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Applied Chemistry

Elixir Applied Chemistry 165 (2022) 56160-56163

ISSN: 2229-712X

# Determination of Physicochemical Properties of Honeybee from Different Regions in Sudan

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**ARTICLE INFO**

**Article history:**
Received: 4 March 2022;
Received in revised form:
29 March 2022;
Accepted: 4 April 2022;

**Keywords**
Sudanese's Honey,
Honeybee,
Diastase,
Physiochemical.

**ABSTRACT**
Honey is a natural product, it is used for different purposes as nutritional, medicinal and industrial and it is an important commodity in the local and international markets. This study has been carried out for honey analysis from four different regions in Sudan to determine the physiochemical characterization. The ash content in range 0.173 % to 1.013% within Codex standard 0.6 %. Moisture content range was 13.936 % to 15.863 % the standard value 20%. The protein content range was 0.875 % to 1.750 %. Insoluble solids content ranged from 0.066 % to 0.337 %, with standard value 0.1%. Diastase activity content not detected in one samples and maximum value 10.241 Schade. The pH and acidity determined where pH ranged 3.81 to 4.77. Free acidity determined, results ranged from 16.00 to 33.50 meq/Kg, obtained within international standards 50 meq/Kg. Lactone content ranged from 118.50 to 297.00 meq/Kg. The total acidity was ranged from 134.5 to 314.5 meq/Kg. Analyzed honey samples are of high quality and conform to international standards. The variation of some compounds which detected in honey sample due to botanical origin of honey and other the parameters influence in honey quality.



## Introduction

Honey is a natural product, it sweet juice substance where is produced by honeybees (*Apis mellifera*) from the nectar of blossoms, from the secretion of living parts of plants or from excretion of plant sucking insects which live on plants (Codex Alimentarius Commission. 2001). Africa is contained with numerous types of wild honeybee (Adjare, S.O. 1990), and more of Africa countries have various forests and woodlands which contain diverse plant species that provide surplus nectar and pollen to foraging bees. The Sudan is one of African country has diverse forests and has diverse types of wild honeybee. The composition and properties of honey samples are highly depending on the type of flowers visited by the bees as well as regional and climatic conditions and on the contribution of the beekeeper (Al ML *et al*., 2009).

Honey is primarily a concentrated solution of sugars, composed mainly of glucose and fructose, together with other components such as organic acids, enzymes, vitamins, acetylcholine, flavonoids, and trace minerals (Molan P.C. 1996). All these minor constituents are known to have distinctive nutritional or medicinal properties and the unique blend accounts for the varied and different applications of natural honeys (James O.O, *et al*. 2009). Honey contained carbohydrates that comprise the major portion of honey 82% (Hak-Gil *et al*.,1988), proteins that include a number of enzymes (diastase, invertase, glucose oxidase, catalase and others), as well as eighteen free amino acids, of which the most abundant is proline, and trace amounts of the vitamins B, riboflavin, niacin, folic acid, pantothenic acid and vitamin B6. It also contains ascorbic acid and the minerals calcium, iron, zinc, potassium, phosphorus, magnesium, selenium, chromium and manganese (White *et al*., 1962). Honey is made by bees and their raw material for nearly all the world's supply of honey is nectar produced in the nectars of flowers; nectars are the main source of an aqueous solution of various sugars. But pollen has an exceptionally high vitamin content specially the water soluble vitamins. The vitamins B complex plus inositol and ascorbic acid are present in pollen (Haydak *et al*., 1944). Also the honey components contain antioxidant compounds, certainly an antioxidants play an important role in food preservation and human health by combating damage caused by oxidizing agents. The antioxidant capacity in honey is the result of the combined activity of a wide range of compounds including phenolic, peptides, organic acids, enzymes, Maillard reaction products and possibly other minor components (Gheldof, N., *et al*. 2002).

Honey has been claimed to have therapeutic properties in the treatment of digestive, respiratory, cardiac and rheumatic disorders, among others. Several studies have reported honey's immunological, antibacterial, anti-inflammatory, antipyretic properties besides its importance in terms of energy intake; where is honey as a healthy natural food, traditionally used as an alimentary supplement to which are attributed many antioxidants and antimicrobial properties (Seraglio, S.K.T., *el al*. 2019). The main objective of this study to determine physiochemical properties for different type of Sudanese honey samples collected from different climatic regions.

## Material and Methods

### Samples Collection

The samples collected randomly from four regions, Boram, Kotom, Aldamazin and South Sudan. All honey samples were directly obtained from beekeepers, the

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beekeepers directly extracted the samples, one kilogram of it was keeping into glass sample bottles with covering, and then the sample bottles encased and stored in a dry and dark place at room temperature (25°C).

