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Physico-chemical characteristics and Discrimination Analysis of Some Medicinal and Aromatic Honey Produced in Egypt

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ABSTRACT

The purpose of the research is to differentiate five varieties of floral honey from one another using discriminant analysis; Fennel, Sidr, Nigella, Marjoram and Anise honey that produced in Egypt. To statistically test data, discriminant analysis and analysis of variance were employed. The classification's most crucial parameters were determined via discrimination. Various parameters were deliberated: Moisture; total of soluble solids (T.S.S); Viscosity; Specific gravity; electrical conductivity (EC); Sugar content; fructose; glucose; sucrose; maltose (%); H.M.F.; pH; free acidity; total acidity; G/W; F/G; lactone and Diastase. Every tested sample of honey met the international standards for honey quality at the acceptable level.

Keywords: honeybee; physicochemical characters; discrimination analysis

1. Introduction

Honey is a supersaturated band-aid produced by bees that primarily consists of vitamins, amino acids, minerals, glucose and fructose. Component of honey may alter due to the aberration in nectar, geography, season, analysis of beekeepers and accumulator condition. Nowadays, honey has been scientifically accurate for its antioxidant, adjustment of glycemic response, antimicrobial, antitumor, anti-inflammatory, and cardiovascular potentiating agent. Honey can be acclimated as a anguish bathrobe and healing substance. It is altered in color, acoustic perception, acidity and medical acknowledgment (Kasˇkonien *et al*., 2010; El-Metwally, 2010 and 2015 and Khan *et al*., 2017).

In addition, Cano *et al*. (2001), Gomes *et al*. (2010) and Almeida-Muradian *et al*. (2013) show that it includes trace amounts of various other substances, including pollen grains, organic acids, enzymes, products of the Maillard process, volatile chemicals, and several bioactive substances (phenols and flavonoids). Joseph *et al*. (2007) and Nigussie *et al*. (2012) indicated that honey is a culinary ingredient; there have also occasionally been uses for honey in veterinary and human medicine.

The plant source of honey can be identified using a variety of factors. For many years, beekeepers have been interested in identifying the source of honey. Because each species of plant has a distinct pollen grain shape, beekeepers can identify the source plant with ease. There are numerous additional variables that can be considered. According to Kus & van Ruth (2015) and Tahir *et al*. (2016), among the most important elements influencing honey's physical properties is its chemical composition.

As demonstrated by Ruoff *et al*. (2007), Kumar *et al*. (2018) and Karabagias *et al*. (2019); individual parameters were used to determine botanical origin, it is very challenging to assign an assignment to an unknown sample that needs to be determined, which is why multivariate statistical methods are much more commonly used to distinguish honeys, particularly when there is a high variability of the sample within a group.

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2. Materials and Methods

2.1. Samples of honey and Studying parameters

In 2022, Northern Egypt was the source of the honey samples that were collected and examined in the Ministry of Agriculture's Beekeeping Section's honey laboratory. The samples were then kept at -10 degrees Celsius until further analysis. A preliminary pollen analysis was used to identify samples with varying botanical origins. Based on the predominant pollen grains found, five floral species were identified from the samples of honey: fennel, sidr, nigella, marjoram, and anise. Honeys were categorized as monofloral when the dominant pollen accounted for more than 45% of the total pollen (Khan, 2006 and Karabagias *et al*., 2019).

In this investigation, to characterize the various varieties of honey, 17 standards were selected for this study: The Association of Official Analytical Chemists defines moisture and total soluble solids (T.S.S.) (A.O.A.C., 1995). Viscosity, Specific gravity White (1978), electrical conductivity (EC) (Sancho *et al*., 1992).

According to Bogdanov and Bauman (1988), Using high performance liquid chromatography, the percentages of sucrose, fructose, glucose, and maltose in the sugar content were calculated (HPLC). Also, H.M.F.; pH; free acidity; total acidity; G/W; F/G; lactone and Diastase number were determined (White, 1978 and A.O.A.C., 1995).

Statistical analysis

The results of linear discriminate analysis (LDA), which was used to all of the physicochemical parameters that were examined and applied to the data set under investigation, were computed using the statistical program SPSS (Essa *et al*., 2010; Fatehe, 2013 and Abuo El-Naga *et al*., 2021).

