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Classical classification and discrimination analysis of physicochemical characters of Sidr honey produced in some Arab countries

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Abstract:

Sidr honey is one of the best and most expensive monoflora honeys world wide. The aim of this work is to use discriminate analysis for classifying of blossom Sidr honey from Egypt, Libya, Algeria and Yemen countries. All data were statistically tested using analysis of variance. Discriminate analysis was used to identify the most important parameters in the classification. The development of partial least square discriminate analysis (PLS-DA) model on validation gave 100% correct classification of the samples. The results showed that, Sidr honey from Egypt and Algeria could not be assigned by 100% into their actual groups even when all parameters were used simultaneously in the analysis as well as Libya and Yemen. Different parameters were discussed in the light, electrical conductivity (EC), pH, free acidity, lactone, fructose, glucose, sucrose and maltose contents accounted for the most discrimination parameters between the different Sidr honey in Arab countries and the effectiveness of the chosen parameters to characterization and discrimination. All tested honey samples were within the level allowable by the international standards for honey quality. The application of the discriminate technique (PLS-DA) presented the best for discriminating Sidr honey in Arab countries.

Introduction

Sidr honey is famous in Arab countries, it was used for both nutritional and therapeutic purposes and its price attains quite high levels, the quality control of local and imported honey is completely inadequate. Properties and compositions of bee honey depend on its geographical floral origin, season, environmental factors and treatment of beekeepers (Kasˇkonien *et al*., 2010 and El-Metwally, 2015). Honey consists primarily of sugars, at most fructose (40–50%) and glucose (32–37%), little amounts of sucrose (<2%) and mineral constituents (ash less than 0.1%). Honey also contains water (13–20%) (Alvarez-Suarez *et al*., 2013). Moisture content of bee honey represents a major importance to its stability against granulation and fermentation. The low moisture content conserves honey from microbiological activity and thus it can be preserved for longer periods (Buba *et al*., 2013; Akhtar *et al*., 2014 and El-Metwally, 2015).

Honey contains at least 181 components (White, 1975). Although the major fundamental of honey are nearly the same in all honey samples, physical properties and the precise chemical composition of natural honeys differ according to the plant species on which the bees forage (James *et al*., 2009 and Cantarelli *et al*., 2008). This situation does not allow a sufficient protection of the consumer and facilitates possible frauds. Indeed, at the scientific level, only a few data are available: a research carried out on Sidr honey samples, to contribute more to the knowledge of Sidr honey produced in some Arab countries (Egypt, Algeria, Libya and Yemen), further investigated the subject, with the aim of evaluating, the quality of Sidr honey, verifying their compliance with international standards (Codex Alimentarius Commission , 2001). This paper was carried out to classify and indication Sidr honey types produced at Egypt, Algeria, Libya and Yemen countries by using discriminate analysis.

Materials and methods

1.Honey samples:

Twenty Honey samples were collected from different geographical regions Sidr plant of Egypt ,Libya, Algeria and Yemen.

1.1. Physical properties:

1.1.1. Moisture determination:

Association of official Analytical Chemists (A.O.A.C.) (1990) official method 969.38 was emphasized by refractometer (Digital refractometer a tago, Germany). All measurements were performed at 20 °C, after waiting for 6 min for equilibrium and obtaining the analogous % moisture (g/100 g honey) from the refractive index of the honey sample by consulting a standard table for the purpose.

1.1.2. The specific gravity and viscosity:

The gravity and viscosity were estimated according to White (1978). Electrical conductivity was determined by conducterimetric experiment (WTW Inolabconducterimeter), from a solution containing 10 g of honey in 75 mL of distilled water, (Sancho *et al*., 1992) the total soluble solids (TSS%) was measured according to A.O.A.C. (1990).

1.1.3.Electrical conductivity (EC) and total soluble solids(TSS):

The EC and TSS were measured using a conductivity meter HI 98311 (Hanna Instruments, Mauritius) in a 20% (w/v) solution of honey suspended in Milli-Q water as recommended by Bogdanov *et al.* (1999). The EC and TSS of each sample were analyzed and the means are expressed as mS/cm and ppm, respectively. The EC of milli-Q water alone was less than 10 µS/cm.

