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FULL PAPER

Physicochemical Properties and Quality Evaluation of some Sudanese Honey

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Abstract
The purpose of this research sought to analyse the physicochemical properties of eight local honey samples obtained from diverse flora and geographical regions in Sudan. The physicochemical characteristics, such as moisture content, refractive index, Brix (total sugar), pH, total acidity, ash content, HMF level, and electrical conductivity, were assessed according to the International Honey Commission guidelines. The pH of honey samples ranged from 4.40 to 6.00, moisture content varied from 13.4% to 25%, and the refractive index extended from 1.4636 to 1.5034. The brix concentration ranged from 68.7% to 84.3%; total acidity varied between 20 and 96 meq/kg; ash level oscillated from 0.1950% to 0.4975%; HMF levels spanned from 8.99 to 1140.28 mg/kg; and electrical conductivity measured between 0.54 and 2.14 mS/cm. The findings were juxtaposed with prior research, and most of the examined samples adhered to these norms. These findings highlight the accuracy and quality of the samples in comparison to established standards. The research emphasises the necessity of continuous monitoring to ensure these indicators stay within permissible ranges, therefore protecting consumer health and upholding industry standards. The findings of this investigation demonstrated that honey samples predominantly met acceptable quality standards. The quality of honey samples varies according to several factors, including the period, process circumstances, floral provenance, geography of origin, and storage period.
Keyword: Honey, HMF, refractive index and electrical conductivity

Introduction

Honey it is natural sweet material made by Apis mellifera bees from plant nectar or secretion of live plant components, which the bees convert, store, and dehydrate in the honeycomb (Codex Alimentarius Commission 2001). It's been valued since ancient times not only as a nutritious food but also as a therapeutic agent due to its diverse biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Bogdanov et al 2008). Several variables impact the content and quality of honey, including its botanical source, geographical location, climatic conditions and processing methods (Khalil et al., 2012). These variations result in significant differences in the physicochemical properties, sensory characteristics, and bioactive components among honeys produced in different regions.

In Sudan, beekeeping is a significant agricultural endeavour contributing to rural livelihoods and the national economy. The country possesses diverse floral resources ranging from acacia species in arid zones to tropical vegetation in the southern regions, leading to the production of honeys with distinct floral and geographical signatures (Mohamed et al., 2018). Despite this potential, limited scientific data are available on the physical and chemical properties and quality attributes of Sudanese honey, particularly in comparison with international standards. Systematic evaluation of honey from various regions of Sudan is therefore essential to assess its compliance with global quality parameters and to promote its competitiveness in local and export markets.

Physicochemical parameters play a key role in determining honey quality and authenticity. Parameters such as moisture content, pH, free acidity, electrical conductivity, ash content, total sugars, and hydroxymethylfurfural (HMF) levels are commonly used as indicators of honey purity and freshness (Terrab et al., 2003). For instance, high moisture content may enhance fermentation and microbial growth, while excessive HMF content indicates heat damage or prolonged storage (White, 1979). Electrical conductivity and ash content reflect the mineral composition, which varies with botanical and geographical origins (Alqarni et al., 2012). Moreover, color intensity and refractive index are often associated with the concentration of phenolic compounds and antioxidant potential, providing further insight into honey's nutritional and therapeutic value (Azeredo et al., 2003).

Quality evaluation of honey extends beyond physicochemical testing to include sensory, microbiological, and adulteration assessments. Adulteration through the addition of sugar

syrups or other sweeteners has become a growing concern worldwide, affecting consumer confidence and market value (Da Silva et al., 2016). Spectroscopic and chromatographic techniques, including UV–Vis spectrophotometry, FTIR, and HPLC, are increasingly used to detect adulteration and verify floral or geographical origin (Ruoff & Bogdanov, 2004). Establishing reliable quality profiles for Sudanese honeys using internationally recognized analytical methods will thus provide a scientific basis for authentication and standardization.

Comparative studies of honey from different countries, such as Saudi Arabia, Egypt, and Ethiopia, have demonstrated considerable variation in physicochemical parameters and antioxidant capacities (Alqarni et al., 2012; Gebremedhin et al., 2020). These differences underscore the influence of floral source and environmental conditions on honey composition. Evaluating Sudanese honey within this broader regional context can reveal its unique characteristics and potential competitive advantages. Furthermore, linking physicochemical properties with sensory quality and consumer acceptability may assist in establishing a scientific basis for branding and geographical indication labeling.

