Australian Journal of Basic and Applied Sciences, 4(4): 552-556, 2010
ISSN 1991-8178

Identification of the Floral Origin of Honey by Amino Acids Composition

[1]Seif Eldin Abdel Rahman Mohammed and [2]Elfadil Elfadl Babiker

[1]National Centre for Research, P.O. Box 6096, Khartoum-Sudan
[2]Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Khartoum North, Shambat, 13314, Sudan

Abstract: A study was performed to find a correlation criterion between the amino acid composition and the botanical origin, in order to classify honey. The amino acids analyzer was used to determine the amino acids of honey samples from five different single botanical origins. Aspartic acid, phenylalanine, glutamic acid and proline were predominant ones, but not unique for honey types. Cystine was not detected in *Acacias* and *Azadirachta* honey. For all honey types the reset of amino acids determined, differed among them. Principle component analysis (PCA) was applied to data for pattern recognition. Discriminant function analysis was applied to four selected amino acids (Asp, Glu, His, and Phe) based on the result of PCA. Five groups of honey are shown in Dendrogram using cluster analysis. Discriminant function analysis showed high correct classification rate (93.33%). *Ziziphus*, *Helianthus*, *Acacia seyal* and *Azadirachta* honeys were 100% correctly classified and *Acacia nilotica* honey was 66.67% correctly classified.

Key words: amino acid composition; principle component analysis; honey classification

INTRODUCTION

Honey is nature's original sweetener. It has been used as food for at least six thousand years and from that time it was the sole source of sweet for much of the world's population. Interestingly, honey has been cited in the Quran, a Holy book of Muslims, in reference to its medicinal properties (Khan, S.Z. and T. Maqbool, 2008). It has been reported that amino acids in honey amount for 1% (w/w), and proline is the major contributor (Babendreier, D., 2004). Besides proline, there are 26 amino acids in honey, their relative proportions depending on the honey origin (nectar or honey dew). Some investigations proved a correlation between pollen, amino acid composition and the foraging behavior of honeybees (Cook, S.M., 2003). Statistical tests have become powerful tools for determining the geographical origin of honey. The application of linear discriminant analysis and the use of certain ratios between amino acid contents found in honeys allowed both geographical and botanical differences to be established (Iglesias, M.T., 2006). Both geographical and botanical origins of honey by applying different statistical tests such as Principle Component Analysis, Cluster Analysis, Partial Least Squares, and Support Vector Machine can be determined based on honey rheological data (Wei, Z., 2010). Also analysis of proline, leucine and phenayalalanine has been proposed to assess the effect of storage, ageing and processing on honey quality. Thus the use of multi-parametric studies, associated with chemo-metrics, yields satisfactory results for honey classification (Baroni, M.V., 2002). Interest in knowledge of the amino acids profile for honey has centered on three fields: first, as a potential tool for the botanical and geographical differentiation of honeys secondly, from a nutritional point of view and third, for quality control as indicator of freshness, based on the content of a few free amino acids (Gonza´lez Parama´s, A.M., 2006). The purpose of the present study was to investigate the amino acid profile of selected honeys types of single botanical origins and to predict their usefulness in honey classification by means of multi-parametric statistics.

MATERIALS AND METHODS

Materials:

Honey samples from five floral sources: *Ziziphus sp, Helianthus annuus, Acacia nilotica, Acacia seyal,* and *Azadarichta indica*, were collected during the season 2006/2007 from different states in Sudan. All reagents

Corresponding Author: Seif Eldin Abdel Rahman Mohammed, National Centre for Research, P.O. Box 6096, Khartoum-Sudan
Phone: +923232937579; E-mail:seifo169@hotmail.com.

used in this study are of reagent grade.

Determination of Amino Acids Composition:

To 200 mg honey sample in the hydrolysis tube 5 ml 6 N HCl was added and the tube was thematically sealed, then incubated at 110 ºC for 24 hours. The solution was filtered using Whatman No 1 filter paper. 200 µl of the filtrate was evaporated at 140 ºC for one hour. 1.0 ml of 0.12 N buffer with a pH 2.2 (11.8 g Tri-sodium citrate dehydrate, 6.0 g citric acid, 14 ml thiodiglycol, 12 ml of 32% HCl and 2 g phenol; dissolved in a litre distilled water) was added to the dried sample. Amino acid of the prepared samples was determined following the manufacturer's instructions (Sycam amino acid analyzer, S433-Sycam-Germany).

Statistical Analysis:

Data were statistically analyzed using Microsoft ware Statistica version 9.0 (StatSoft, Inc. 1984-2009, USA).