1.2.The chemical properties:

1.2.1. Measuring of pH, free acidity, lactones and total acidity:

The pH, free acidity, lactones and total acidity were measured, with a combined pH glass electrode connected to pH meter Basic 20, in a solution prepared with 10 g of honey in 75 mL of distilled water (NP 1309/1976). Free acidity was determined by potentiometric titration (A.O.A.C. ,1990). Official method 962.19. Honey samples were homogenized in a water bath and filtered through gauze, prior to analysis. Ten grams of honey were then dissolved in 75 mL of distilled water and alcoholic solution of phenolphthalein added. The solution was titrated with 0.1 N NaOH. Acidity (milli equivalent of acid per kg of honey) was determined as 10 times the volume of NaOH used in titration.

1.2.2. Estimation of the sugar content:

The honey tested was similar with the findings of other previously studied by High Performance Liquid Chromatography (HPLC) measured the concentration of fructose, glucose, sucrose and maltose (%) according to Bogdanov and Bauman (1988).

2. Statistical analysis:

Analysis of variance (ANOVA) and linear discriminate analysis (LDA) were used to the investigated set of data and calculated by the statistics software SPPS (version.20.0 for windows). ANOVA was applied to all the investigated physicochemical parameters, as a pre-treatment p rocedure, in order to point out the significant parameters that could discrimination Sidr honeys of Arab countries ($P< 0.05$). LDA, which is a supervised statistical technique, was then applied only to the significant parameters ($P< 0.05$) (independent variables) to determine a linear amalgamation of these group of subjects, which could provide a discrimination rate of Sidr honey according to different countries. The original and cross validation methods were considered. In the cross validation method, each case is classified by the functions derived from all cases other than that case. Lastly, the statistical criterion of Wilk's Lambda was also considered, since it evaluates the statistical significance (discriminatory ability) of the discriminate functions derived (Karabagias *et al.,*2017).

Results and Discussion

1. Physicalchemical properties of Sidr honey produced in Arab countries:

The measured values of physicochemical properties of Sidr honey in four Arab countries (Egypt, Algeria, Libya and Yemen) were shown in Table (1). Moisture content, a parameter related to the ripening degree, was ranged 17.70 ±0.224% to 18.00 ±0.447%, there were no significant differences, between Sidr honey samples, hence these results are indicated of good storage ability of these honeys. The sidr plants grow in same the approximately environmental condition nearly .The average moisture of the Sidr honey samples from Arab countries under study were found to be within the limit of not more than 20.0 g/100 g as prescribed by Codex Alimentarius Commission (2001). Moisture content is an important quality parameter, important above all for honey shelf-life (Bogdanov *et al*., 2008). Thus, honey having high water content is more probable to ferment (Bogdanov *et al*.,1999). A maximum value of 20.0 g/100 g was established by Codex Alimentarius Commission (2001) and European Commission (2002) commission as the international standard for honey moisture contents. The specific gravity were 1.415 ±0.018 to 1.417 ±0.073 of all samples were found this value meet honeys quality European Legislation, European Commission (2001). Viscosity,shows no significant difference between examined samples ($P<0.05$) were 69±0.08 to 69±0.36. But the electrical conductivity (EC%) is one of the most important factors for determining the physical characteristics of honey (Serrano *et al.,* 2004). It is also an important physical measurement for the authentication of unifloral honeys (Mateo and Bosch-Reig, 1998). The EC % values of the honey samples of Egypt and Algeria 0.046±0.009 and 0.036±0.004, respectively, were significantly different higher than honey samples of Libya and Yemen (P=0.0005***) which recorded 0.011±0.002 and 0.015±0.003, respectively. The EC% values of all tested honey samples were reported to be 0.21–1.61 mS/cm, in a previous study by Ouchemoukh *et al.* (2007). EC is a basic physicochemical parameter for the authentication of unifloral honeys (Mateo and Bosch-Reig, 1998). The EC value depends on the ash and acid content in honey in which the higher the content, the higher the resulting conductivity (Bogdanov *et al*., 2002). This parameter was recently included in the international standards, replacing the determination of ash content (Codex Alimentarius Commission, 2001). However, the results were similar to the findings previously reported by Saxena *et al.*(2010) and Alvarez-Suarez *et al.* (2010).