**Physicochemical analysis**

Physicochemical compositions of honey samples were tested for determination of ash content, moisture content, protein content water insoluble solids, pH, free acidity, diastase activity, total acidity, lactone.

**Ash content**

Ash content was determined by burning honey samples in an electric muffle furnace, where 10 g of honey samples weighted into ignited and weighed Platinum dish. Were heated slowly until test sample became black and dry. Placed an ash in furnace at 600°C to constant weight after that cooled and weighed (AOAC 2000).

**Moisture content**

The moisture content of honey samples determined by Means of Refractometer method (AOAC 2000). the digital refractometer used for reading of honey samples at 20°C and obtain corresponding percent moisture from the refractive index table.

**Protein content**

The protein content was determined by using the micro-kjeldahl method according to (AOAC 2000) method**.**

**Water insoluble solids**

20 grams of honey weighed and dissolved in 200 ml distilled water at 80 °C and mixed well. The test sample was filtered through a previously dried and weighed fine sintered glass crucible and washed thoroughly with warm water until free from sugar. The crucible was dried at 135 °C for an hour, cooled and weighed. It returned to the oven for 30 minute intervals until constant weight is obtained. The calculation and expression of results as percent water insoluble solids by following equation (Bogdanov. 2009).

$$\text{Insoluble solid \%} = \frac{m}{m_o} \times 100$$

Where, m is mass of dried insoluble solid, and $m_o$ is mass of honey taken.

**Determination of pH and acidity**

10 g of honey sample dissolved in 75 ml of carbon dioxide-free water in a 250 ml beaker. The solution stirred carefully with the magnetic stirrer, and then immersed the pH electrodes in the solution and record the pH. Titrated with 0.5M NaOH at rate of 0.5 ml/min, the addition of NaOH stopped at pH 8.50. Immediately pipeted in 10 mL 0.05M NaOH, and without delay back-titrate with 0.05M HCl from 10 mL burette to pH 8.30. Calculate as milliequivalent/kg: (AOAC 2000)

$$\text{Free acidity} = (\text{mL 0.05M NaOH from buret - mL blank}) \times 50/\text{g test portion}$$

$$\text{Lactone} = (10.00 - \text{mL 0.05M HCl from burette}) \times 50/\text{g test portion}$$

$$\text{Total acidity} = \text{free acidity} + \text{lactone}$$

**Diastase activity**

The reagents required to estimate the diastase activity in honey samples were prepared and standardized according to official AOAC methods, where reagent used Iodine solution fresh preparations, Acetate buffer solution adjusted pH 5.30, Sodium chloride solution 0.5 M, and Starch solution. 5 g of honey sample dissolved in 15 mL distilled water and 2.5 mL buffer solution, and transferred to 25 mL volumetric flask containing 1.5 mL NaCl solution. Diluted to volume. Pipetted 5 mL starch solution into side arm of reaction tube and 10 mL test solution into bottom of tube, with care not to mix. Placed the tube in water bath about 15 min at 40 °C, and then mix contents by tilting tube back and forth several times. At 5 min, removed 1 mL aliquot with 1 mL serological pipet and added rapidly to 10.00 mL dilute $I_2$ solution in 50 mL graduate. Mixed, and diluted to previously determined volume, and determine *A* in photometer. The absorbance was recorded and plotted *A* against time (min) where calibration curve was obtained. According to the official AOAC method the number 300 was divided by the time needed to reach the absorbance value of 0.235 and expressed as DN or diastase number (AOAC 2000).

**Results and Discussion**

All honey samples were directly obtained from beekeepers which collected from four different regions in Sudan, analyzed for determination physical properties and chemical compassion, where parameters detected pH, ash content, moisture content, protein content and insoluble solids, shown in table 1, and following figures.

**Table 1. physicochemical results of honey samples analysis**

| Samples region | Ash % | moisture % | Protein % | Insoluble solids % |
|---|---|---|---|---|
| Kbmb | 0.359 | 15.863 | 0.875 | 0.223 |
| Boram | 0.207 | 13.936 | 1.750 | 0.066 |
| South Sudan | **1.013** | 15.581 | 0.875 | 0.274 |
| Damazin | 0.173 | 15.669 | 0.875 | 0.337 |

An ash contents of honey samples results shown in table 1, and figure 1, obtained at range 0.173 % to 1.013 %, where the range of contents in Sudanese honey between 0.12 to 1.21 g/100 (Yousif Mohamed. *et al* 2011), standard value of Codex standard for honey 0.6 % (Codex Alimentarius 2001), the ash content of all honey samples in range, the variation of ash contents refers to botanical origin of honey.