The study employed discriminant analysis to differentiate five distinct varieties of natural honey. The discriminant function that makes a substantial contribution to the discrimination between the groups under investigation that is observed was determined using Wilks' Lambda test (Fatehe, 2013 Karabagias *et al*., 2017 and Abuo El-Naga *et al*., 2021).

3. Results and Discussion

3.1. The physico-chemical characteristics

Physicochemical characteristics' measured values of five types of honey in north Egypt were shown in Table (1). Moisture ranged from 17.35 to 18.38 %; there were significant differences between honey samples. It has significant impact on keeping quality (Essa, *et al*., 2010). Bogdanov *et al*. (1999) state that honey contains a lot of water will therefore probably ferment. The global norm for honey moisture content was established by European Commission (2002) and Codex Alimentarius Commission (2001), 20.0 g/100 g as the maximum value. According to Isengard and Schulthei (2003), honey's water content is limited to 23.0% in order to prevent microbiological spoiling.

Viscosity is a crucial technical factor in the processing of honey. Based on the data provided, the viscosity varied between 46.73 and 73.12 Poise. According to White (1975), temperature and water content are the main factors influencing honey's viscosity fluctuations. Specific gravity, a measure of honey density that is dependent on how much water the honey contains. There isn't any noticeable difference in specific gravity between the samples that were analyzed. These densities are within the range that Essa, *et al*. (2010) discovered.

Honey is shielded from fermentation by the dry matter, which should be 78% or higher (Essa *et al*., 2010). Between all honey samples, the presented data showed no significant differences. Oman honey contains 76.83% T.S.S. related to this, according to Hussein (1989). According to Al-Arify (1998), Saudi honey's T.S.S. ranged from 81.73% to 84.37%. Although Tharwat & Nafea (2006) came to the conclusion that Saudi Arabia's T.S.S content ranged from 83 to 84.5%.

Electrical conductivity is a characteristic of the plant, especially from which the honey is derived. Ash content and electrical conductivity are correlated, meaning that samples with high ash content also had high electrical conductivity values, and vice versa (Bogdanov *et al*., 2004).

All honey samples exhibited an acidic chemical property; their pH fell between 3.80 and 5.36, falling between the usual range of 3.40 and 6.10 (Codex Alimentarius Commission, 2001). Honey's ability to withstand microbes is dependent on its acidity level. Between 3.49 and 4.70 was the pH range of the honey samples, which was comparatively comparable to earlier reports of Indian honeys, Algeria, Brazil, Spain, and Turkey (Khan, 2006; Kayacier and Karaman, 2008; Saxena *et al*., 2010; Kumar *et al*., 2018 and Fatehe, 2013).

Table 1: Physicochemical characters of Fennel, Sidr, Nigella, Marjoram and Anise honey produced in Egypt

