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# Microbiological Characterization and Physicochemical Properties of Sudanese Honeys

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***Authors' contributions***

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## ABSTRACT

**Aim:** Honey is not always a safe product and in some instances it is spoiled by the growth of micro-organisms. The aim of the study was to characterize honey on biological and physicochemical basis.
**Study Design:** Determination of the microbial loads in the Sudanese honey brands and characterization of their physicochemical properties.
**Methodologies:** Several microbiological tests were employed for the determination of the microbial loads. The methods of Association of the Official Analytical Chemists were employed for the physicochemical properties.
**Results:** The microbiological tests were negative for *Escherichia coli,* and total coliforms (mpn/ml). Few honey brands contained *Clostridium botulinum, Salmonella, Staphylococcus aureus,* yeasts and moulds. The maximum total viable bacteria count was > 3.77 log. cfu/ml. The results of the physical parameters tested were as follows: pH 3.6, specific gravity 1.2, viscosity 120.7 Poise, and refractive index 1.4., moisture 18.2%, acidity 54.2 (meq/kg), total sugars 70.5%, fructose 32.1%, glucose 32.8%, and sucrose 5.5%.
**Conclusion:** The physicochemical properties of the investigated samples comply with the *Codex Alimintarius* Standards for honey. However, some honey brands contained yeasts, moulds, as well as some pathogenic bacteria such as *Salmonella spp, Staphylococcus aureus, Clostridium botulinum.* Thus the study justified the importance of the proper

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processing condition of the honey such as the hygiene of beekeepers and beekeeping tools, pasteurization or irradiation of the honey.

*Keywords: Honey composition; Clostridium botulinum; Staphylococcus aureus; Salmonell spp.*

## 1. INTRODUCTION

Honey is a popular sweetener throughout the world; it is made by bees generally from nectars extracted from the nectarines of flowers. The chemical composition of honey mainly consists of water, glucose, fructose, sucrose, dextrin, vitamins, minerals and small quantities of microelements and proteins [1].

Since ancient times honey was used both as natural sweetener and healing agent [2], and is widely used as a topical antibacterial agent for treatment of wounds, burns and skin ulcers. Manuka honey has been reported to exhibit antimicrobial activity against pathogenic bacteria including multi-drug resistant *Staphylococcus aureus* strains and *Helicobacter pylori* isolates from gastric ulcers [2]. As an alternative to conventional antibiotic therapy, γ-irradiated Manuka honey is commercially available as a topical ointment for burned or wounded skin to protect from opportunistic bacterial infections or to cure chronic wounds [3]. Also there is clinical evidence of application of Sudanese honeys to infected wounds and chronic ulcers, the results showed advantages over conventional antibiotics [4].

There is a hypothetical risk of infection of wounds resulting from the application of honey, as honey sometimes contains viable spores of Clostridia [5]. However, any concern about risk of infection can be overcame by the use of honey that has been treated by gamma-irradiation, which kills *clostridia* spores in honey [6] without loss of any of the antibacterial activity.

In many societies honey has an important place in traditional food preparation and is also viewed as a source of special nutrition for children, although it should not be fed to young babies due to fear from infant botulism. Honey is widely used as a medicine and is highly prized for this reason. It is often regarded as a special tonic food to be eaten during illness [7].

The biological factors which contributes for honey's antagonistic activity against array of pathogenic microorganisms include its water absorption ability which makes it capable of killing bacteria by dehydrating them [8]; also honey is sterile as most micro-organism cannot survive in it; presence of enzymes which produces hydrogen peroxide that kills bacteria and its acidic nature [9]. Moreover, this antimicrobial activity is due to availability of plant polyphenolics [10] and most probably due to macromolecules assemblages such as proteasomes and IgM as well as peptides/glycopeptides, and proteoglycan - like molecules [11,12]. It is believed that the strong antibacterial activity of Manuka honey is due to the presence of the specific antibacterial substance methylglyoxal [13].