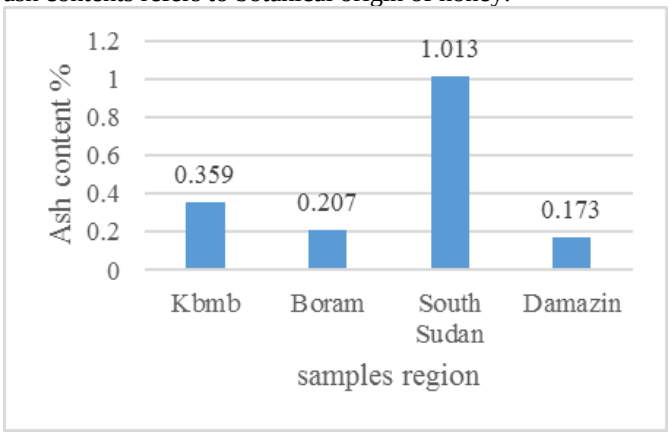


**Fig 1. Ash content in honey samples**

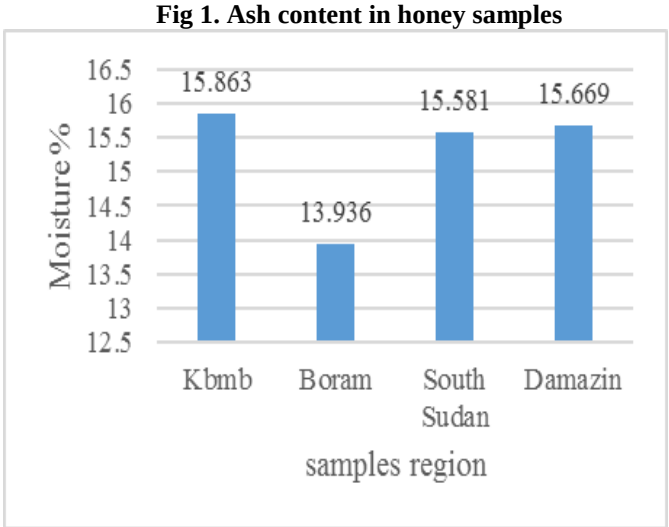


**Fig 2. Moisture content in honey samples**

Moisture content is an important parameter in honey quality, in this study the moisture content of all honey samples determined had been found at range between 13.936 % to 15.863 % were shown in table 1, and figure 2. The moisture continents of all honey samples within below international standard value not more than 20% (Codex Alimentarius. 2019). Variation of honey's water content depends on various factors, such as the harvesting season, the degree of maturity reached in the hive, and the geographic and environmental factors (Acquarone. *et al* 2007).

The protein content of honey samples found with range 0.875 % to 1.750 % shown in figure 3. The protein content in honey samples depend on botanical origin of honey. Usually honey contains a trace amount of protein originated from pollens which is a natural and protein rich food source (Schafer *et al* 2006). Water insoluble solids were determined in all honey samples, ranged from 0.066 % to 0.337 %, in table 1, and figure 4, the maximum acceptable value is 0.1 % (Codex Alimentarius. 2019). Moreover, honey contains water-insoluble solids that are often insoluble in water, which are solid materials of its components such as pollen, wax, honey-comb and particles of debris.