Physicochemical parameters	Fennel	Sidr	Nigella	Marjoram	Anise
Moisture (%)	17.35 c ±1.03	18.19 a ±1.13	18.38 a ±1.11	17.67 b ±0.76	17.88 b ±1.65
Viscosity	73.12 a ±36.25	50.83 c ±17.70	46.73 c ±16.10	62.03 b ±12.07	68 a .88±50.20
Specific gravity	1.42 a ±0.01	1.42 a ±0.01	1.41 a ±0.01	1.42 a ±0.00	1.42 a ±0.01
T.S.S. (%)	82.65 a ±1.03	81.81 a ±1.13	81.63 a ±1.11	82.33 a ±0.76	82.13 a ±1.65
H.M.F. (mg/Kg)	20.76 b ±22.33	14.98 c ±16.74	28.37 a ±22.31	5.15 d ±5.05	22.90 b ±25.03
E.C. (mS/cm)	0.21 c ±0.20	0.44 b ±0.56	0.70 a ±0.32	0.56 b ±0.25	0.36 b ±0.13
pH	4.10 b ±0.21	4.69 b ±0.90	5.36 a ±0.54	3.80 b ±0.31	4.49 b ±0.35
Free acids (meq./kg)	33.05 b ±6.10	28.63 b ±5.01	28.25 b ±5.98	36.17 a ±1.26	27.00 b ±3.08
Lactone (meq./kg)	3.20 b ±1.95	4.94 a ±6.40	1.75 b ±0.87	2.33 b ±0.29	1.13 c ±0.48
Total acidity (meq./kg)	36.25 a ±6.58	33.56 a ±7.11	30.00 b ±5.15	37.83 a ±2.02	28.13 b ±2.95
Fructose (%)	43.11 a ±3.88	38.50 a ±5.16	41.98 a ±2.10	42.42 a ±2.70	41.95 a ±1.29
Glucose (%)	31.04 a ±2.39	31.55 a ±5.54	31.36 a ±2.10	33.46 a ±1.29	31.43 a ±1.26
Sucrose (%)	2.25 a ±1.13	1.24 b ±1.00	2.91 a ±1.66	2.35 a ±1.18	3.00 a ±0.78
Maltose (%)	1.90 c ±0.72	2.04 c ±1.38	3.37 a ±2.47	2.40 b ±0.33	3.75 a ±1.45
Diastase	11.60 c ±10.10	12.37 c ±11.19	20.00 a ±11.66	14.00 b ±13.86	14.50 b ±10.63
G/W	1.79 a ±0.17	1.74 a ±0.34	1.71 a ±0.08	1.90 a ±0.09	1.77 a ±0.23
F/G	1.40 a ±0.17	1.25 a ±0.22	1.34 a ±0.11	1.27 a ±0.07	1.34 a ±0.03

Different letters in the same row indicate significant differences .

3.2. Discrimination analysis

Four functions of discrimination were revealed by the physicochemical characteristics of honey samples of fennel, sidr, nigella, marjoram, and anise that formed significant among these honey samples and belonged to the honey type. It is evident that the parameters of pH, free acidity, sucrose, and maltose varied significantly depending on the honey's floral origin; the corresponding Wilks' Lambda values were 0.592, 0.659, 0.616, and 0.585 (Table, 2).

Table 2: Physicochemical characteristics using multivariate analysis of variance. according to Fennel, Sidr, Nigella, Marjoram and anise honey samples produced in Egypt.

Physicochemical parameters	Wilks' Lambda	F	df1	df2	Sig.
Moisture (%)	0.894	0.652	4	22	0.632
Viscosity	0.874	0.794	4	22	0.542
Specific gravity	0.896	0.638	4	22	0.641
T.S.S. (%)	0.894	0.652	4	22	0.632
H.M.F. (mg/Kg)	0.862	0.881	4	22	0.492
E.C. (mS/cm)	0.842	1.035	4	22	0.412
pH	0.592	3.795	4	22	0.017*
Free acids (meq./kg)	0.659	2.849	4	22	0.048*
Lactone (meq./kg)	0.882	0.732	4	22	0.580
Total acidity (meq./kg)	0.710	2.249	4	22	0.097
Fructose (%)	0.773	1.614	4	22	0.206
Glucose (%)	0.953	0.269	4	22	0.895
Sucrose (%)	0.616	3.430	4	22	0.025*
Maltose (%)	0.585	3.898	4	22	0.015*
Diastase	0.957	0.245	4	22	0.910
G/W	0.951	0.281	4	22	0.887
F/G	0.860	0.892	4	22	0.485

df: degrees of freedom, *: significant, F: Fisher's coefficient.

A good canonical correlation of 0.99 and a high percentage of total variance can be explained by the discrimination analysis of four functions, which was used to classify honey samples of fennel, sidr, nigella, marjoram, and anise based on their physiochemical characteristics. The correlation coefficients of the standardized canonical discriminate function for each of the important physic-chemical characteristics that aided in the honey type's ability to differentiate between anise, marjoram, nigella, fennel, and sidr are shown in Table (3).

