MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (3): 488-491, 2009
ISSN: 1680-5593

Determination of Honey Botanical Origin by Using Discriminant Analysis

Suat Sahinler, Nuray Sahinler and Aziz Gul
Department of Animal Science, Faculty of Agriculture, Mustafa Kemal University, Hatay, Turkey

Abstract: One of the most important criterions is determination of honey botanical origin in point of the sense of taste, the force of habit and quality of honey and the results of this, in preference of honey. The purpose of this study, is to discriminate the honey type using a linear discriminant function on the basis of honey chemical characteristics by using discriminant analysis, which is one of the multivariate techniques and has recently been much used in applied sciences. In this study, independent variables, mineral content (%), moisture (%), pH, acidity (meq), invert sugar (%), sucrose (%), diastase level and HMF (mg) belonging to 5 types as sunflower, citrus, cotton, pine and high plateau honeys were used. The discriminant functions from different combinations of variables on the basis of honey chemical characteristics were given and the cases from the original honey botanical origins, sunflower, citrus, cotton, pine and high plateau, could be classified 100% correctly by using these functions.

Key words: Honey, honey type, honey classification, chemical characteristics, botanical origin, discriminant analysis

INTRODUCTION

Various honey identification procedures have been studied by many researchers (Persona *et al*., 1988; Barbara, 1991; Popek, 2002). The main criteria of honey classification are physical attributes, which are measured simply and they have a good information value. A pollen analysis appeared to be the most frequently used method and electrical conductivity was found to be the most useful chemical characteristic for identifying honeys in some of these studies (Pridal and Vorlova, 2002). Optical rotation is a parameter in relation to determination of botanical origin and adulteration of honey but the limit values have not been harmonized so far (Bogdanov *et al*., 1997; Pridal and Vorlova, 2002). Microscopic analysis is another analytical method for the identification of botanical origin. Quantitative and qualitative content of honey particles and pollen grains is studied for the identification of honey group and the blossom origin. The microscopic analysis is able to detect the botanical origin more exactly than other analytical methods. However, it is difficult to correctly interpret results of melissopalynology and it needs a lot of experiences (Pridal and Vorlova, 2002). Nevertheless, with these methods, it is impossible to classify all honeys according to their individual types and varieties. In addition, some statistical methods such as canonical analysis, principle component analysis were suggested for choosing some of chemical and physical characteristics to identify of honey botanical origin (Krauze and Zalewski, 1991).

The aim of this study, was to find how exactly, it is possible to classify honey in relation to its botanical origin if chemical characteristics are used. Therefore, it was needful to find how close relations exist between the results of chemical characteristics in 50 honey samples from the Turkey.

In order to develop a procedure to determinate honey botanical origins based on the chemical characteristics of honey quality, 2-phase calculations were conducted. The first phase involved a one way ANOVA and MANOVA, while in the second phase, a method of discriminate function analysis, which is one of the multivariate techniques and has been much used in applied sciences recently was used. Moreover, a model to use determination of honey botanical origin was proposed.

MATERIALS AND METHODS

Honey samples: This research was carried out by using 50 honey samples freshly harvested and provided by various beekeepers in different local areas of Turkey. The honey samples were analyzed immediately.

Bee races: The African haplotypes and their ecotypes in mt-DNA studies of these races were observed in Hatay region (Kence, 2003). Besides, common bee races of Hatay region are *Apis mellifera anatoliaca*, *Apis mellifera caucasica*, *Apis mellifera syriaca* and their hybrids

Corresponding Author: Suat Sahinler, Department of Animal Science, Faculty of Agriculture, Mustafa Kemal University, 31034, Hatay, Turkey

(Sahinler and Sahinler, 1996). Common bee races of South Anatolia (Mugla, Kahramanmaraþ, Cukurova) region are *Apis mellifera anatoliaca* (Guler and Kaftanoglu, 1999; Kence, 2003).

