International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://www.theaspd.com/ijes.php

Physicochemical Characteristics Of Abu Dhabi Honey

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Abstract
The physicochemical properties of Sidr and Damas honey produced in Abu Dhabi were evaluated. A total of 57 honey samples produced in the Emirate of Abu Dhabi were utilized in the study. Physicochemical parameters such as moisture content, acidity, diastase activity, Hydroxymethylfurfural analysis, sugar content, Electrical conductivity and heavy metals were assessed. The moisture content of both Sidr and Damas honey were in the range of 16.6 to 20.8 % while the total acidity levels varied from 6.2 to 46.6 meq/l. The total reducing sugar ranged from 58-67%. The diastase activity and HMF values suggest the freshness and proper storage of the samples. Three Sidr samples failed to comply with total reducing sugar content and Damas honey exhibited significantly higher levels of total reducing sugars. In conclusion, determination of physicochemical characteristics of honey is a valuable tool that reflects both quality and authenticity.
Keywords: *Sidr honey, Damas honey, physiochemical analysis, multivariate, honeybees, hives*

Background

Honey holds a special space having deeply rooted in the Emirati culture and tradition due to its religious and medicinal significance (1). It is a blend of sugar substances predominantly monosaccharides, organic acids, enzymes and other micronutrients (2). Given the nutritive value, therapeutic significance, and cultural heritage, bee keeping (apiculture) has been practiced in the United Arab Emirates mainly for honey and beeswax since ancient times.

The Emirate of Abu Dhabi in the United Arab Emirates (UAE) is home for more than 400 native plant species playing pivotal role in the natural ecosystems which includes Ghaf, (*Prosopis cineraria*), Mangroves (*Avicennia marina*), Sidr (*Ziziphus spina-christi*), Samar (*Acacia tortilis***)** and damas trees (*Conocarpus lancifolius*) that are generally either heat and/or drought tolerant in nature. The pastures of Abu Dhabi are conducive for beekeeping, having dual benefits of ecology and sustainable agriculture.

Sidr tree is widespread in Abu Dhabi and the floral origin has been found to influence the quality characteristics of honey. For instance, the glucose fructose ratio was relatively higher in Sidr than Samar in some regions of Saudi Arabia (3). The honey harvested by bees from the nectar of the Sidr is considered as one of the best honeys (4). Likewise, Samar is yet another most common melliferous tree in the region which blooms during the hottest season (April to July). This small to medium sized tree is known to exhibit medicinal properties and used in the treatment of dry cough and diarrhea (5). In the case of damas plant extract, immense therapeutic potential which include anti-inflammatory, and antioxidant properties were confirmed through invitro studies (6). It is evident from previous studies that these plant species have immense phytochemicals and the nectar sipped by the bees have a profound influence on the vegetation and foraging areas.

Because of the nutritive value and therapeutic properties, the demand for authentic honey is surging across the world and according to a study in Europe, more than 40% of honey marketed were suspicious to be adulterated (7). In particular, the sugar substitution of honey has negative health impact on consumers necessitating more stringent regulation and vigilance on the product.

In the UAE honey is regulated as per the Cabinet Resolution No 49 of 2016 and requires product registration as a prerequisite for the sale in the local market. In addition, honey shall fulfill quality and safety criteria in accordance with the UAE standard (8).

As per the standards, honey is defined as a natural sweet substance produced by honeybees from the nectar of plants or from secretion plants, which the bees collect, transform by combining it with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature. With this background, the current study reports the Physicochemical properties of honey produced in the Emirate of Abu Dhabi named "Abu Dhabi Honey".

MATERIALS AND METHODS

Honey Samples

A total of 57 honey samples produced in the Emirate of Abu Dhabi were utilized in this study.

Pollen analysis

For pollen analysis, 10 ± 0.1 g of each honey sample was taken in a 50 ml conical glass tube. Twenty milli liters of warm deionized water was added to the tube and mixed well to dissolve the honey and centrifuged at 1000 g for 10 minutes. After discarding the supernatant, another 20 mL of deionized water added and mixed. The samples were centrifuged for 5 min at 1000 *g*. The supernatant was discarded.

The remaining sediments were mixed thoroughly with a Pasteur pipette and transferred to a glass slide and visualized under microscope. Pollen types were identified by comparison with reference database. For quantification, at least 300 pollen grains were counted from each sample. The percentage frequency of the pollen taxa in all the samples was calculated.

Moisture analysis

The moisture content was calculated by the determination of the refractive index of the honey at specific temperature using an automated digital refractometer. Briefly, a drop of honey was placed on the surface of the meter and the displayed reading was recorded and converted to moisture content using the Chataway table (AOAC, 2006).