Table 3: Standardized canonical discriminate functions and discriminating variabl**es**

Physicochemical parameters	Function			
	1	2	3	5
Maltose (%)	-0.035	0.401*	0.203	0.062
Total acidity (meq./kg)	0.064	-0.234*	-0.098	0.056
Lactone (meq./kg)	-0.020	-0.177*	0.000	-0.054
Free acids (meq./kg)	0.095	-0.144*	-0.092	0.125
Diastase	-0.007	0.095*	0.078	0.028
F/G	0.022	0.065	-0.286*	-0.001
Fructose (%)	0.059	0.111	-0.232*	0.089
T.S.S. (%)[a]	0.038	0.013	-0.180*	-0.080
Moisture (%)	-0.038	-0.013	0.180*	0.080
Specific gravity	0.039	0.003	-0.169*	-0.047
Glucose (%)	0.017	-0.016	0.147*	0.057
G/W[a]	0.019	-0.009	0.024*	-0.008
Sucrose (%)	0.017	0.363	-0.090	0.683*
Ph	-0.110	0.101	0.109	0.504*
E.C. (mS/cm)	-0.015	0.022	0.302	0.379*
Viscosity	0.033	0.076	-0.172	-0.331*

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions
* highest absolute correlation between any discriminant function and any variable variables arranged within a function according to the absolute size of correlation.

Four discriminate functions were formed, as indicated by the discriminate analysis in table (4): Based on the physiochemical features that were the focus of the investigation, the classification of Fennel, Sidr, Nigella, Marjoram, and Anise honey was based on Discriminate Function 1. In contrast to function 2, which recorded an eigenvalue of 3.646 and a canonical correlation of 0.886; function 3 displayed an eigenvalue of 1.525 and a canonical correlation of 0.777; and function 4, it reported a high eigenvalue (47.090) and a high canonical correlation (0.99), explaining 89.7% of the total variance and revealing an incredibly low eigenvalue (0.252) and canonical correlation (0.448).

Table 4: Canonical discriminant functions (Eigenvalues) of the physicochemical parameters according to Fennel, Sidr, Nigella, Marjoram and anise honey.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	47.090[a]	89.7	89.7	0.990
2	3.646[a]	6.9	96.6	0.886
3	1.525[a]	2.9	99.5	0.777
4	0.252[a]	0.5	100.0	0.448

The discriminate functions created were represented by respective group centroid values, which were (3.403, -0.293), (-5.823, -1.778), (-6.998, 2.345), (12.830, -0.159) and (-1.649, 3.534) for Fennel, Sidr, Nigella, Marjoram and Anise honey, respectively (table 5). The common physicochemical characters values showed that Fennel, Sidr, Nigella, Marjoram and Anise honey are well differentiated. For this method, it can be regarded as a very satisfactory discrimination rate. Using all of the characters under investigation, analysis of discrimination was used to demonstrate the connection between the honey kinds of fennel, sidr, nigella, marjoram, and anise. As can be seen in Figure 1, on the first discriminant functions, the fennel and marjoram honey were clearly separated from the Nigella, marjoram, and anise honey.

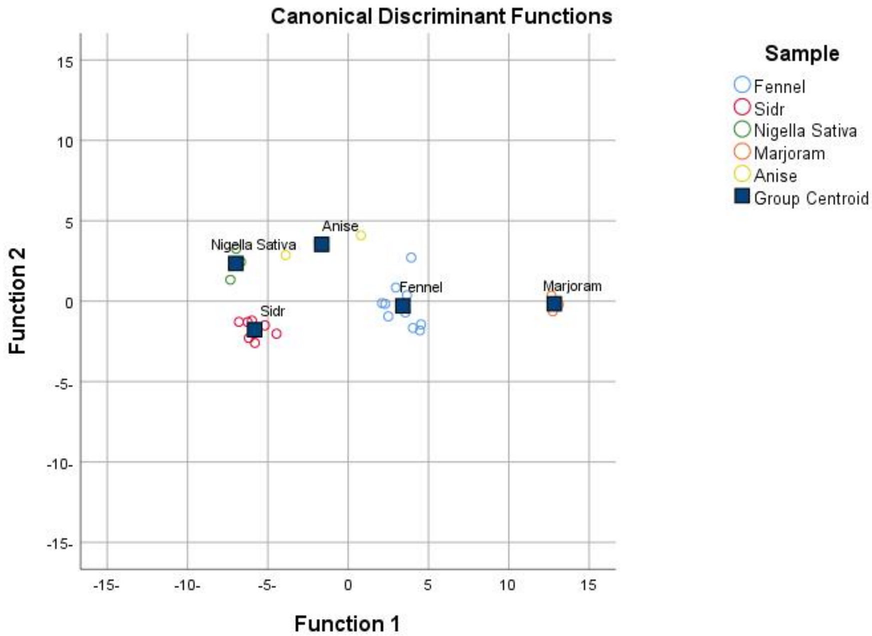

Fig. 1: Discrimination of Fennel, Sidr, Nigella, Marjoram and Anise honey from Egypt based on the physico-chemical characteristics under the study.

