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**Research Article** 

# Physicochemical evaluation to assess the quality of honey samples marketed in Oman

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**Abstract:** The study aimes to investigate the physicochemical properties of seven honey samples to assess their quality as per GCC Standardization Organization (GSO) and international standard parameters. Seven honey samples, four marketed honey samples, and three locally produced Omani honey were collected and analysed for the pH, acidic content, % of insoluble matter, moisture content, proline, hydroxyl methyl furfural (HMF) and total reducing sugar contents. The results showed that pH of the tested honey samples are within the limit however acidity of the three samples did not comply with the prescribed limits. The moisture, proline, and hydroxy methyl furfural (HMF) contents of the honey samples tested are found to be within the acceptable range. However, the % of insoluble matter expressed for locally produced Sidr, Sumer, and Zah'r honey samples was below the maximum limit (0.5%) while marketed honey samples exceeded the limits of GSO (0.1%). The total reducing sugar concentration was below the limit in terms of four samples. Most of the tested honey samples meet the International/GSO standards for quality while a few failed to comply with acidity limits, the total reducing sugars content, and % of insoluble matter.

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#### **KEYWORDS**

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#### **1. INTRODUCTION**

Honey, characterized by its sweet taste, is a natural product that does not contain artificial substances. It is a semi-liquid sugar solution that is used as a popular substitute for sugars. Honey is collected by bees from the nectar from flowers and plants and stored in combs. Honey has been known since ancient times for its nutritional and therapeutic properties (Ahmed *et al.*, 2014; Tariq *et al.*, 2022). Honey is a composition of sugars (mainly glucose and fructose) as well as 20% of water and a small percentage of proteins, enzymes, vitamins, minerals, amino acids, and volatile compounds (Mokaya *et al.*, 2020). Honey is rich in many health-promoting nutrients, which are carbohydrates, potassium, iron, and zinc (Moniruzzaman *et al.*, 2013). It has therapeutic properties *i.e.*, anti-fungal, anti-bacterial, and anti-inflammatory, and is also a powerful natural antioxidant. In Oman, Honey is produced by two types of bees, *Apis florea* (Omani Dwarf bees) and *Apis mellifera* (Omani domesticated bees). The good quality among the different varieties of honey is Al-Baram or Al-Sumer (*Acacia tortilis*) and Sidr (*Ziziphus*)

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honey (Shafeeq, 2016). The composition of honey varies according to the diversity of bees, the region, the season, the source of the nectar, as well as the method of harvesting and storage (Al-Farsi et al., 2018). As honey is an essential daily-life dietary supplement, it is important to evaluate its quality to ensure its purity and to make sure that it has all nutritional components present in it. This quality control evaluation can be done by assessing physicochemical parameters such as the pH, moisture, ash value, hydroxyl methyl furfural (HMF) and colour, etc., to evaluate and test the purity of marketed honey (Gela et al., 2021; Lewoyehu & Amare, 2019; Sabir & Mohammed, 2011). The pH and the total acidity measure the quality of the honey product and pH is not directly related to the acidity due to buffering action by the minerals and acids present in the honey (Singh & Singh, 2018). Moisture test helps in determining water content inside honey components which affects honey stability against fermentation and granulation. In addition, moisture testing can help the honey producer to maintain the proper storage and transport conditions (Sereia et al., 2017). Ash content which is another physicochemical parameter is used to evaluate richness of the honey in minerals content and it is widely used in nutritional aspects (Boussaid et al., 2018; Tigistu et al., 2021). Honey contains a lot of bioactive compounds including, phenols, flavonoids, vitamins, organic acids, and carotenoids, that may potentiate the antioxidant effects of honey (Mokaya et al., 2020). The bioactive compounds showed antioxidant activity by the mechanism of either scavenging free radicals, quenching singlet oxygen, or chelation of the radicals or metal ions. Honey is used since ancient times for both domestic consumption and fulfilling medical needs. The rich antioxidant properties of honey due to its high phenolic contents, flavonoids, and presence of catalase, glucose oxidase, ascorbic acid, organic acid, amino acids, and carotenoids derivatives make it more popular recently as a source of antioxidants. The honey a rich source of antioxidants works both orally or topically as a cough suppressant, to treat burns and infections. It can be used for immune boosting, antimicrobial, and relieving stress and anxiety. It has a preventive role in the progress of neurological diseases, cancers, cardiovascular diseases, and aging (Ratiu et al., 2020). The current research work involves the evaluation of the physicochemical parameters in the marketed honey and verifying their compliance with GSO standards. The evaluation of different quality control parameters was performed according to the Harmonised methods of the International Honey Commission (Afshari et al., 2022; Bogdanov et al., 2002; Rysha et al., 2022).