Acidity analysis

Acidity is determined by neutralizing the acid present in a known sample using a standard base. Briefly, 3 mg sample of homogenized honey was dissolved in water and titrated against 0.1 N NaOH solution until the formation of a pink color and the titer value was recorded and the results are expressed as milliequivalents (meq) per kg of honey.

Diastase Activity analysis

To determine diastase activity, one gram of honey sample was dissolved in acetate buffer solution (0.1M, pH 5.20 ±0.02) and transferred to a 100 ml volumetric flask and made to 100 ml mark with acetate buffer solution. From this, 5 ml of the honey sample solution is transferred to a test tube. Simultaneously blank solution of the acetate buffer (5 ml) was also taken on a test tube, and both tubes were warmed in a water bath at (40.0 ± 0.2 °C) for at least 5 minutes. After warming, Phadebas tablet was added and stirred until complete disintegration of the tablet (~10 seconds). The tubes were returned to the water bath for 30 minutes. To terminate the reaction, 1 ml of sodium hydroxide solution (pH 7.2) was added and mixed and filtered using filter paper to measure the absorbance of both solutions (Sample & Blank) in 1 cm cuvettes at 620 nm using water as reference.

Hydroxymethylfurfural (HMF) analysis

Briefly, 4-8 grams of honey sample was taken in a 50 ml volumetric flask to which 3-5ml of each Careez I & Careez II solutions were added and made to 50 ml using distilled water. The solution was shaken using Wrist shaker or by vortex mixture, filtered using 0.45 µm filter paper and filled in vials for injection into HPLC.

Sugar content

Sugar determination fructose, glucose and sucrose (Standard sources Fructose USP Grade 125 mg CAT 1286504, Glucose Merck KGaA cat 1.08337.1000, Sucrose USP Grade 100 mg CAT 1623637) performed using a Shimadzu SIL-20AC HT Prominence Auto Sampler on a Shimadzu LC-20AT Prominence Liquid Chromatograph, ChromScinecs NH2 Amino, 5-um HPLC Column and a

Shimadzu RID-10A Refractive Index Detector. The quantitative estimation was calculated using known reference standards of at least five calibration points.
Standard solutions were prepared for each sugar and injected into the equipment to create a calibration curve. A 20 µl of the sample was injected into the column and the separation was conducted at 40°C with the flow rate of 1.5 ml/min. The identification of sugars in honey was calculated by comparing retention times of individual sugars in reference to tested solution for quantitative determination.

Heavy metals analysis

The samples were subjected to microwave-assisted acid digestion and the metal elements were identified by inductively coupled plasma mass spectrometry (ICP-MS).

Data analysis

Descriptive statistics which include the mean, standard deviation, minimum, maximum, and median values for each group was employes to assess the central tendency and variability of each component (fructose, glucose, sucrose, total reducing sugars, moisture, and total acidity). An independent two-sample t-test was performed to assess whether the observed differences in means between Sidr and Damas honeys were statistically significant. Principal Component Analysis (PCA) was conducted to reduce the dimensionality of the dataset and identify the main sources of variation. PCA loadings and scatterplots were used to determine the variables. All models were scaled and extracted at a confidence level of 95%.

RESULT AND DISCUSSION:

A total of 57 (32 Sidr and 25 Damas) honey produced in the Emirate of Abu Dhabi were evaluated in the current study. The mean, standard deviation, minimum and maximum values of the studied parameters of the honey samples are presented in Table 1. The microscopic observation of the floral study suggests that the samples are predominantly monofloral as more than 45% of the total pollen count appears to be from single source. The representative micrographs of pollen study are presented in fig. 1.