Biochemical analysis: The samples of honey were analyzed by the standard methods of the association of official analytical chemists (Anonymous, 1990; Bogdanov *et al*., 1997). Moisture content of honey was determined by refractometer, protein content of honey was determined by using kjeldahl apparatus (Anonymous, 1990), mineral percentage was measured by calcinations at 600°C to mineral furnace. HMF was determined calorimetrically after dilution with distilled water and addition of p-toludine solution. Absorbance of solution was determined at 550 nm using 1 cm cells in an LKB-Biochrom Spectrophotometer. The acidity, 10 g of the honey samples were dissolved in 75 mL CO_2 with free distilled water and titrated with 0.1 N NaOH; pH of the honey solution was measured with a pH meter. The diastase activity was measured according to Anonymous (1990).

Statistical analysis: In order to develop a procedure to identify the type of honeys, based on the chemical characteristics of honey quality, 3 phase calculations were conducted. The 1st phase involved single factor variance analysis and averages calculated were grouped in homogeneous groups with use of a DUNCAN test (at the level of a = 0.01) (Bek and Efe, 1988). The 2nd phase involved multivariate variance analysis, which is a technique for assessing group differences across multiple metric dependent variables simultaneously, based on a set of categorical (nonmetric) variables acting as independent variables (Sahinler *et al*., 2005). In the 3rd phase, the discriminant analysis method was used. Moreover, a model to estimate honey botanical origin was proposed.

Discriminant function analysis is used to determine, which variables discriminate between 2 or more naturally occurring groups and to predict group membership based on a linear combination of the interval variables (Tabachnick and Fidell, 1996). The procedure begins with a set of observations were both group membership and the values of the interval variables are known. The end result of the procedure is a model that allows prediction of group membership when only the interval variables are known. A discriminant function, also called a canonical root, is a latent variable, which is created as a linear combination of discriminating (independent) variables. This is analogous to multiple regression, but the b's are discriminant coefficients, which maximize the distance between the means of the criterion (dependent) variable (Overall and Klett, 1972). The statistical tests were performed using SPSS 11.0.

RESULTS AND DISCUSSION

The variance analysis and multiple comparison test results of chemical parameters were summarized in Table 1. On the basis of results in Table 1, it can be inferred that the level of moisture content (%) is unvarying in all of honeys whereas the other parameters differentiate subgroups of honeys with different letters. Multivariate variance analysis applied to the data and the results matches the univariate results: there is an overall difference among the chemical compositions of samples from different types ($p<0.01$). Several different discriminant analyses were run on the questionnaire data using statistical package for the social sciences. The results were given in Table 2.

All chemical characteristics using one by one don't allow for the distinction of all honey botanical origins 100% except pH (Table 2). Some of them (e.g., Ash content) make it possible to distinguish 2 honey botanical origins (Citrus and Cotton) 100%. Sucrose content is not a distinctive parameter for distinction of honey botanical origins. Because, any of honey botanical origins could not be discriminate 100% by using sucrose content. Thus, it was obvious that the discriminant function analysis basing on the determination of the pH or Ash + pH, Moisture + pH, Moisture + Acidity will enable to identification of honey botanical origins. Statistically, so pH, of which classification rate is 100%, is sufficient to identify honey botanical origin that we do not need other chemical characteristics of honey here. Because, adding the other characteristics to the model will not increase the correct classification rate. Anyway, the classification rate can not be more than 100% (Table 2).

In the discriminant analysis, classification functions are fitted for estimating the group, which was drawn the sample. The number of classification functions will be the number of original groups. Thus, 5 classification functions were fitted for each honey botanical origin separately (Table 3). The function given in third row and included pH as independent variable is used. Because, that function has 100% classification rate and Canonical Correlation Coefficient being 1 approximately.