The frequent contamination of honey by micro-organisms comes from the nectar, pollen, and beekeeping tools. Thus the presence of micro-organisms in the honey can influence, sometimes, the stability of the product and its hygienic quality [14]. The aim of the present study was to characterize Sudanese honeys on biological and physicochemical basis

## 2. MATERIAL AND METHODS

### 2.1 Collection of Honey Samples

Nine genuine and commercial honey samples were randomly collected from different markets in Khartoum. The samples were labeled and unprocessed honeys. All samples are extracted or squeezed honeys. None of the samples was further processed by any technique to kill bacteria.

### 2.2 Microbial Counts in Honey Samples

Total viable counts and total yeasts and moulds as expressed in cfu/ml (colony forming units/millilitre) were performed according to Harrigan [15]. Total coliforms (mpn/gm) were carried out using most probable number (mpn) technique following Harrigans [15]. Detection of food - borne pathogens: *Staphylococcus aureus* was detected by transferring 0.1 ml of honey dilutions [10ml of the honey samples were added aseptically and homogenized in 90 ml of sterile 0.1% peptone solution and mixed well to give dilution ($10^{-1}$). Preparation of serial dilutions was continued until the dilution ($10^{-6}$)] to the growth media (Baird Parker agar) and incubated at 37ºC for 24 hours [15].Similar procedure was followed for detection of *Salmonella spp.* 25 ml of honey sample were mixed with 250 ml sterile nutrient broth and incubated at 37ºC for 24 hours. Then 10 ml were drawn aseptically and added to 100 ml selenite cystine broth and incubated at 37 for 24 hours. Then with sterile loop streaking was done on bismuth sulphite agar plates. The plates were incubated at 37ºC for 72 hours. Black metallic shiny discrete colonies indicate the presence of *salmonella*. *Clostridium botulinum* was detected on glucose-peptone-beef infusion broth; the culture was heat-shocked at 80-100ºC before incubation at 25-35ºC for 5-7 days. *E. coli* was detected by the presumptive test and confirmed by streaking positive presumptive into eosin methylene blue agar [15].

### 2.3 Determination of Honey Physical Parameters

The pH was measured by a digital pH meter. The specific gravity of the honey samples was determined by direct weighing procedure (Pycnometry) of Joslyn [16]. The viscosity was measured by viscotester model (Haake 6 plus thermo) following the manufacturer's instructions. The refractive index measurement was done with an Abbe refractometer (Hilger, M 64.315/56304 England). The refractometer's readings were adjusted to (20ºC).

### 2.4 Determination of Honey Chemical Parameters

Reducing sugars was determined by titration against Soxehlet modified Fehling's solutions. Sucrose was calculated by difference between reducing sugars before and after inversion following AOAC procedures [17]. Glucose was determined by iodometric method of the FAO [18]. Fructose was estimated by deducing glucose from total sugars. Moisture contents of the honey samples were determined from refractive indices using the Wedmore table [17]. Honey acidity was determined by dissolving 10 g honey in 75 ml carbon dioxide free distilled water and by titration against 0.05 M NaOH, the end point was adjusted to pH 8.5. Then 10 ml NaOH was added and back titrated against 0.05 M HCl till end point at pH 8.3. Acidity was expressed as miliequivalents/kg honey.

## 3. RESULTS AND DISCUSSION

Honey can be characterized in three ways through physical, chemical, and biological methods, although the physical and chemical tests usually predominated. There are almost many reports on honey microbial activities like antibacterial, antifungal and antiviral. However, few of them indicated contamination of honey with microorganisms. Nevertheless, this is the first report on the microbial loads in Sudanese honeys.

Honey is not normally processed, so it would be expected to contain a diverse microbial population originating from flowers, plants, and hives, as well as honeybees themselves [14].