## **2. MATERIAL and METHODS**

## **2.1.** Collection of Honey Samples

A total of seven honey samples were collected from different groceries, supermarket, and hypermarkets in Oman. The list of samples coded as AFS1 to AFS7 and presented in Table 1, which shows the location, and type of honey. The honey was evaluated for moisture contents, pH, insoluble matter, proline contents, HMF, and total reducing sugars values. Each experiment was repeated thrice. All the chemicals including formic acid, ninhydrin and the proline used were of analytical grade, and purchased from Thermo Scientific chemicals.

S. No	Code Assigned	Types	Location
1	AFS1	Pure Australian honey	Al-Batinah North -Liwa Hypermarket
2	AFS2	Natural bee honey Saudi Arabia	Al-Batinah North -Liwa Hypermarket
3	AFS3	Pure bee honey Dubai	Al-Batinah North -Liwa Hypermarket
4	AFS4	Sumer (Acacia tortilis)	Ad-Dakhiliyah Governorates
5	ASF5	Sidr (Ziziphus)	Ad-Dakhiliyah Governorates
6	ASF6	Zah'r (Flower)	Ad-Dakhiliyah Governorates
7	ASF7	Australian origin honey	Al-Batinah North -Liwa Hypermarket
	Total Samples		07

 Table 1. Code, location, and the marketed honey samples.

#### 2.2. Measurement of Moisture Content of Samples

A refractometer is used to measure the refractive index (RI) and Brix at 20°C, from which the humidity value is calculated. Moisture content is defined as the amount of water present in honey which affects honey stability against fermentation and granulation. The high-water content in honey is related to its storage and harvesting, as the high percentage of moisture leads to the growth of mould and yeast, decreases the shelf life, and loss flavour. It is measured according to the International Honey Commission (IHC) (Bogdanov *et al.*, 2002). The susceptibility to honey increases towards microorganisms with water contents above 17% (Puścion-Jakubik *et al.*, 2020). Lower moisture contents (20%) increase the shelf life of the honey(Al-Farsi *et al.*, 2018). The Abbe refractometer was used (Bellingham + Stanley Ltd. Abbe 60/DR refractometer) to calculate the moisture contents in the tested honey sample taking water as the reference material. The sample was homogenised and was heated in a water bath at 50 °C to dissolve honey crystals, if any. It was Cooled and stirred again at room temperature. The prism surface was covered with the sample and the refractive index (RI) reading was calculated at 20 °C using the following equation.

RI at 20 °C = RI at measured temperature (T) + 0.00045 (T-20 °C) provided T is above 20 °C.

#### 2.3. Measurement of pH of Samples

The pH expresses the concentration of hydronium in honey and it can affect further the percentage of HMF contents. The most common organic acids present in the honey samples are citric and gluconic acid and others (malic, butyric, lactic, formic, acetic, and succinic acid, etc). The pH analysis is useful to estimate the quality of the honey. The increase in pH may indicate fermentation or adulteration (Al-Farsi *et al.*, 2018).

Acidity by the titrimetric method: The variation in the content of some organic acids and phosphate (inorganic ions) depends on different sources of nectar, acidity, and the activity of the enzyme glucose oxidase. The increase in acidity affects the development of yeast and mould in the honey product. Free acidity should not be more than 50 milliequivalent/kg of honey (Puścion-Jakubik *et al.*, 2020). A pH meter was calibrated to pH 4, 7, and 10. 10 g of the honey sample was dissolved in 75 mL of water. It was stirred with the help of a magnetic stirrer and then a pH electrode was immersed in it to measure the pH directly (Digital pH meter Martini instruments). The solution was slowly titrated with 0.1 M NaOH until the pH reached 8.30. The reading was recorded, and free acidity was calculated in meq/kg. The experiment was performed in triplicate.