Table (1): Physicalchemical properties of Sidr honey produced in Arab countries (Egypt, Algeria, Libya andYemen).

Parameter	Egypt	Algeria	Libya	Yemen	F	P
Moisture (%)	17.70 ±0.224a	17.70 ±0.235a	18.00 ±0.292a	18.00 ±0.447a	1.54	0.243ns
Specific gravity	1.417 ±0.073a	1.417 ±0.043a	1.415 ±0.039a	1.415 ±0.018a	0.003	1.000ns
Viscosity(Poise)	69.00 ±0.316a	69.00 ±0.292a	69.00 ±0.100a	69.00 ±0.381a	0.000	1.000ns
EC (%)	0.046 ±0.004a	0.036 ±0.003a	0.011 ±0.002b	0.015 ±0.003b	184.04	0.000***
TSS (%)	82.00 ±2.345	82.00 ±2.236a	82.00 ±1.871a	82.00 ±2.121a	0.000	1.000ns
PH	5.30 ±0.332A	4.30 ±0.292b	3.500 ±0.200c	3.90 ±0.255bc	39.78	0.000***
Free Acidity(meq/ kg)	34.00 ±2.641b	47.00 ±1.797a	34.00 ±1.766b	49.90 ±1.105a	97.63	0.000***
Lactone(meq/ kg)	10.00 ±0.954a	3.50 ±0.381b	10.00 ±0.731a	2.000 ±0.283b	214.32	0.000***
Total acidity (meq/ kg)	43.14 ±4.384	49.76 ±2.483	43.66 ± 1.557	51.72 ±1.484	12.43	0.000***
Fructose(g/100g)	39.14 ±0.51b	39.70 ±0.39b	41.90 ±0.86a	38.00 ±0.89b	27.60	0.000***
Glucose(g/100g)	30.1 ±0.9b	32.0 ±0.40a	29.60 ±0.90b	30.00 ±0.60b	10.12	0.001**
Fructose/ Glucose Ratio	1.31 ± 0.066	1.24 ± 0.044	1.44 ± 0.104	1.28± 0.049	7.29	0.003**
Sucrose(g/100g)	1.10 ±0.25c	3.00 ±0.38b	5.10 ±0.29a	5.50 ±0.29a	218.38	0.000***
Maltose(g/100g)	6.00 ±0.39a	3.00 ±0.23b	6.30 ±0.33a	6.50 ±0.35a	123.18	0.000***
Glucose/Water Ratio	1.67 ±0.037	1.78 ±0.028	1.644 ±0.104	1.660 ±0.066	8.86	0.001**

Different letters in the same row indicate significan t differences.