In recent years, international standards such as those issued by the Codex Alimentarius and the European Commission have emphasized the significance of physicochemical testing as a prerequisite for honey quality assurance (Codex Alimentarius Commission, 2001). Meeting these standards not only ensures product safety and authenticity but also enhances market access and consumer trust. Therefore, this study aims to determine the physicochemical properties and evaluate the quality of selected Sudanese honey samples collected from different ecological zones. The findings are expected to provide valuable data for national quality specifications, support local beekeepers, and contribute to the global knowledge base on honey characterization.

Materials and Methods

Samples

Eight samples of honey were collected from different geographical regions and botanical origins in Sudan. The samples were stored at room temperature(table 1).

Table 1: Honey samples Collection and Dates

Sample No	Date of harvest	Botanical origin	Geographical Origin
1	2/2015	**Talih** *(Acacia nilotica , subsp niloticca ,family)*	Blue Nile state- East of Aldmazeen
2	3/2015	**Sunflower** *(Helinathus annuus , family Asteraceae)*	East Sudan- Alfao
3	10/2015	**Sunnut** *(Acacia nilotica, subsp nilotica, family)*	Sennar state- singa
4	11/2015	**Sedir** *(ziz phus spina-christi family rhannaceae)*	Blue Nile state- Aldinder
5	1/2016	**Sedir** *(ziz phus spina-christi family rhannaceae)*	Um dafoog-
6	1/2016	**Sunut** *(Acacia nilotica, subsp nilotica, family)*	North of kordofan
7	1/2010	multifloral	East Sudan- Alfao
8	1/2010	**Sunut** *(Acacia nilotica, subsp nilotica, family)*	East Sudan- Algadarif

Determination of refractive index and brix

The refractive index and brix were determined according to Bogdanov, 2009. The ABBE' Refractometer was adjusted first, using distilled water (RI=1.333). A few drops of sample were placed on the dispersion prism, the Refractometer was then closed, and the sample RI was read.

Determination of water content

Water content is a quantity ascertained from honey refractive index (Bogdanov, 2009) with reference to table of standard (International Honey Commission). The standard table derived using a graph showing the water content plotted against the logarithm of the refractive index minus one.

Determination of free acidity and pH

The free acidity and pH were assessed following Bogdanov (2009). Ten grammes of sample were dissolved 75 ml of water free from carbon dioxide, agitated using a magnetic stirrer and the pH was documented. The solution titrated with 0.1 M sodium hydroxide to a pH of 8.3 using a 10 mL burette. The acidity of honey refers to the total concentration of free acid, measured in milliequivalents per kilogramme of honey.

F.A = ml of 0.1M NaOH x 10

Determination of ash content

Five grams of the sample (Bogdanov, 2009) was weighed into a crucible (M_0). The water evaporation and ashing procedure started at a low temperature, gradually increasing to 350-400°C. Subsequent to the initial ashing, The crucible was placed in the furnace and exposed to a temperature of 600°C for 4 hours. The crucible was cooled in the desiccator and then weighed. The ashing technique was repeated until a consistent weight was reached. The fraction of ash WA in 100g honey may be determined using the following formula:

$$WA = (M1-M2)/M0 \times 100$$

where M0 represents the mass of honey obtained.
M1 represents dish mass plus ash, while M2 = dish mass alone.

hydroxymethylfurfural(HMF)

This method was done according to Bogdanov, 2009. Five grammes of sample were dissolved in about 25 ml of deionised water, diluted to 50 ml with deionised water, filtered through a 0.45 µm filter, and injected through HPLC.

Chromatographic condition:

Mobile phase: 80% water (5% acetic acid):20% methanol, **Column:** C_{18} (25 x 0.46)cm,(5 µm)

Flow rate 1ml\min, **injection volume** 20 µl, λ_{max}: 285 nm

Determination of conductivity

Ten gramme of sample were dissolved in 75 ml of carbon dioxide-free water, the cell was immersed in solution and the electrical conductance was recorded(according to Bogdanov, 2009).