#### 2.4. Determination of Insoluble Matter of Samples

The insoluble matter may be present in the final product during preparation and indicate how well the honey is strained during extraction from the honeycombs or during processing and packaging. The honey sample should not contain more than 0.1 g/100 g (0.1% w/w) of insoluble ingredients except for pressed honey where it should be no more than (0.5% w/w)(Puścion-Jakubik *et al.*, 2020). In this 20 gm of honey was dissolved in 200 mL of water at 80 °C. It was filtered with a dried crucible and washed thoroughly with warm water until it was free from sugars. The residue left was dried at 135 °C to a constant weight.

*Calculation:* The % insoluble matter calculated as  $g/100 \text{ g} = m/m_1 \text{ x } 100$ 

Where, m = mass of dried insoluble matter

 $m_1 = mass of honey taken$ 

#### 2.5. Proline Content

The proline content is used to check honey ripeness. It is the major amino acid present in bee honey. The amount of proline present in the honey sample is determined by the formation of the coloured complex with the ninhydrin and is directly related to the reading of absorbance at 510 nm as per the Ough method of Proline determination in honey (Bogdanov *et al.*, 2002; Ough, 1969). The proline content should not be less than 25 mg/100g (Puścion-Jakubik *et al.*, 2020). Honey with a proline content of less than 180 mg/kg is considered unripe in Germany (Bogdanov *et al.*, 2002). A 0.5 mL of the honey sample was taken in a test tube (5g in 50 mL of water and then dilution it to 100 mL) and 0.5 mL of water (blank) in the second test tube and 0.5 mL of proline standard (stock solution 40mg/50mL and 1 mL diluted to 25mL to get 0.8mg/25mL) in three other test tubes. In each test tube, 1 mL of ninhydrin and 1 mL of formic acid were added. After shaking it was placed in a boiling water bath for 15 min and remained in the water bath for another 10 minutes at 70 °C. In all the test tubes, 5 mL of isopropanol and water were added. The absorbance of the sample and standard was measured at 510 nm using a UV spectrometer (Spectrum Instruments SP-UV 500DB spectrophotometer) (Bogdanov *et al.*, 2002).

Calculation: The proline content (mg/kg) in honey sample is calculated from the formula:

$$\frac{\text{Es}}{\text{Ea}} \ge \frac{\text{E1}}{\text{E2}} \ge 80$$

Es = Absorbance of the sample solution

Ea = Absorbance of proline standard solution (average)

 $E_1 = mg$  proline taken for standard solution

 $E_2$  = weight of honey in grams

80 dilution factor

#### 2.6. Hydroxy Methyl Furfural (HMF) Content

The hydroxy methyl furfural (HMF) is formed due to an increase in the temperature and during the reaction of dehydration of sugar. It results from the Millard reaction (results from the reaction of reducing sugar and amino acids to form complex compounds). It is determined by taking the difference in the UV absorbance of the clear aqueous honey and the same honey sample after the addition of bisulphite (to avoid interference from other components) at 284 nm. The value is then subtracted from the background absorbance at 336 nm. Honey for sale should not contain more than 40 mg/kg (Puścion-Jakubik et al., 2020). The 5g of the honey sample was dissolved in 25 mL of the water and 0.5 mL of Carrez solution I (15 g of potassium hexacyanoferrate (II) in 100 mL of water) and 0.5 mL of Carrez solution II (30 g of zinc acetate in 100 mL of water) was added and made up the mark to 50 mL with water. The first filtered 10 mL was rejected and the next 5 mL each in two test tubes (I and II) were taken. Then 5 mL of water was added to test tube I (sample solution) and 5 mL of the sodium bisulphite solution (0.2% freshly prepared) to test tube II (reference solution). The absorbance of the sample solution against the reference solution was measured at 284 and 336 nm, respectively using a UV spectrophotometer (Spectrum Instruments SP-UV 500DB spectrophotometer) (Bogdanov et al., 2002).