The microbial loads of some Sudanese honey brands were investigated through presence/ absence detection methods. Fig. 1 represents the total viable counts in each honey sample. The average total viable bacteria were 3.73 (log. cfu/ml) and the maximum loads were found in samples F and G 5.4 log. cfu/ml and 4.7 log. cfu/ml, respectively. The results of the presumptive test shown in table 1 have revealed detection of some food-borne micro-organisms. Moulds and yeast were detected in samples D and E. Same observation was reported earlier on Sudanese honeys. Mohammed [19] detected the presence of yeast, moulds, and bacteria in both authenticated and commercial honey samples. *Staphylococcus aureus* was detected in samples B, E, G and H. Also *Clostridium botulinum* has detected in sample B and G. It was reported that moulds, yeasts and spore forming bacteria commonly exist in honey [20]. However, some reports indicated that honey has been very often incriminated as a source of spores of *Clostridium botulinum* responsible for causing the infant botulism [21]**.** The pathogenic bacterium *Salmonella* was also detected in sample B and I. Sudanese commercial honeys sometimes get fermented and it can be speculated mostly due to the presence of these pathogenic and non pathogenic food borne microorganisms. The studied samples were free from some virulent pathogenic bacteria such as coliforms, and *E. coli*. This might be due to susceptibility of these bacteria to honey (Table 1).

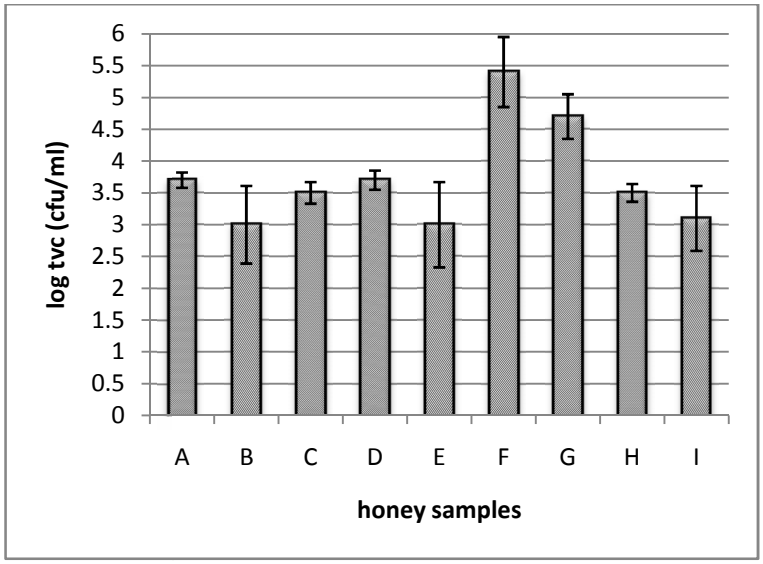


**Fig. 1. Total viable bacteria count in Sudanese honey samples**

**Table 1. Microbial loads of some Sudanese honeys**

| Organism sample Code | *Clostridium botulinum* | *Staphylococcus aureus* (cfu/ml) | *Salmonella spp.* | *E.coli* | Total *coliforms* (mpn/gram) | Total yeasts and moulds (cfu/ml) |
|---|---|---|---|---|---|---|
| A | - | - | - | - | - | - |
| B | + | + | + | - | - | - |
| C | - | - | - | - | - | - |
| D | - | - | - | - | - | + |
| E | - | + | - | - | - | + |
| F | - | - | - | - | - | - |
| G | + | + | - | - | - | - |
| H | - | + | - | - | - | - |
| I | - | - | + | - | - | - |

*Key: +: microbe was detected, -: microbe was not detected*

The physiochemical properties of the studied sample are consecutively presented in Table 2 and Table 3. The average pH (3.6), specific gravity (1.2), viscosity (120.7), and refractive index (1.4) are consistent with previous results of Sudanese honeys [22,23]. The average glucose (32.8 %), fructose (31.1 %), sucrose (5.5 %), and total sugar (70.5%) reported in the present study are similar to other reports [22-24]. Moisture is the most critical factor when considering honey fermentation. Honey is mostly prone to spoilage when its moisture is more than 20%. In the present study, samples H and I has > 20% moisture contents. The average acidity was 54.2 (meq/kg) which corresponds to value reported by other authors [22,23]. Generally, the physio-chemical properties of the investigated samples comply with the *Codex Alimintarius* Standards for Honey [25].