Results and Discussion

Determination of water content, refractive index and brix

The results of the refractive index and brix of the honey samples are shown in **table 2** The brix content ranged from (68-81.8%), water content ranged from(16.6-25%) and refractive index from (1.4622-1.5021). tow sample out of 8 were found without the limit for these tests' guidelines established by the Codex Alimentarious Commission in 2001 That is, not greater than 20%. There is no discernible difference in moisture, refractive index, or brix between the various kinds of floral and geographical origin. Several factors impacted moisture content, including climate, season of year, maturity or ripeness, processing processes, storage conditions, botanical origin of the sample, and moisture content of the primary plant nectar. (Nandaa, V et al 2003; Costa, L. et al 1999; Ozcan, M.D. et al 2006).

Sample 8 (table 2) was found to have higher water content (25%) and lower refractive index(1.4636) and brix(68.7%); sample 7 was found to have lower water content(13.3%) and refractive index (1.5034) and brix(81.8%); there is a negative correlation between water content and brix or refractive index. The samples' higher sugar content (brix) might be the cause of the low moisture content that was noticed and or a dry harvest season with low humidity or due to early harvesting from comb.

The water content in the studied Sudanese samples was found to be similar than study of Adgaba, N. (**1999)**

Table 2: The Water content, Refractive index and Brix in honey samples collected from different regions:

Sample No	Water content %	Refractive Index / 26^0C	**Brix %** 26^0C
1	16.0	1.4967	81.8
2	17.0	1.4942	80.9
3	21.4	1.4854	77.5
4	17.8	1.4919	80.0
5	16.8	1.4946	81.0
6	19.6	1.4875	78.3
7	13.4	1.5034	84.3
8	25.0	1.4636	68.7

Determination of free acidity and pH

Regardless of their geographical origin, all of the samples tested were acidic. Their pH varied from 4.40 to 6.00 (Table 3), which is nearly identical to the pH ranges of Algerian honeys (3.49 to 4.53) (Ouchemoukh, S. et al 2007) and Portuguese honeys (3.70 to 4.30) (Gomes, S. et al 2010). Honey pH levels are critical during extraction and storage because they affect texture, stability, and shelf-life (Baroni, M et al 2009). Honey's low pH prevents bacteria from surviving and growing. The pH of Sample (4) was found to be higher than the Codex Alimentariou Commission's (2001) range of 3.2 to 5. These pH increases were ascribed to Depending on the environment, time of year, level of ripeness, and handling methods, and storage conditions. These pH variations are mostly attributable to the diversity of various acids and minerals contained in honey. Likewise, the floral difference may also cause different ranges of pH. The total acidity of seven analysed samples was determined to be well below the specified limits of maximum 50 meq/kg suggested by the Codex Alimentarious Commission (2001); only one sample exceeded these limits (table 3).

Honey's high acidity indicates that the sugars in the honey have been turned into organic acid, which gives honey its taste and keeps it from going bad due to microbes (Bogdanov S. et al 2008). Honey is acidic because it has gluconic acid and other organic acids (lactones or esters) in balance with metal ions (phosphate and chloride). (Nandaa V. et al 2003). Acidity differences amongst honey kinds can be linked to variations in these elements caused by the extraction season (Perez-Arquillue C. et al 1994). The free acidity and lactonic acidity values were collected from several places in Ibadan; however, the acidity values from 17.4 to 33.4 meq kg-1, while the lactonic acidity values ranged from 5.7 to 12.3 meq kg-1 (Adenekan et al 2010).

Table 3: pH and Total Acidity in honey samples collected from different regions:

Sample No	pH	Acidity**(meq/kg)**
1	5.20	45
2	4.70	34
3	4.73	50
4	6.00	20
5	4.30	30
6	5.10	35
7	4.40	45
8	4.50	96

Determination of Ash

Honey often has low ash concentration (de Rodríguez et al. 2004). The ash concentration of all honey samples analysed was confirmed to be below the Codex Alimentarious Commission's specified limits of 0.6% (2001). Their ash levels ranged from 0.1950 to 0.4975% (table 4). The talih sample has a low ash level (0.1950%). Honey's ash composition can change depending on harvesting procedures, beekeeping practices, and the material acquired by bees while feeding on flora (Ouchemoukh, S. et al. 2007). The ash content reflects the wide variety of honey formulations. Ash concentration serves as a quality criterion for botanical honey; floral honeys include lower ash levels compared to honeydew honeys. (Ouchemoukh, S et al. 2007).