Dilution, D = Final volume of the solution/10 Calculation: HMF (mg/kg) =  $(A_{284} - A_{336}) \ge 149.7 \ge 5 \ge D/W$ D = dilution W = wt. in g of the honey sample

#### 2.7. Total Reducing Sugars Determination by Titration Method

The sugars present in honey are mainly fructose, glucose, and low concentration of other sugars such as sucrose and maltose. The Fehling A and B titration method was used for estimating the total reducing sugars. Reducing sugar reduces Fehling's solution. The titration methods were used for the determination of glucose using methylene blue as an indicator (De Beer et al., 2021; Puścion-Jakubik et al., 2020). However, according to the GSO standard, the total reducing sugars present in the honey samples should be above 45g/100g (Al-Farsi et al., 2018). The carbohydrate concentration was used for the estimation of botanical origin and its proper classification(Puścion-Jakubik et al., 2020). Lane and Eynon's method was used to determine the total reducing sugar contents of honey (LANE, 1923). Fehling's solution A (7 g of CuSO<sub>4</sub>. 5H<sub>2</sub>O in 100 mL of distilled water) and Fehling's solution B (35 g of potassium sodium tartrate in 12 g of NaOH diluted to 100 mL distilled water) were freshly prepared. A standard inverted sugar solution was prepared. Briefly, the burette was filled with standard inverted sugar solution (0.985 g dried sucrose dissolved in 500 mL of water, then added 2 mL of concentrated sulphuric acid). The solution was boiled for 30 minutes and kept for 24 hours). After a day, it was neutralized with sodium carbonate, and the final volume was made up to 1000 mL. A blank titration was performed.

In a 250 mL conical flask, 5 mL of each Fehling solution A and B were pipetted and heated to boiling, then 48 mL of standard sugar was added which changed the colour of solution from blue to orange-brown. 2-3 drops of methylene blue indicator were added with continuous boiling and stirring. The titration was carried out until the colour changed to reddish brown due to cuprous oxide formation (endpoint).

 $V (blank) = V_f - V_i$ = 41.8 - 0 = 41.8 mL

From this step Fehling factor (strength of copper sulphate solution) was calculated:

Fehling factor = titrated value x 0.001

= 41.8 x 0.001 = 0.0418

• Sample titration

1 gm of the honey sample was accurately weighed in a 250 volumetric flask. It was diluted with 250 mL of distilled water and mixed well. In a conical flask, 5 mL of each Fehling solution A and Fehling solution B were added. From the burette around 12 mL of honey, the solution was added and then boiled (the solution colour was orange-brown). After adding 2-3 drops of methylene blue indicator with continuous boiling and stirring, the solution was titrated within minutes until the colour changed from blue 3 to reddish brown colour (endpoint)("https://law.resource.org/pub/in/bis/S06/is.4941.1994.pdf,"). Reducing sugar content was calculated as follows.

Reducing sugar = $(250 \times 100 \times S) / (H \times M)$ 

S = Fehling factor.

H = volume of honey solution required (burette reading).

M = mass of honey in gm.

#### **3. RESULTS and DISCUSSION**

A total of seven samples were collected that includes four marketed honey products collected from hypermarkets in Oman and 3 locally produced samples viz., Omani Sumer, Sidr, and Zah'r. The physicochemical parameters of these samples were evaluated and compared with the GCC standardization organization (GSO), International Honey Commission (IHC) limits, and the literature data.

#### **3.1. Moisture Content**

Moisture contents of the honey sample were measured by an Abbe refractometer. The honey moisture content depends on the methods of extraction, preservation, and storage. The moisture contents above the limits may cause microbial growth and further loss in taste and low shelf life. The percentage above 17% increases the chance of microbial growth however the moisture contents below the limit of 20% increase the shelf life of honey samples. The water contents may vary with the interaction of sugars present in the honey and low water contents prevents the microorganism attack as hyperosmotic honey will draw the water from the microorganism and kill them (Malika *et al.*, 2005). The normal range of moisture content is between 13.7-18.8% for Sidr, 14.9-18.3% for Sumer, and 14-17.2% for Zah'r (Al-Farsi *et al.*, 2018). None of the samples tested exceeds the limits as approved by the GSO. The results of the percentage moisture contents of the tested honey samples measured in triplicate are presented in Table 2.