**Table 2. Physical properties of Sudanese honeys (Mean ± SD)**

| Sample code | pH | Specific gravity | viscosity (Poise) | Refractive index |
|---|---|---|---|---|
| A | 2.91 ± 0.08 | 1.23 ± 0.00 | 119.4 ± 0.3 | 1.49 ± 0.00 |
| B | 2.82 ± 0.03 | 1.25 ± 0.00 | 131.5 ± 0.6 | 1.5 ± 0.00 |
| C | 3.35 ± 0.05 | 1.32 ± 0.00 | 132.8 ± 0.6 | 1.5 ± 0.00 |
| D | 4.14 ± 0.06 | 1.22 ± 0.00 | 121.9 ± 0.7 | 1.49 ± 0.00 |
| E | 3.49 ± 0.03 | 1.10 ± 0.00 | 116.9 ± 0.4 | 1.49 ± 0.00 |
| F | 4.84 ± 0.03 | 1.34 ± 0.00 | 112.9 ± 0.6 | 1.49 ± 0.00 |
| G | 3.78 ± 0.03 | 1.19 ± 0.00 | 129.2 ± 0.8 | 1.5 ± 0.00 |
| H | 3.55 ± 0.06 | 1.82 ± 0.00 | 108.2 ± 0.1 | 1.49 ± 0.00 |
| I | 3.55 ± 0.00 | 1.40 ± 0.00 | 113.8 ± 0.9 | 1.49 ± 0.00 |
| Mean | 3.6 ± 0.61 | 1.2 ± 0.20 | 120.7 ± 8.7 | 1.49 ± 0.01 |

**Table 3. Chemical properties of Sudanese honeys (Mean ± SD)**

| Sample code | Glucose (%) | Fructose (%) | Sucrose (%) | Total sugars (%) | Moisture (%) | Acidity (meq/kg) |
|---|---|---|---|---|---|---|
| A | 33.5 ± 0.01 | 32.9 ± 0.09 | 4.0 ± 0.9 | 70.1±0.3 | 19.1±0.1 | 54.0 ± 0 .02 |
| B | 39.2 ± 0.00 | 27.7 ± 0.05 | 5.8 ± 0.7 | 72.1±0.0 | 15.1±0.1 | 61.0 ± 0.00 |
| C | 35 ± 1.10 | 31.9 ± 0.07 | 4.0 ± 0.5 | 70.1±0.1 | 15.4±0.0 | 83.0 ± 0.01 |
| D | 31.3 ± 0.02 | 32.9 ± 0.02 | 4.9 ± 0.7 | 69.2±0.3 | 16.5±0.1 | 39.0 ± 0.01 |
| E | 28.9 ± 0.03 | 32.1 ± 0.06 | 7.2 ± 0.0 | 68.0±0.7 | 20.0±0.0 | 37.0 ± 0.01 |
| F | 31.1 ± 0.00 | 32.1 ± 0.06 | 8.0 ± 0.5 | 70.1±0.2 | 19.1±0.1 | 81.0 ± 0.01 |
| G | 33.5 ± 0.02 | 31.1 ± 0..07 | 8.7 ± 0.2 | 75.0±0.1 | 16.4±0.1 | 43.0 ± 0.01 |
| H | 31.7 ± 0.01 | 33.1 ± 0.01 | 2.4 ± 0.7 | 69.3±0.7 | 21.3±0.1 | 52.0 ± 0.00 |
| I | 31.8 ± 0.01 | 35.6 ± 0.05 | 5.3 ± 1.3 | 68.7±0.6 | 20.3±0.1 | 36.0 ± 0.01 |
| Mean | 32.8 ± 2.90 | 31.1 ± 2.10 | 5.5 ± 2.1 | 70.5 ± 2.10 | 18.2 ± 2.30 | 54.2 ± 17.90 |

## CONCLUSION

Honey is not always a safe product and in some instances it is spoiled by the growth of micro-organisms. The study constitutes an investigation regarding microbial and physicochemical characteristics of Sudanese honey form difference sources. TVCs for bacteria, moulds and yeasts have been identified. The growth of food-borne pathogens have also been detected, *Salmonella spp, Staphylococcus aureus, Clostridium botulinum.*

Thus the study justified the importance of the proper processing (condition) of the honey with collection – packaging – proper handling techniques in order to destroy the microbial loads.

## COMPETING INTERESTS

All authors have declared that no competing interests.

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