Table 4: The Ash content in honey samples collected from different regions:

Sample No	Ash content (%)
1	0.1950
2	0.2494
3	0.4436
4	0.2476
5	0.4975
6	0.2985
7	0.1990
8	0.3960

Determination of hydroxymethylfurfural(HMF)

Hydroxymethylfurfural (HMF) is an aldehyde and furan chemical generated during the thermal degradation of sugars and carbohydrates. HMF is a significant indicator of honey purity, since it is well recognised as a criterion for assessing honey freshness (Terrab A et al 2002). Elevated HMF concentrations in honey indicate excessive heating and inadequate storage conditions. The Codex Alimentarius Commission (Bogdanov S. 2009) advises that the HMF concentration in honey should not surpass 80 mg/kg (Alimentarius C. 2001). In addition to storage factors, such as temperature, the age of the honey and its floral sources may affect HMF levels (Terrab A. 2002). Research has revealed that HMF levels in honey held for extended durations (12-24 months) significantly increased (Khalil et al., 2010), beyond the threshold deemed suitable and secure for consumption by people. The amounts of HMF in the examined Sudanese honey samples varied as 8.99 to 1140.28 mg/kg. (table 5). Five of eight samples met the Codex Alimentarius Commission's limit. Two samples had greater HMF level than the Codex Alimentarius Commission's limit; this might be due to sugars

being converted into HMF during long-term storage. HMF may also be created by heating-catalyzed breakdown of hexoses, according to prior research (Bicchi C. et al 1983). Another element that might alter the HMF concentration of honey is Sudan's tropical environment, since hot weather has been shown to raise HMF levels in bee colonies (Ajlouni S. et al 2010). This is why the European Union (2001) set a higher limit of 80 mg/kg for honey produced in tropical climates. The HMF levels of the examined honey sample wereequivalent to the previously reported HMF content of Malaysian tualang and gelam honey (ranging from 6.65 to 1131.76 mg/kg) (Khalil MI et al 2010).

Table5: the HMF level in honey samples collected from different regions:

Sample No	Level of HMF (mg/kg)
1	45.46
2	12.66
3	80.66
4	8.99
5	111.70
6	ND
7	640.631
8	1140.281

Determination of Electrical Conductivity

The conductivity values ranged from 0.54 to 2.14 (**Table** 6), the Honey standard of the Codex Alimentarius Sandard it is not more than 0.8 mS/cm . Our results exceeded the minimum limits set by Codex Alimentarius (2001). Atanassova et al. (2009) estimated the EC values (0.117-0.428 mS/cm) for honey from Bulgaria. Rehman et al. (2007) found an EC value of 0.05 mS/cm for honey from Pakistan. Omafuvbe and Akanbi (2009) revealed that Nigerian honey had an average EC value of 0.03409mS/cm.

All of the ionisable organic and inorganic substances in honey are evaluated by electrical conductivity. It is commonly used in routine honey control instead of ash content and has been connected to the botanical origin of honey (Malika et al., 2005; Adenekan et al., 2010).

Table 6: the Conductivity in honey samples collected from different regions:

Sample No	Conductivity mS\cm
1	2.09
2	0.83
3	2.00
4	1.27
5	0.54

6	0.92
7	1.12
8	2.14

Conclusion

This investigation found that most honey samples were of excellent grade. The study discusses the diversity of various physicochemical features of Eight honey samples were gathered from various geographical locations and botanical origins in Sudan. The results met international norms. Honey samples vary in quality due to a variety of factors, including season, honey origin, bee activity, bee feeding, honey extraction duration and procedure, storage conditions, and honey freshness. To increase honey quality, care must be taken to standardise and rationalise beekeeping operations, production procedures, and storage processes. The comparison of these honey varietals to established worldwide criteria gave us an indication of the quality of our honeys produced in various parts of Sudan. More research on specific characteristics could be more useful to characterise honey properties in Sudan.

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