S. No	Sample	Temperature (°C)	Refractive index	Refractive index (20°C)	Water content (g/100g)
1	AFS1	22.8	1.4936	1.4948	16.8
2	AFS2	23	1.4973	1.4986	15.2
3	AFS3	23.2	1.4973	1.4987	15.2
4	AFS4	22.7	1.4973	1.4985	15.2
5	ASF5	23.5	1.4970	1.4985	15.2
6	ASF6	22.8	1.4973	1.4985	15.2
7	ASF7	22.6	1.4973	1.4984	15.4

Table 2. The moisture contents of the tested honey sample by Abbe refractometer.

AFS1; Pure Australian honey, AFS2; Natural bee honey Saudi Arabia, ASF3; Pure bee honey Dubai, ASF4; Sumer (*Acacia tortilis*), ASF5; Sidr (Ziziphus), ASF6; Zah'r (Flower), ASF7; Australian origin honey.

## 3.2. Acidity, pH, and % Insoluble Matter

The pH corresponds to the quality of the honey sample (stability, texture, flavor, and shelf life), and an increase in acidity may affect the growth of mould and yeast in the honey sample. The pH ranges for the Sidr, Sumer and the Zah'r honey is 4.71-7.51, 4.12-4.90, and 3.46-4.79 (Al-Farsi *et al.*, 2018). The minimum and maximum pH range reported by White was between 3.5 to 4.5 (WHITE, 1975). The presence of gluconic acid in all honey is due to the oxidation of glucose by the glucose oxidase activity added by honeybees during ripening. The acidification fastens the healing process by releasing oxygen from haemoglobin and makes a less favourable environment for the destructive proteases (Molan & Rhodes, 2015). The increase in the acidity of the sample might be due to inappropriate storage (duration and temperature) and processing conditions. The accepted range for free acidity according to GSO should not be more than 50 millimoles/kg (Al-Farsi *et al.*, 2018). The results of the pH showed that all the honey tested was acidic and in conformity with results carried out by another research group 3.40-6.10 (El Sohaimy *et al.*, 2015). The acidity values are within the limit for most of the tested samples. The sample AFS1 has a marginal increase in acidity with 51 millimoles/kg. The acidity results for the Sumer (AFS4) and Zah'r (ASF6) showed increased acidity with 78 and 61 mM/kg. The

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acidity adds to the flavours and protection against the microorganism. The acidity is due to the contents of gluconic acid and glucolactone. The insoluble matter expressed for Sidr, Sumer, and Zah'r was 0.45, 0.4, and 0.35% below the maximum limit of GSO (0.5%). The insoluble matter present in other honey samples is above the limits of GSO (0.1%)(Al-Farsi *et al.*, 2018). The results of the pH, acidity, and the % insoluble matter measure three times are shown in Table 3.

S. No	Sample	pН	mL of 0.1 M NaOH after titration	Acidity (mM/kg)	weight of honey after filtration (g)	% Insoluble matter (g/100 g)
1	AFS1	3.69	5.1	51	0.06	0.3
2	AFS2	4.25	2.7	27	0.05	0.25
3	AFS3	4.32	1.3	13	0.06	0.3
4	AFS4	5.12	7.8	78	0.08	0.4
5	ASF5	4.80	1.9	19	0.09	0.45
6	ASF6	3.96	6.1	61	0.07	0.35
7	ASF7	4.68	0.8	8	0.06	0.3

Table 3. Acidity in (mM/kg), pH and % insoluble matter (g/100g) of the honey samples.

AFS1; Pure Australian honey, AFS2; Natural bee honey Saudi Arabia, ASF3; Pure bee honey Dubai, ASF4; Sumer (*Acacia tortilis*), ASF5; Sidr (Ziziphus), ASF6; Zah'r (Flower), ASF7; Australian origin honey.

#### **3.3. Proline Content**

The proline is the main amino acid and its content directly indicates the honey ripeness. It measures the quality and antioxidant activity of the honey (Truzzi *et al.*, 2014). The proline contents should not be less than 25mg/100g (Puścion-Jakubik *et al.*, 2020). Although there is no limit for the proline contents in the GSO standard, however, a proline content of less than 180 mg/kg is considered unripe or adulterated by sugar addition in Germany (Al-Farsi *et al.*, 2018). Our measured proline content in the tested honey samples showed that none of the samples has lower proline contents as prescribed by different countries. The proline contents of the measured honey samples measured in triplicate are shown in Table 4.