The values of independent variables, which the equations include, of a honey sample (pH value) put into the equation fitted for each honey origin and a value is calculated. The biggest value, which of them gives, the sample belongs to that honey origin. For example, if it is put the pH (5.63) value into the classification functions, the highest value (4951.03) obtains from Eq. 1. This means that the sample can belong to first honey group, which is

Table 1: The variance analysis and multiple comparison test results of chemical parameters (mean±SE)

	Group of Honey botanical origin				
Chemical characteristics	Sunflower	Pine	Citrus	Cotton	High plateau
Total ash (%)	0.37±0.23	0.57±0.06*	0.32±0.01*	0.47±0.02	0.47±0.01
Moisture	18±0.20a*	17.21±0.06b	18.43±0.45a	18.47±0.12a*	16.99±0.06b*
pH	5.63±0.03a*	4.45±0.02b	3.57±0.01c	3.93±0.05d	3.44±0.03e*
Acidity	40.73±2.46a*	25.73±1.02b	34.97±0.16c	25.25±0.83b*	37.67±0.63ac
Invert sugar	71.67±0.67a	67.50±1.03a	67.43±0.40a	59.95±1.98b*	76.11±1.78c*
Sucrose	6.47±0.78a*	3.99±0.16bc	3.34±0.34c*	4.67±0.23b	3.91±0.16bc
Diastase level	23.43±3.33a	29.40±0.00b*	10.90±0.00b*	23±0.00a	23±0.00a
HMF	2.17±0.00a*	5.44±0.65c*	3.77±0.47abc	4.60±0.89bc	2.76±0.51ab

*Units indicate the honey botanical origin, which has the lowest and the highest values of concerning chemical characteristics (Sahinler *et al*., 2004); Other letters also indicate the different honey botanical origins concerning chemical characteristics

Table 2: Results of the discriminant function analysis method

	Groups of honey botanical origin (Properly qualified samples (%))				
Measure	Sunflower	Pine	Citrus	Cotton	High plateau
Ash	0.0	66.7	100.0	100.0	0.0
Moisture	66.7	100.0	0.0	33.3	100.0
pH	100.0	100.0	100.0	100.0	100.0
Acidity	66.7	33.3	100.0	33.3	100.0
Invert sugar	100.0	33.3	66.7	66.7	66.7
Sucrose	66.7	33.3	66.7	66.7	66.7
Diastase level	0.0	100.0	100.0	100.0	0.0
HMF	100.0	33.3	33.3	33.3	33.3
Ash + pH	100.0	100.0	100.0	100.0	100.0
Moisture + pH	100.0	100.0	100.0	100.0	100.0
Moisture + Acidity	100.0	100.0	100.0	100.0	100.0
Ash + Moisture + pH	100.0	100.0	100.0	100.0	100.0
Ash + pH + Acidity	100.0	100.0	100.0	100.0	100.0
Moisture + pH + Acidity	100.0	100.0	100.0	100.0	100.0

Table 3: Classification function coefficients include the variables, which are selected according to the Table 2 for determination of honey botanical origins

	Groups of honey botanical origin								
Function	Sunflower	Pine	Citrus	Cotton	High plateau	Eigen value	Percentage of Var.	Cum. Var (%)	Can. Cor. Coef.
Ash	10.54	16.386	9.10	13.42	13.42	0.335	100.0	100.0	
(Constant)	-3.54	-6.279	-3.05	-4.74	-4.74				0.501
Moisture	143.58	137.250	147.04	147.30	135.55	4.514	100.0	100.0	
(Constant)	-1293.82	-1182.420	-1356.79	-1361.69	-1153.33				0.905
pH	1760.42	1390.630	1116.67	1229.17	1075.00	296.080	100.0	100.0	
(Constant)	-4960.12	-3095.750	-1996.72	-2418.97	-1850.61				0.998
Ash	29.30	31.220	21.01	26.52	24.88	296.401	99.9	99.9	
pH	1763.81	1394.230	1119.10	1232.23	1077.88	0.334	0.1	100.0	0.998
(Constant)	-4975.03	-3112.680	-2004.39	-2431.19	-1861.36				
Ash	-111.84	-99.480	-116.52	-111.68	-101.51	6.255	97.4	97.4	
Moisture	166.40	157.550	170.81	170.09	156.26	0.168	2.6	100.0	0.929
(Constant)	-1478.72	-1328.730	-1557.49	-1546.06	-1305.64				