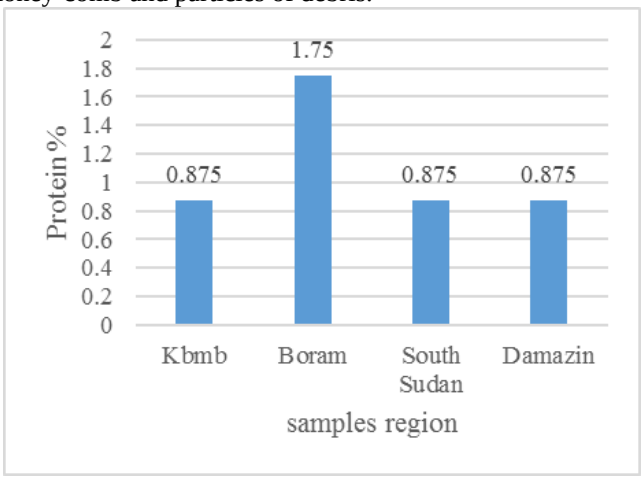


**Fig 3. Protein content in honey samples**

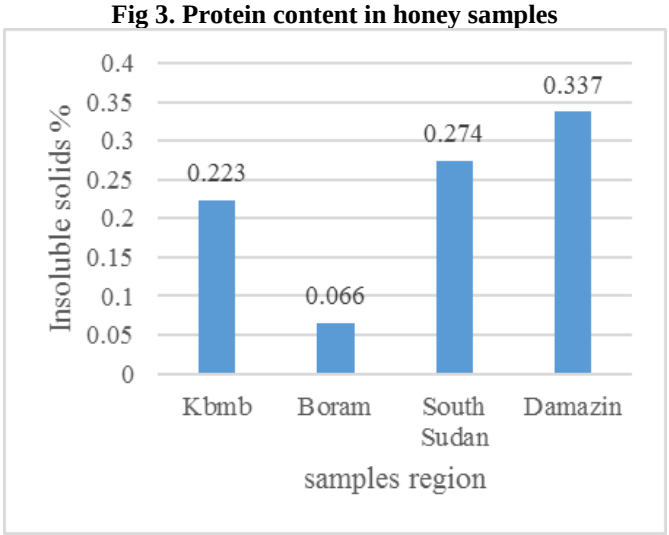


**Fig 4. Insoluble solids content in honey samples**

**Table 2. Diastase activity and acidity of honey samples analysis**

| Samples region | Diastase Activity | pH | Free Acidity meq/Kg | Lactone meq/Kg | Total Acidity meq/Kg |
|---|---|---|---|---|---|
| Kbmb | 10.241 | 3.81 | 33.50 | 218.00 | 251.50 |
| Boram | 6.338 | 3.98 | 23.00 | 136.00 | 159.00 |
| South Sudan | 6.461 | 4.77 | 17.50 | 297.00 | 314.50 |
| Damazin | ND | 4.17 | 16.00 | 118.50 | 134.50 |

In present study of the honey samples analyzed to determine diastase activity, free acidity, lactone and total acidity obtained results shown in table 2 and following figures. Diastase activity of the samples measured, were obtained in varies value shown in figure 5; Diastase activity is a quality factor influenced by honey storage and heating. The diastase activity of honey, in general not less than 8 Schade units and in the case of honeys with a low natural enzyme content not less than 3 Schade Units (Codex Alimentarius. 2019).

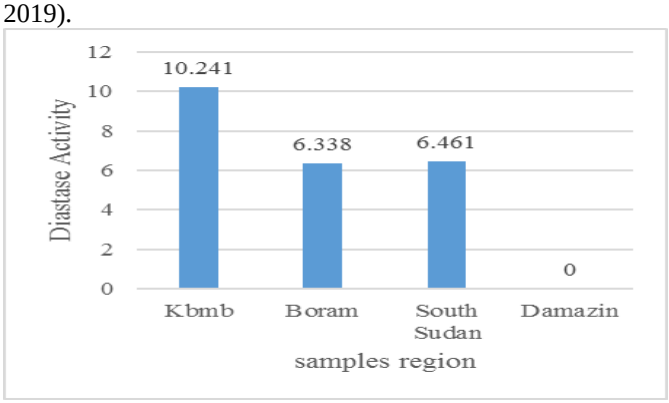


**Fig 5. The diastase activity of honey samples**

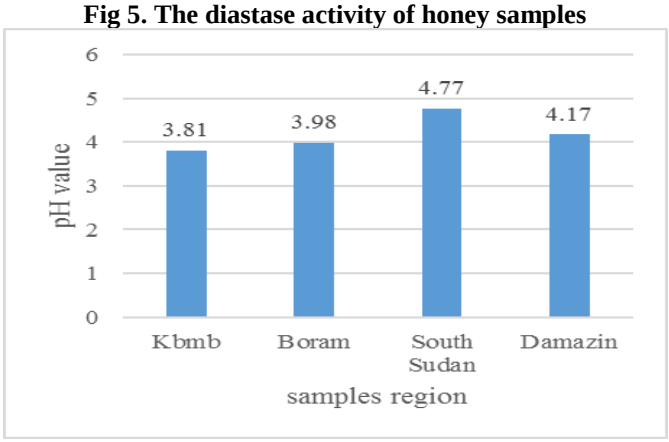


**Fig 6. pH values of honey samples**

pH measured in all samples were acidic, the result obtained at varies values ranged between 3.81 to 4.77 as showed in table 2 and figure 6. Where the standard range between 3.6 to 4.3 according to trial of the International Honey Commission (Bogdanov. 2009). The pH values of honey reported at Indian, Portugalian, Spanish, and Turkish with varies ranges as 3.70 to 4.40, 3.45 to 4.70, 3.63 to 5.01, and 3.67 to 4.57, respectively (Saxena, S. *et al* 2010., Kayacier, A. 2008). The pH values of Sudanese honey range reported between 3.70 to 4.80 (Yousif Mohamed. *et al* 2011), the measured pH values were in range.

