A viscosity function shows the viscosity over the shear rate. For a Newtonian liquid, this function is a straight line but If its viscosity changes with the shear rate, a liquid is non-Newtonian and for exact definition apparent viscosity need to be specified. The viscosity of honey decreases as shear rate increases with respect to water content in the individual samples. In multiple published reports on the rheological properties of honey, there is a common observation that it is in fact a Newtonian fluid but current data observed disagree with them<sup>34</sup>. The thixotropy (time depended) behavior was observed in honeys sample.

To the author knowledge there are some papers on the rheological behavior of honey bee using a falling ball viscometer based on water content, different temperature and shear rate. It can be observed that all the parameters are influenced primarily by the type of honey bee, followed by the moisture content. This fact is in agreement with other studies, which revealed the high influence of moisture content on the rheological parameters. In the case of glucose and fructose, they had less influence on the rheological parameters than the moisture content.

#### CONCLUSION

This study investigated the physicochemical characterization and rheological properties of different honey samples to confirm its economical and nutritional quality. Determination of rheological behavior of substances is particularly important for designing of equipments, for transport, pumping, storage of substances and an indirect measure of product consistency and quality. Because of pure honey exhibits thixotropy time dependent flow pattern so outcome of this study can be applied in processing, quality

improvement and product storage of honey it was also established that rheological properties of honey is a newly useful tool for detecting honey adulteration.

#### SIGNIFICANCE STATEMENT

This study discover a newly and accurate protocol that can be beneficial for assessment the quality improvement and storage of Egyptian honey. Pure honey exhibits thixotropy time dependent flow pattern so this study will help the researcher to uncover the critical areas of honey adulteration that many researchers were not able to explore. Thus a new theory that pure honey is a Non Newtonian fluid may be arrived at.

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