S. No	Sample	Abs of sample	Abs of proline (Average)	Proline contents (mg/kg)
1	AFS1	0.176		370.52
2	AFS2	0.160		336.84
3	AFS3	0.121		254.73
4	AFS4	0.299	0.152	629.47
5	ASF5	0.169		355.78
6	ASF6	0.229		482.10
7	ASF7	0.118		248.42

**Table 4.** The proline contents (mg/kg) of the tested honey sample.

AFS1; Pure Australian honey, AFS2; Natural bee honey Saudi Arabia, ASF3; Pure bee honey Dubai, ASF4; Sumer (*Acacia tortilis*), ASF5; Sidr (Ziziphus), ASF6; Zah'r (Flower), ASF7; Australian origin honey.

## 3.4. Hydroxy Methyl Furfural (HMF) Level

The hydroxy methyl furfural formation in the honey samples takes place at high temperatures in acidic conditions. The HMF is a major intermediate product of the Maillard reaction and is a browning reaction between sugars and free amino acids on prolonged storage and heating conditions (Chou *et al.*, 2020). According to the reports, high HMF values may alter the flavour

and colour of the honey samples due to caramelization and degradation of honey samples. The average HMF value for the honey samples for sale should not exceed 40 mg/kg and may be affected by pH, acidity, moisture, and storage. The EU standard and Codex Alimentarius have also fixed the maximum HMF value for honey should not exceed 40 mg/kg (tropic ambient temperature honey should not be more than 80 mg/kg) (Chou *et al.*, 2020). The GSO limits for the HMF contents are not more than 80 mg/kg. Our results for the HMF values for the tested honey samples are within the recommended range according to the GSO limit (80 mg/kg). The HMF contents are measured in triplicate and are represented in Table 5.

S. No	Sample	Absorbance at 336 nm	Absorbance at 284 nm	HMF (mg/kg)
1	AFS1	0.306	0.492	41.766
2	AFS2	0.441	0.465	8.982
3	AFS3	0.248	0.401	34.35
4	AFS4	0.377	0.486	24.47
5	ASF5	0.383	0.477	21.10
6	ASF6	0.235	0.486	18.78
7	ASF7	0.315	0.517	45.35

**Table 5.** The hydroxy methyl furfural (HMF) contents present in the honey samples.

AFS1; Pure Australian honey, AFS2; Natural bee honey Saudi Arabia, ASF3; Pure bee honey Dubai, ASF4; Sumer (*Acacia tortilis*), ASF5; Sidr (Ziziphus), ASF6; Zah'r (Flower), ASF7; Australian origin honey.

#### **3.5. Total Reducing Sugars**

According to the GSO honey standard, the total reduced sugars contents should be above 45%. Among the seven tested samples, four of the samples were below the limit in terms of total reducing sugar. The sugar levels in the honey influence the efficacy of the honey and may be affected by long storage during processing. The factors such as moisture levels, area of the honey harvested, and harvest time affect the sugar contents. The high glucose ratio allows honey to crystallize whereas other sugars present inhibit it (Ayubi, 2017). The total reducing sugar in the honey samples is measured in triplicate and represented in Table 6.

S. No.	Sample	Initial reading	Final reading	Difference	Reducing sugars g/100 g
1	AFS1	0 mL	29.5	29.5	35.42
2	AFS2	0 mL	15.1	15.1	69.21
3	AFS3	0 mL	29.3	29.3	35.67
4	AFS4	0 mL	33.7	33.7	31.01
5	ASF5	0 mL	21.7	21.7	48.16
6	ASF6	0 mL	24.2	24.2	43.18
7	ASF7	0 mL	18.9	18.9	55.29

**Table 6.** Determination of reducing sugars in the honey samples.

AFS1; Pure Australian honey, AFS2; Natural bee honey Saudi Arabia, ASF3; Pure bee honey Dubai, ASF4; Sumer (*Acacia tortilis*), ASF5; Sidr (Ziziphus), ASF6; Zah'r (Flower), ASF7; Australian origin honey.