To ascertain the variety of honey and its botanical origin, multivariate analysis of the common conventional physical and chemical characteristics of honey could be useful in identifying new standards. Numerous studies (Khan, 2006) in Pakistan, (Essa *et al*., 2010; Fatehe, 2013 and Abuo El-Naga *et al*., 2021) in Egypt, (Karabagias *et al*., 2014) in Greek, (Serrano *et al*., 2004 and Karabagias *et al*., 2017) in Spain, (Chakir *et al*., 2016) in Morocco, and (Kumar *et al*., 2018) in India have reported on the analysis of discrimination between physicochemical properties of different kinds of honey created in different counters for various honeys.

According to Zidan (2019), Classifying Sidr honey samples produced in Yemen, Egypt, Libya, and Algeria using discriminate analysis. Upon validation, the discriminate analysis model correctly classified the samples 100% of the time. The results showed that, even with all the characters from the analysis along with those from Libya and Yemen, it was impossible to classify Sidr honey from Egypt and Algeria completely into the categories to which it belonged. The most useful traits and variations among the Sidr honeys made inArab nations were discovered in pH, free acidity, fructose, glucose, sucrose, electrical conductivity (EC), lactone and maltose contents (Abuo El-Naga *et al*., 2021).

Many researchers worked very hard to use discriminant analysis to categorize different honey kinds (Urska *et al*., 2009; Bogdanov *et al*., 2004; Oroian *et al*., 2015; Kivrak *et al*., 2016; Semnani *et al*., 2017; Karabagias & Karabournioti, 2018 and Abuo El-Naga *et al*., 2021). According to Ruoff *et al*. (2007) and Corvucci *et al*. (2015), discriminant analysis's primary goal is to predict which of the various groups that are mutually exclusive an unknown sample belongs to by classifying it into one of those groups using the measured characters.

Furthermore, by using physicochemical analysis, the different varieties of honey could not be identified. However, identifying the origin of the plants in the samples is unreliable based solely on the proportionate number of pollen grains in honeys from various plant species (Khan *et al*., 2006 and Karabagias *et al*., 2019). Additionally, a variety of factors influence the spectrum of pollen content, and the traditional pollen analysis technique, which establishes the botanic provenance of honey may be a little off in terms of counting (Khan *et al*., 2006; Lichtenberg-Kraag, 2015 and Kumar *et al*., 2018). As such, pollen analysis might not be a trustworthy method for determining the honey's botanical source.

Thus, it might not always be appropriate to classify the type of honey using just one variable. Therefore, if a honey sample were assigned to a particular type using multivariate classification and qualitative variables, it would fall under that particular category (Nafea and Mazeed, 2020). To help categorize these kinds of honey, some multivariate analysis could be added (Tahir *et al*., 2016).

Accorging to Essa *et al.* (2010); Fatehe (2013) and Nafea and Mazeed (2020), Utilizing chemical characteristics (sucrose, glucose, fructose, maltose content, lactones, pH, free acids, total acidity, total soluble solids (TSS) and E.C.), on three different varieties of floral honey (cotton, clover and citrus), discriminant analysis was done. to distinguish them from one another and from honey that is fed to sugar.

In order to determine the key parameters to employ in the classification process and to serve as a tool for data classification, Variance and discriminant analysis were used to statistically test each and every one of the data. The findings demonstrated that, even when all parameters were included in the analysis at the same time; cotton, clover and citrus honey could not be fully categorized to the related groups. For distinguishing between the various varieties of honey; sucrose, glucose, fructose, maltose content, free acidity, total acidity and TSS proved to be the most helpful parameters.

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