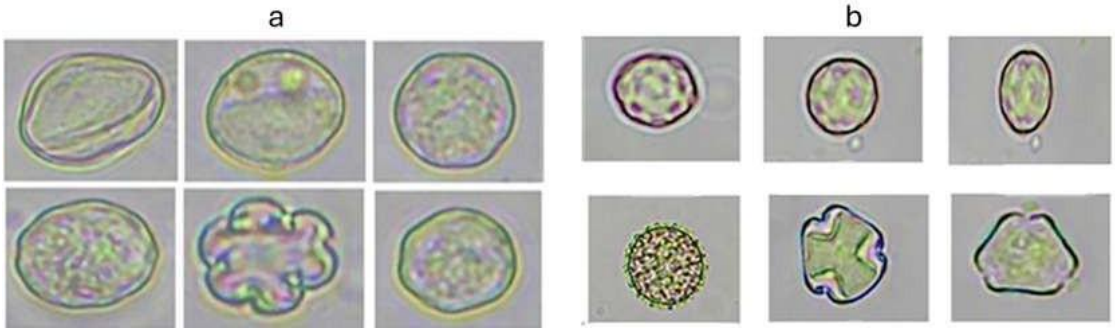

Fig. 1. photo micrographs of pollen analysis; a: Sidr, b: Damas

The hygroscopic property of honey plays a vital role in maintaining the quality of the honey. For instance, high moisture content can result in fermentation leading to quality deterioration of honey (9). The standard requirement states that the moisture level should not exceed 20%. The moisture content of both Sidr and Damas honey were in the range of 16.6 ± 0.42 to 20.8 % ± 0.77% within the range prescribed in the standard. Honey is naturally acidic in nature as it contains many organic acids such as formic acid, oxalic acid, butyric acid, gluconic acid etc. The total acidity levels varied from 6.2 ± 0.5 to 46.6 ± 0.4 meq/l in Sidr honey while Damas was in the range of 23.8 ± 0.2. Acidity levels above the threshold (50 milli equivalents acid /1000g) may trigger fermentation (10).

Parameters	**Sidr n=32**	**Damas n=25**
Moisture		
Mean	**18.32**	**18.27**
Standard deviation	**0.6**	**1.23**
Minimum	**17.2**	**16.6**
Maximum	**19.4**	**20.8**
Free Acidity		
Mean	**19.28**	**23.88**
Standard deviation	**14.89**	**2.21**
Minimum	**6.54**	**19.81**
Maximum	**51.4**	**28.18**
Diastase Activity		
Mean	**25.9**	**49.65**
Standard deviation	**9.8**	**16.98**
Minimum	**12.1**	**28**
Maximum	**19.4**	**87.41**
Fructose		
Mean	**35.82**	**38.2**
Standard deviation	**1.18**	**0.49**
Minimum	**33.03**	**36.88**
Maximum	**37.63**	**38.92**
Glucose		
Mean	**27.2**	**28.8**
Standard deviation	**1.04**	**0.48**
Minimum	**24.98**	**28.1**
Maximum	**28.99**	**29.98**
Sucrose		
Mean	**2.4**	**0.24**
Standard deviation	**2.0**	**0.12**
Minimum	**0.02**	**0.02**
Maximum	**4.9**	**0.72**
Total reducing sugar		
Mean	**63.02**	**67.01**
Standard deviation	**1.94**	**0.84**
Minimum	**58.49**	**65.61**
Maximum	**65.64**	**68.9**

Table. 1. Physicochemical parameters of honey

Some of the unique properties of honey are determined by both floral compounds from the nectar and their conversion by bees. For instance, enzymatic conversion of nectar to honey by thermolabile diastase is an important quality parameter to determine the impact of temperature (11). It is well known that honey is primarily composed of sugars and crystallization is a natural process. Hence, to melt the crystallized honey, heating is frequently applied during processing which may result in the loss of thermolabile and aromatic compounds. Furthermore, heating may also trigger the formation of undesirable products such as hydroxymetylfurfural (HMF). Determination of diastase activity and HMF values are regarded as important quality criteria for honey (12).

In the current study, the diastase activity of Sidr honey was 26.0 ± 9.8 while Damas showed higher enzyme activity of 49.6 ± 17.0. The legal compliance mandates diastase activity not less than 8 Schade units and HMF values less than 80 mg/kg. The HMF values of the samples studied were below the detection limit for Sidr samples while damas was in the range of 0.7 ± 0.3. The diastase activity and HMF values were well within compliance, suggesting the freshness and proper storage of the samples. In general, the diastase activity is around 8- 20. Higher diastase activity in Damas may be due to foraging behavior of bees and seasonal variations requiring further evaluation on this type of honey.