## 4. CONCLUSION

A total of seven honey samples (three Omani honey and four marketed honey) were collected from local markets in Oman. The Omani honey includes Sumer, Sidr, and Zah'r types. All seven honey samples confirm the test limits (moisture, pH, proline, and HMF) approved either by International or the GSO standards. The acidity of the three samples was found to be above the limits that may affect the growth of moulds and yeast. The insoluble matter expressed for Sidr, Sumer, and Zah'r was below the maximum limit of the GSO (0.5%) while the insoluble matter present in other honey samples was above the limits of the GSO (0.1%). The total reducing sugar of four samples was below the limit which indicates adulteration according to the GSO. The adulteration in terms of microbial or non-microbial, heavy metal, pesticides, and antibiotics contamination of the honey products may cause health hazards. Thus, the correct physicochemical analysis supports the originality and safety of honey products. In our study, most of the tested parameters of honey samples were within the limits still monitoring is required to improve processing and storage conditions for better honey quality to conform with the international standard limits in terms of both quality and quantity.

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## **Declaration of Conflicting Interests and Ethics**

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s). **Ethics Committee Number**: EBS/18/2021-2022, Ethics and Biosafety Committee, College of Pharmacy, National University of Science and Technology, Azaiba, Muscat, Sultanate of Oman.

# **Authorship Contribution Statement**

Al Zahraa Mohammed Said Al Hadhrami: Investigation; Methodology. Fatema Rashid Abdullah Al Mazrooei: Investigation; Methodology. Sheikha Mohammed Ali Al Mamari: Investigation; Methodology. Sakina Habib Juma AL Humaid: analyzed and interpreted data. Shah Alam Khan: Formal analysis, supervision. Md Jawaid Akhtar: Conceptualization, Data curation. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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# REFERENCES

- Afshari, A., Ram, M., & Mohamadi, S. (2022). Quality Evaluation of Iranian Honey Collected from Khorasan Province, Iran. *International Journal of Food Science*, 2022, Article ID 3827742. https://doi.org/10.1155/2022/3827742
- Ahmed M, Khiati B, Meslem A, Aissat S, Djebli N (2014) Evaluation of hysicochemical and Antioxidant Properties of Raw Honey from Algeria. *J Microb Biochem Technol.*, S4:006. https://doi.org/10.4172/1948-5948.S4-006
- Al-Farsi, M., Al-Belushi, S., Al-Amri, A., Al-Hadhrami, A., Al-Rusheidi, M., & Al-Alawi, A. (2018). Quality evaluation of Omani honey. *Food Chemistry*, 262, 162-167.
- Ayubi, A.N. (2017). Analysis of Total Reducing Sugar Content, Acidity Value, and Hydroxymethylfurfural (HMF) Content of Various Honey Types. *Indonesian Journal of Chemistry and Environment*, 1(1), 21-28.