Canonical correlation coefficient measures correlation between classification function and variables in equations

sunflower honey. If all average pH values in Table 1 put into the equations in Table 3, it is seen that all honey origins could be classified 100% correctly by using the classification function includes only pH as independent variable.

CONCLUSION

These results has indicated that:

- HMF, ash and sucrose contents are not useful alone as a predictor to determine the honey origin
- The most useful predictors are pH, invert sugar content and acidity or combinations of them, respectively
- It can be said that statistically, so pH, of which classification rate is 100% and Canonical Correlation Coefficient is 1 approximately, is sufficient to identify honey origin that we do not need other chemical characteristics of honey
- Being the most useful predictors are the chemical characteristics of honey, which can be measured easily such as pH and acidity will provide an important advantages to researchers

It should be expected giving the reliable estimations by using the classification functions include pH as independent variable for the honey samples, which are suspicious being from one of these 5 honey origins.

REFERENCES

Anonymous, 1990. Association of Official Analytical Chemists. 15th Edn. Methods of Analysis.

Barbara, S.B., 1991. Using pollen to identify honey. Am. Bee J., 131 (4): 242-243.

Bek, Y. and E. Efe, 1988. Experimental Design I. University of Cukurova, Faculty of Agriculture, Lecture Notes Adana, Turkey, 51: 395.

Bogdanov, S., P. Martin and C. Lullmann, 1997. Harmonised methods of the European Honey Commission, Apidologie, Extra Issue, pp: 1-59.

Guler, A. and O. Kaftanoglu, 1999. Determination of Performances of some important races and ecotypes of Turkish honeybees (*Apis mellifera* L.) under migratory beekeeping condition. Turk. J. Vet. Anim. Sci., 23 (3): 577-581.

Kence, A., 2003. Genetic variety and saving of honeybees in Turkey. Second Marmara Conference of Beekeeping, Yalova, Turkey, pp: 22-23.

Krauze, A. and R.I. Zalewski, 1991. Classification of honeys by principle component analysis on the basis chemical and physical parameters. Eur. Food Res. Technol., 192 (1): 19-23.

Overall, J.E. and C.J. Klett, 1972. Applied Multivariate Analysis. 1st Edn. McGraw-Hill, New York.

Persona, O.L., R., Stefanini, M.G. Piazza and M. Accorti, 1988. Diagnosis of unifloral honeys: Application of a statistical approach to honey classification. Apicoltura, 4: 27-38.

Popek, S., 2002. A procedure to identify a honey botanical origin. Food Chem., 79: 401-406.

Pridal, A. and L. Vorlova, 2002. Honey and its physical parameters. Czech J. Anim. Sci., 47 (10): 439-444.

Sahinler, N. and S. Sahinler, 1996. A study on the present situation and problems of apiculture and some recommendations in Hatay province. J. Agric. Faculty of MKU, 1 (1): 17-28.

Sahinler, S., A. Sahin and O. Gorgulu, 2005. Assessing the possible effects of ferula eleaochytris powder effects in layer diet on feed intake and some egg parameters by using a multivariate analysis method for repeated measures. J. Applied Anim. Res., 28: 29-33.

Sahinler, N., S. Sahinler and A. Gul, 2004. Biochemical composition of honeys produced in Turkey. J. Apicultural Res., 43 (2): 53-56.

Tabachnick, B.G. and L.S. Fidell, 1996. Using Multivariate Statistics. 3rd Edn. Harper Collins College Publishers, New York.