Total soluble solids is a measure of the combined content of all inorganic and organic substances in honey in the molecular, ionized or micro-granular (colloidal solution) suspended forms. The present data in Table (1) revealed non-significant between all Sidr honey samples, were 82.0 ±1.41 to ±2.45, these results demonstrated a good correlation between EC and TSS, indicating that both parameters can be used to determine honey purity European Commission (2001). Chemical characteristic of all Sidr honey samples were acidic, the pH ranged 3.5±0.2 to 5.3±0.332, it was agreement with the standard limit pH 3.40-6.10 (Codex Alimentarius Commission , 2001). The acidic of Libyan Sidr honey was high significantly (pH 3.5±0.2) (*P* =0.0022**), than other samples followed by Yemen (3.9± 0.255) and Algeria honey (4.3± 0.292). The lowest acidity was detected in Egypt honey (5.3± 0.332). High pH value (6.23) was recorded for Sidr Aseer honey, while Sidr Albaha had a pH of 3.93 (Al-khalifa and Al-Arify, 1999). The pH values of honey samples were close to those previously reported in Indian, Algerian, Brazilian, Spanish and Turkish honeys (between pH 3.49 and 4.70) (Kayacier and Karaman, 2008 and Saxena *et al*., 2010). Acidity in honey is calculated as free, lactonic and total acidity. Specifics a free acidity of not more than 50 meq/1000 g (meq/kg) (European Commission, 2002). Some factors affecting bee honey acidity e.g. harvest seasons and floral types (El-Sherbiny and Rizk 1979 and Pe´rez-Arquillue´ *et al*., 1994).The average values for free acidity in samples were between 34.0±2.288 and 49.9±1.159 meq/kg and were highly significant among Yemen, Algeria Sidr honey samples and Egypt, Libya (*P*=0.0002***). Lactonic acidity ranged from 2.0±0.356 to 10.0±1.034 meq/kg and found highly significant between all samples (*P*=0.0000***). Total acidity detected highly significant between all samples (*P*=0.0000***), it's ranged from 44.03±5.02 to 51.93±1.59 meq/kg; The present invitigations are quite in agreement with Ouchemoukh *et al.* (2007). The high acidity of honey correlates with the fermentation of sugars present in the honey into organic acid, which is responsible for two important characteristics of honey: flavor and stability against microbial spoilage (Bogdanov *et al*.,2008). Illustrated

HPLC chromatography of the fructose, glucose, sucrose and maltose analysis of all Sidr honey samples in different Arab country under studies. The results indicated that there were significant differences of fructose value between Libyan Sidr honey (42.0±1.18) and others examined samples (*P*= 0.0204*).Furthermore, glucose content no significant in all Sidr honey samples. The glucose content was lower than the fructose content which indicated the natural feeding of honey colonies. In addition, the clear sucrose contents of Yemeni Sidr honey(5.5±0.29 g/100 g) and Libyan honey (5.1±0.29 g/100 g) were statistically significantly (*P*= 0.000***) higher than Algeria (3.0±0.29 g/100 g) and Egyptian honey (1.1g/100g).While maltose contents of Sidr honey samples shows highly significant (6.0±0.374, 6.3±0.37 &6.5±0.24 g/100 g) for Egypt, Libya and Yemen , respectively and Algeria Sidr honey recorded 3 ±0.22 g/100 g.(*P*= 0.000***).These results supported the previous several studies on different honey types (Buba *et al*., 2013 and EL-Metwally, 2015). Fructose/ glucose ratio indicates the ability of honey to crystallize. White and Doner (1980) reported that even though honey has less glucose than fructose, the honey were granulated because glucose less soluble in water than fructose. When the fructose/glucose ratio is high, honey remains liquid. Honey crystallization is slower when the fructose/glucose ratio is more than 1.3 and it is rapid when the ratio is below 1.0 (Amir *et al*., 2010). However, because honey contains others sugars (sucrose, maltose, turanose, etc.) and insoluble substances (like dextrin, colloids, etc) which can influence the crystallization process, the glucose/water (G/W) ratio is considered more suitable than the fructose/glucose (F/G) ratio for the forecast of honey crystallization. It has been stated that when the glucose/water ratio is <1.3 honey crystallization is very slow or even zero and it is complete and rapid when the ratio is >2.0 (Amir *et al*.,2010). Glucose, which is a major sugar in honey, can spontaneously crystallize from honey solutions in the form of its monohydrate (White and Doner,1980). This sometimes occurs when the moisture level in honey is allowed to drop below a certain level; i.e., when the musture content is very low. The international normal established by Codex Alimentarius Commission (2001) that a good quality honey should not contain more than 5 g/100 g sucrose. The apparent sucrose contents of the honey samples studied were in the range of 1.1 to 5.5 g/100 g. The values obtained for sucrose contents of the honey samples were all within the limits of international standards. According to White and Doner (1980) even though honey contains an active sucrose incision enzyme (sucrose and glucosidase), the sucrose level in honey never arrives at zero. The sucrose contents obtained in this realization are within the range of values stated for Argentine and Turkish (Cantarelli *et al*.,2008), Venezuelan (Vit *et al*.,2009), American (White and Doner,1980), Algerian (Makhloufi *et al*., 2007), Pakistani (Zafar *et al*.,2008) and Spanish (Cavia *et al*., 2006) honeys.