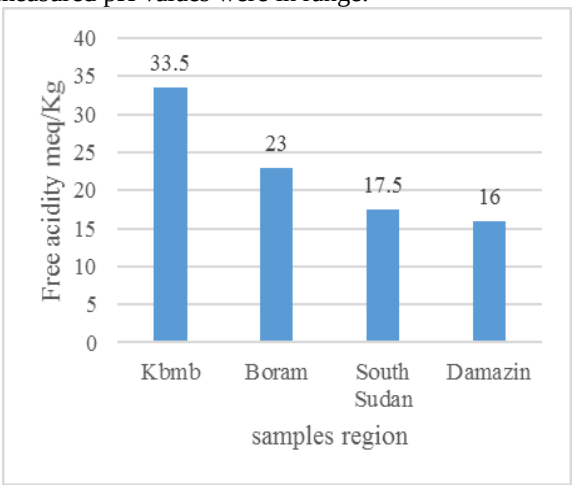


**Fig 7. The free acidity of honey samples**

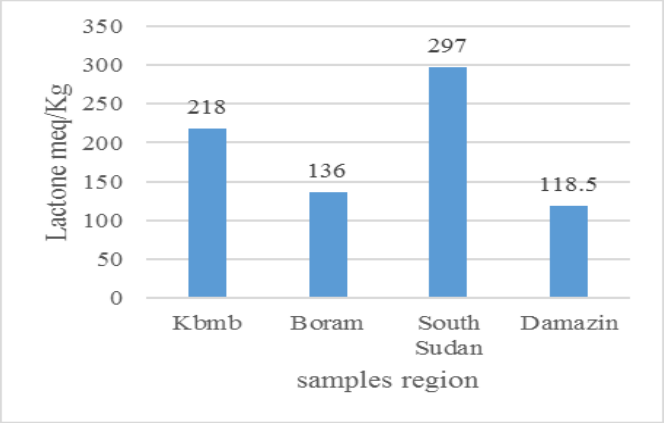


**Fig 8. The lactone of honey samples**

Free acidity of honey samples determined, results ranged from 16.00 to 33.50 meq/Kg shown in table 2, and figure 7, all obtained free acidity values were within international standards value was 50 meq/Kg at maximum free acidity (Codex Alimentarius 2001). Know free acidity is an important parameter related to the deterioration of honey. The higher free acidity values of honey samples may be indicative of fermentation of sugars into organic acids. There many parameters influence in honey quality, therefore the presence of different organic acids, geographical origin, botanical origin and harvest season can affect in the honeys' acidity. Lactone content of honey samples analyzed in this study ranged from 118.50 to 297.00 meq/Kg. The total acidity calculated from free acidity and lactone content in honey samples were ranged from 134.5 to 314.5 meq/Kg, we observed the total acidity levels are depending on lactone content and free acidity, the higher values may be indicative of fermentation of sugars into organic acids.

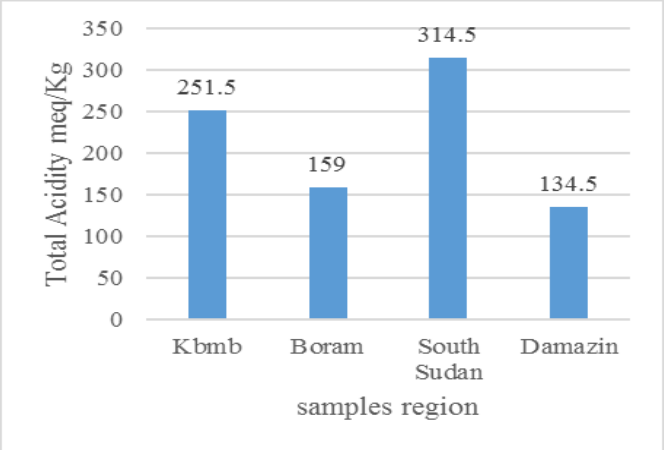


**Fig 9. The total acidity of honey samples**

## Conclusion

The results of this study referred to physio-chemical analysis of honey samples from four different geographic regions in Sudan. The values of quality parameters for all honey samples within international standard of honey. The results indicated honey purity, freshness, and good storage practices by beekeepers. The variation in total acidity because of fermentation of sugars into organic acids, and variation of other parameters in honey samples due to botanical origin of honey and difference of botanical diversities.

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