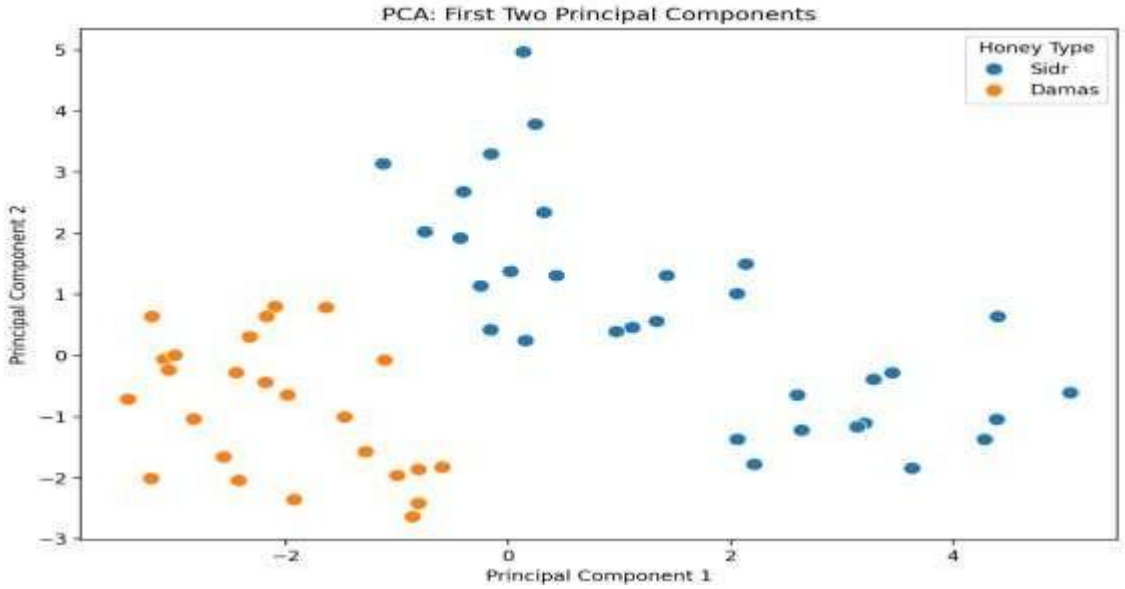

Fig. 2. Principal component analysis

The exposure of honeybees to contaminants such as pesticides and heavy metals from the foraging environment near their hives has significant impact on honey quality (13). The concentrations of different metals in the honey samples were in accordance with the standard requirements and corroborate with previous studies (14). The sweetness of honey is predominantly derived from monosaccharides which constitute more than 80% of the total sugar composition. Honeybees collect nectar from flowering plants containing mostly sucrose which is then mixed with invertase enzyme in the stomach to convert it into glucose and fructose. Sucrose content is a quality parameter to assess honey maturity and indicates whether harvesting is done before the completion of enzymatic process, often referred to as immature honey (15). The Fructose content of Sidr honey was 36. 0 ±1.2 while the glucose content was in the range of 27.1 ± 1.0 and sucrose was 2.4 ± 2.0. In the case of Damas samples, fructose was 38. 2 ± 0.5, glucose was 28.8 ± 0.5 and 0.1% sucrose were observed. The total reducing sugar of Damas was 67.0 ± 0.8%. Out of the 32 Sidr samples, 3 samples (9%) were not complaint for total reducing sugar (sum of glucose and fructose) having 58% against the regulated value of 60%. Almost 33% of commercial honey samples from Abu Dhabi studied previously failed to comply with the standard requirement (16). Nevertheless, the sucrose content was below the prescribed level of less than 5% in both sample types. In freshly harvested honey, it is possible that the enzyme invertase has not yet broken down all the sucrose. The Fructose to Glucose ratio of Sidr and Damas samples were almost in the same range (1.32 ± 0.04 for Sidr and 1.33 ± 0.02 for Damas) suggesting a lesser tendency to granulation (17). The glucose to water ratio has also been considered as an indicator for crystallization which was 1.5 and 1.6 for Sidr and Damas respectively suggesting non granulating type (18). Both Sidr and Damas honeys have similar levels of fructose and glucose, and total reducing sugar with Damas honey having slightly higher averages. The strong correlations among sugar components suggest that these variables are closely linked in honey composition, while moisture and acidity are more independent. Multivariate analysis using Principal component analysis (PCA) further confirmed

that sugar-related variables are the main contributors to the differentiation between Sidr and Damas honey. The first two principal components capture a large portion of the variance and provide good separation between Sidr and Damas honey (Fig. 2). For instance, diastase activity, HMF, fructose, and some metals have a strong influence on the main components. The PCA scatterplot and loadings heatmap help identify which variables drive the differences between honey types. To assess the magnitude and significance of differences between Sidr and Damas honeys, Cohen's d (effect size) and t-tests were performed for each component. Fructose, glucose, sucrose, and total reducing sugars all show very large and statistically significant differences (Cohen's $d > 1$, $p < 0.001$) while moisture and total acidity do not show significant differences between the two honey types.

CONCLUSION

In conclusion, determination of physicochemical characteristics of honey is a valuable tool that reflects both quality and authenticity. Out of the total 57 samples, 3 Sidr samples (5%) were not complaint for total reducing sugar. Differences were evident between Sidr and Damas samples. Damas honey exhibited significantly higher levels of fructose, glucose, and total reducing sugars, with very large effect sizes and highly significant p-values while Sidr honey had higher sucrose content. The moisture and total acidity did not differ significantly between the two honey types, indicating that these properties are not distinguishing factors.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support and encouragement of the Abu Dhabi Quality and Conformity Council. Gratitude is also extended to the management of Central Testing Laboratories (CTL) and Department of Municipalities and Transport (DCT) for invaluable support and assistance.

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