2. Discrimination of Sidr honey producing in Arab countries on selected physicochemical parameter values:

Results discriminate analysis showed that two discriminate functions were formed significant among these honey samples belong to country, hence, chemical parameters analysis showed significant differences on pH, lactone, total acidity, fructose , glucose , sucrose and maltose according to floral origin of honeys Wilks' Lambda between 0.028 to 0.345 (P = < 0.001 to 0.000) (Table, 2). The discriminate two functions was used for the classification of Sidr honey according to belong countries, since it explained 100% of total variance and a good canonical correlation equal to 0.999. In addition, the standardized canonical discriminate function coefficients correlation for each of the significant physicochemical parameters that contributed to the country discrimination of Sidr honey show in Table (3). In the end summary regarding the identification of the variables with the highest discriminatory power, higher the absolute value of a standardized canonical coefficient, the more significant the variable is for the determination of honey origin. Remarkable, discrimination ability of conventional physicochemical parameters, ease of application and reproducibility, have been previously reported in the literature in studies involving Spanish (Serrano *et al*., 2004 and Karabagias *et al*., 2017), Moroccan (Chakir *et al*., 2016) and Greek (Karabagias *et al*., 2014) unifloral honeys, in agreement with the present results. Ruoff *et al*. (2007) stated that several exceptions are listed in the above-mentioned standards, thus indicating the limited value of this measure and for the discrimination of honey types. Thus multivariate data evaluation of traditional physical and chemical measures and may also be helpful to establish new criterion for a more reliable description of the honey types and for the determination of their botanical origin. The chemo-metric analysis of physical and chemical data demonstrated that the botanical origin of honey can be determined without considering pollen analytical results.

Table (2): Multivariate analysis of variance for testing the equality of the means of investigation of the physicochemical parameters according to Sidr honey of Arab countries.

Tests of Equality of Group Means					
Physicochemical Parameters	Wilks' Lambda	F	df1	df2	P
Moisture (%)	0.776	1.538	3	16	0.243 ns
Specific (gravity)	0.999	0.003	3	16	1.000ns
Viscosity (poison)	1.000	0.000	3	16	1.000ns
EC (%)	0.028	184.044	3	16	0.000***
TSS(%)	1.000	0.000	3	16	1.000 ns
PH	0.118	39.778	3	16	0.000***
Acidity	0.052	97.631	3	16	0.000***
Lactone	0.024	214.321	3	16	0.000***
Fructose (g/100g)	0.162	27.59 5	3	16	0.000***
Glucose (g/100g)	0.345	10.115	3	16	0.001**
Sucrose (g/100g)	0.024	b218.377	3	16	0.000***
Maltose (g/100g)	0.041	123.182	3	16	0.000***

F: Fisher's coefficient, ns: not significant, df: degrees of freedom, p: probability.

3. Summary regarding the identification of the variables with the highest discriminatory power:

The higher the absolute value of a standardized canonical coefficient, the more significant the variable is for the determination Sidr honey of countries. Table (3) showed physicochemical parameters of groups correlations between discriminating variables and standardized canonical discriminate functions. Variables ordered by absolute size of correlation within function. The standardized canonical discriminate function coefficients obtained in the developed statistical model for the discrimination of Sidr honey from Arab countries under study. Based on the aforementioned, the variables that most contributed to the discrimination of Sidr honey according to country origin .

Table (3): Physicochemical parameters of groups correlations between discriminating variables and standardized canonical discriminate functions. Variables ordered by absolute size of correlation within function.

Physicochemical parameters	Function	
	1	2
Moisture (%)[a]	0.064	0.496*
Specific (gravity)[a]	-0.050	0.007
Viscosity	0.000	0.000
EC (%)	0.055	-0.328*
TSS(%)[a]	0.004	0.069*
PH[a]	0.033	0.078*
Acidity	-0.185*	-0.001
Lactone	0.274*	0.001
Fructose(g/100g)[a]	0.342*	-0.184
Glucose(g/100g)[a]	-0.375*	-0.006
Sucrose(g/100g)	-0.120	0.332*
Maltose(g/100g)	-0.120	0.332*

a. This variable not used in the analysis.