- Bogdanov, S., Martin, P., & Lullmann, C. (2002). Harmonised methods of the international honey commission. *Swiss Bee Research Centre, FAM, Liebefeld*, *5*, 1-62.
- Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsì, F., Ferrari, G., & Hamdi, S. (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry*, *11*(2), 265-274.
- Chou, W.-M., Liao, H.-C., Yang, Y.-C., & Peng, C.-C. (2020). Evaluation of honey quality with stored time and temperatures. *J. Food Nutr. Res.*, 8, 591-599.
- De Beer, T., Otto, M., Pretoruis, B., & Schönfeldt, H. C. (2021). Monitoring the quality of honey: South African case study. *Food Chemistry*, 343, 128527. https://doi.org/10.1016/j.foodchem.2020.128527
- El Sohaimy, S.A., Masry, S., & Shehata, M. (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences*, 60(2), 279-287.
- Gela, A., Hora, Z.A., Kebebe, D., & Gebresilassie, A. (2021). Physico-chemical characteristics of honey produced by stingless bees (Meliponula beccarii) from West Showa zone of Oromia Region, Ethiopia. *Heliyon*, 7(1), e05875. https://law.resource.org/pub/in/bis/S06/is.4941.19 94.pdf
- LANE, J.H. (1923). Determination of reducing sugar by means of Fehling's solution with methylene blue as internal indicator. J. Soc. Chem. Ind., 17, 32-37.
- Lewoyehu, M., & Amare, M. (2019). Comparative assessment on selected physicochemical parameters and antioxidant and antimicrobial activities of honey samples from selected districts of the Amhara and Tigray regions, Ethiopia. *International Journal of Food Science*, 2019(1), 1-10, https://doi.org/10.1155/2019/4101695
- Malika, N., Mohamed, F., & Chakib, E. (2005). Microbiological and physicochemical properties of Moroccan honey. *International Journal of Agriculture and Biology*, 7(5), 773-776.
- Mokaya, H.O., Bargul, J.L., Irungu, J.W., & Lattorff, H.M.G. (2020). Bioactive constituents, in vitro radical scavenging and antibacterial activities of selected Apis mellifera honey from Kenya. *International Journal of Food Science & Technology*, *55*(3), 1246-1254.
- Molan, P.C., & Rhodes, T. (2015). Honey: a biologic wound dressing.
- Moniruzzaman, M., Khalil, M.I., Sulaiman, S.A., & Gan, S.H. (2013). Physicochemical and antioxidant properties of Malaysian honeys produced by Apis cerana, Apis dorsata and Apis mellifera. *BMC Complementary and Alternative Medicine*, *13*(1), 1-12.
- Ough, C. (1969). Rapid determination of proline in grapes and wines. *Journal of Food Science*, 34(3), 228-230.
- Puścion-Jakubik, A., Borawska, M.H., & Socha, K. (2020). Modern methods for assessing the quality of bee honey and botanical origin identification. *Foods*, *9*(8), 1028.
- Ratiu, I.A., Al-Suod, H., Bukowska, M., Ligor, M., & Buszewski, B. (2020). Correlation study of honey regarding their physicochemical properties and sugars and cyclitols content. *Molecules*, *25*(1), 34. https://doi.org/10.3390/molecules25010034
- Rysha, A., Kastrati, G., Biber, L., Sadiku, V., Rysha, A., Zogaj, F., & Kabashi-Kastrati, E. (2022). Evaluating the physicochemical properties of some kosovo's and imported honey samples. *Applied Sciences*, 12(2), 629. https://doi.org/10.3390/app12020629
- Sabir, M.S.A.S.S., & Mohammed, M.Y. (2011). Evaluation of physico-chemical properties of honey samples collected in Erbil/Iraq.
- Sereia, M.J., Março, P.H., Perdoncini, M.R.G., Parpinelli, R.S., Lima, E.G., & Anjo, F.A. (2017). Techniques for the evaluation of physicochemical quality and bioactive compounds in honey. In V.A.A. Toledo (Ed.), Honey analysis (p. 193-214). IntechOpen. http://dx.doi.org/10.5772/66839
- Shafeeq. (2016). The unique Omani honey [https://timesofoman.com/article/88881/Lifestyle/ Culture/Oman-Culture-The-Unique-Omani-Honey]. *Times of Oman*.

- Singh, I., & Singh, S. (2018). Honey moisture reduction and its quality. *Journal of food science and technology*, *55*(10), 3861-3871.
- Tariq, K., Iqbal, S., & Ali, M.F. (2022). Quantifying physicochemical properties of honey collected from different floral sources of Islamabad and Mardan regions. *Agrinula: Jurnal Agroteknologi dan Perkebunan*, 5(1), 10-19.
- Tigistu, T., Worku, Z., & Mohammed, A. (2021). Evaluation of the physicochemical properties of honey produced in Doyogena and Kachabira Districts of Kembata Tambaro zone, Southern Ethiopia. *Heliyon*, 7(4), e06803.
- Truzzi, C., Annibaldi, A., Illuminati, S., Finale, C., & Scarponi, G. (2014). Determination of proline in honey: comparison between official methods, optimization and validation of the analytical methodology. *Food chemistry*, 150, 477-481.
- White, J.W. (1975). *Composition of honey*, In: E. Crane, (ed.) Honey, a comprehensive survey. Heinemann Edition; London, 157-206.