*. Largest absolute correlation between each variable and any discriminate function.

4. External validation of the developed statistical model for the differentiation of Arab countries:

In order to investigate the robustness of the statistical model developed for the classification of honeys, data involving specific physicochemical parameters of Sidr honey from Arab countries were introduced into the set of data and a new statistical analysis was carried out. The common physicochemical parameter values taken into account from the present database were EC, pH, total acidity, lactone, fructose, glocose, sucrose, maltose. In Figure (1), it is also shown that Sidr honey Egyptian, Algerian, Libyan and Yemeni are well differentiated. The overall correct classification and cross validation method rate was 100% within and between the countries, which is considered a very satisfactory discrimination rate for this method. Table (4) lists the discriminatory power of the developed statistical model (Egypt, Algeria, Libya and Yemen Sidr honey) was taken as the dependent variable. The total number of honey samples was arrived to 20 prior to discriminate analysis. Based on chemical parameter analysis and electrical conductivity, content values could be classified as honeydew honeys. Discriminate analysis showed that two discriminate functions were formed: discriminate function 1 was the basic function for the classification of Egyptian and Algeria Sidr honey according to physiochemical parameters , since it explained 62.8% of the total variance providing a high eigenvalue (527.29) and a high canonical correlation (0.999) in comparison with those of the discriminate function 2 (eigenvalue of 282.886 and canonical correlation of 0.998). Respective group centroid values, representing the discriminate functions created, were (23.141,-12.870), (-18.676, -17.027), (17.680, 16.119) and (-22.146, 13.779) for Egypt, Algeria , Libya and Yemen Sidr honey, respectively (Figure, 1). The discriminate function 1 was used for the classification of Sidr honey according to countries, since it explained 62.8% of variance function 1 and a good canonical correlation equal to 0.999. In addition, the standardized canonical each of the significant physicochemical parameters that contributed to the country discrimination of Sidr honeys are given in Table (3) and 100% for the cross validation method, considred a satisfactory discrimination rate for this method. Finally ,two methods for the determination of the botanical origin of Sidr honey are used. The first is to find and quantify specific parameters of unifloral honeys. In the second method different quality parameters are first determined and then physiochemical analysis is applied for honey classification. The first method is easier and more straight forward, but due to the major natural variation of honey composition, it may not be always possible to find specific markers for each unifloral honey. The second method yields statistical models for the classification of a known group of Sidr honey. The application of this method in future routine work needs statistical models that are based on the analysis of a representative number of authentic unifloral honeys. In these models polyflor al honeys, should also be included. Also, the physiochemical techniques should be correspond for routine use in control laboratories.

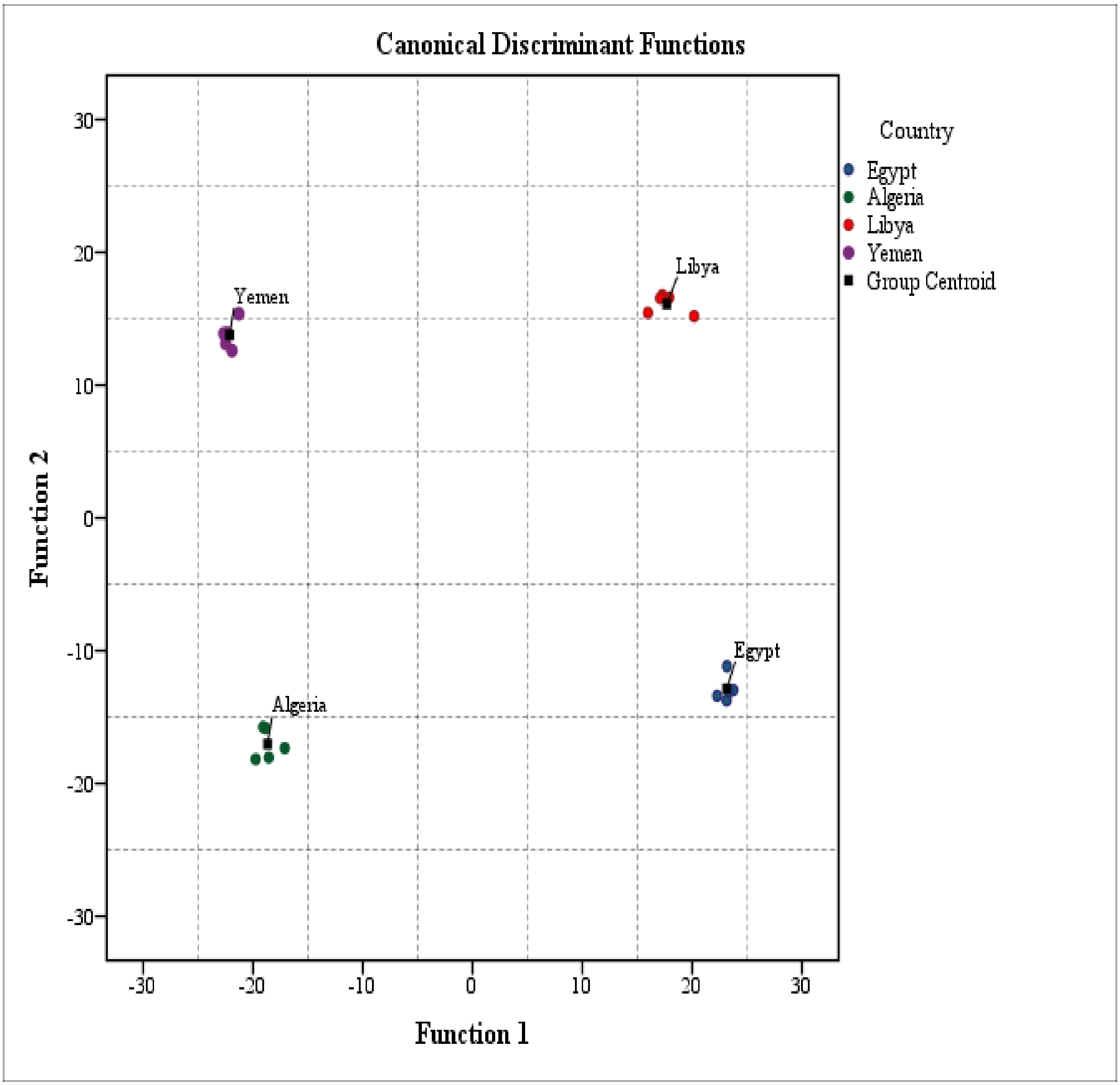

Figure (1): Discrimination of Sidr honey from Egypt, Algeria, Libya and Yemen based on 12 physicochemical parameters.

Table (4): Discriminatory power of the developed statistical model for the classification of Sidr honey from Arab countries

		Country	Predicted Group Membership				Total
			Egypt	Algeria	Libya	Yemen	
Original	Count	Egypt	5	0	0	0	5
		Algeria	0	5	0	0	5
		Libya	0	0	5	0	5
		Yemen	0	0	0	5	5
	%	Egypt	100.0	.0	.0	.0	100.0
		Algeria	.0	100.0	.0	.0	100.0
		Libya	.0	.0	100.0	.0	100.0
		Yemen	.0	.0	.0	100.0	100.0
Cross-validated[a]	Count	Egypt	5	0	0	0	5
		Algeria	0	5	0	0	5
		Libya	0	0	5	0	5
		Yemen	0	0	0	5	5
	%	Egypt	100.0	.0	.0	.0	100.0
		Algeria	.0	100.0	.0	.0	100.0
		Libya	.0	.0	100.0	.0	100.0
		Yemen	.0	.0	.0	100.0	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
b. 100.0% of original grouped cases correctly classified.
c. 100.0% of cross-validated grouped cases correctly classified.
Classification Results b,c

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