RESULTS AND DISCUSSION

Table 1 shows the content of amino acids of different honey types. 17 amino acids were determined in all honey samples. Sulphur-containing amino acid (cystine) was not present in both *Acacias* and *Azadirachta indica* honeys. Hermosin *et al*. (2003) and Echigo *et al*. (1986) reported similar observation for the absence of methionine and cystine in some Spanish and Japanese honeys. Threonine, serine, glycine, cystine, alanine, valine, methionine, isoleucine, leucine, tyrosine, histidine, lysine, and arginine contents were significantly ($p \geq 0.05$) differed between honey types. Proline which derived mainly form the bees secretions was not significantly different ($p \geq 0.05$) in all samples. Similar result for proline content was reported by Hermosin *et al.*(2003). Davies (1975) analyzed honey samples and non-honey sugar products. He concluded that non-honey sugar products could be recognized easily by their lower proline contents. *Acacia nilotica* and *Helianthus* honey were shown to contain high level of aspartic acid. Glutamic acid content (76.19 mg/100g) was found to be high in *Helianthus* honey. High contents of aspartic and glutamic acids could be due to hydrolysis of asparagine and glutamine during derivitization conditions yielding aspartic and glutamic acids, respectively; which agree with our results. Phenylalanine was high in *Azadirachta indica* honey (79.9 mg/100g) superseding proline contents for all honey types. Bogdanov *et al.* (2004) suggested that part of honey free amino acids are added by the bees which leads to high variability in amino acids contents within honey types of the same botanical origin. However, total amino acid rarely exceeds 300 ppm as reported by Ball (2007). In the principle component analysis, 2D or 3D corresponding score plot which shows the relation between the observations, and groupings of observations in the score plot can be used for classification (Wei, Z., 2010). The principle component analysis was applied for 16 amino acids (proline not included). Two principle components (PC) were obtained and were found to describe 83.1% of the common variance. PC1 explains 72.92% of the variables variance, while PC2 explains 10.18% of the total variance. Figure 1 shows 2D loading plots that characterize the main recognition patterns in variables and their explained variance. High positive loadings indicated that such variables have considerable presence in the corresponding PC. All 16 amino acids have shown positive loadings to PC1 and PC2, but aspartic acid and glutamine have higher positive loadings to PC1. On the other hand, histidine and phenylalanine have higher positive loadings to PC2. Positive loadings showed the contribution mostly defined by variables (amino acids) that distinguish honey samples according to floral origins. The amino acids Asp, Glu, His, and Phe were further used for classification of honey groups. Thus, application of discriminant function analysis to these four amino acids enabled 100% identification of *Ziziphus*, *Acacia seyal*, *Helianthus*, and *Azadirachta* honeys (Table 2). *Acacia nilotica* honey was 66.67% correct distinguished because one sample was identified as *Acacia seyal* by discriminant function analysis. Figure 2 illustrates the dendrogram that corresponds to clusters of the observations corresponding to each floral origin of the honey samples using joining (tree clustering) and Euclidean distance as a measure of similarity to classify the honey samples of five floral origins. It was obvious to distinguish five different groups. From the left, respectively first and fifth groups composed *Ziziphus* and *Azadirachta* honeys; the second group comprises *Acacia seyal* honey while *Acacia nilotica* sample misclassified to this group and the rest samples of this honey are confined to group three. Similar results were reported by Wei *et al*. (2010). The fourth group combines *Helianthus* honey which overlaps over the second and third groups. Several authors have applied PCA, discriminant function, and cluster analysis to honey's chemo-metrical parameters in order to predict the flora and/or geographical origins of honey. The present results agree with findings of Cometto *et al.* (2003) and dos Santos *et al.* (2008).

Table 1: Amino acids contents (mg/100g protein) of single floral honey of different origins.

Amino acid	Floral origin				
	Ziziphu ssp. (n=3)	*Acacia nilotica.* (n=3)	*Acacia seyal* (n=3)	*Helianthus annuus* (n=3)	*Azadirachta indica* (n=3)
Asp	46.28(±4.15)[a]	79.83(±9.30)[a]	64.69 (±5.40)[a]	81.07(±4.30)[a]	34.80(±1.80)[a]
Thr	30.00(±3.50)[ab]	16.30(±2.90)[a]	21.90(±1.30)[ab]	40.04(± 1.90)[b]	15.58(±0.36)[a]
Ser	30.31(±2.90)[ab]	19.36(±4.30)[a]	25.47(±2.03)[ab]	42.51(±1.50)[b]	18.45(±1.30)[a]
Glu	51.01(±8.10)[a]	57.95(±2.40)[a]	55.00(±4.70)[a]	76.19 (±3.80)[a]	38.97(±1.20)[a]
Gly	29.34(±6.40)[ab]	15.93(±3.10) [a]	24.38(±3.10)[ab]	42.49(±2.60)[b]	14.89(±2.10)[a]
Ala	32.84(±1.20)[ab]	21.49(±1.60) [a]	34.68(±3.30)[ab]	51.42(±1.00)[b]	21.97(±0.30)[a]
Cys	6.12 (±3.50)[a]	0.00	0.00	17.31(±2.90)[b]	0.00
Val	29.51(±1.8)[ab]	16.71(± 4.60) [a]	30.97(±5.30)[ab]	48.94 (±2.10)[b]	30.69(±1.00)[ab]
Meth	24.10(±1.3)[ab]	12.50(± 2.10) [a]	20.26 (± 8.70) [a]	38.50(±9.60)[b]	15.36(±2.10)[a]
Ile	28.21(±1.9)[ab]	15.63(±0.97) [a]	24.01(±9.10)[ab]	43.61(±4.70)[b]	24.20(±1.70)[ab]
Leu	30.00(±1.6)[ab]	16.56(± 1.20) [a]	29.99(±4.60)[ab]	51.16(±2.20)[b]	40.27(±2.90)[ab]
Tyr	22.19(±7.8)[ab]	12.54(± 1.40) [a]	18.75(±2.90)[ab]	37.63(±1.30)[b]	19.32(±2.80)[ab]
Phe	24.27(±8.0)[a]	31.14(± 3.10) [a]	25.81(±3.70)[a]	43.88(±1.60)[a]	79.90(±6.00)[a]
His	52.19(±5.1)[b]	7.64 (±0.71) [a]	38.72(±2.20)[ab]	6.68 (±1.57)[a]	36.44(±2.20)[ab]
Lys	33.37(±1.2)[ab]	18.66 (±4.50)[a]	27.67(±5.90)[a]	45.83(±3.02)[b]	22.57(±2.14)[a]
Arg	29.29(±4.5)[ab]	10.27 (±0.60)[a]	21.35(±2.00)[ab]	40.51(± 2.90)[b]	17.59(±1.18)[ab]
Pro	70.63 (±3.2)[a]	37.96 (±7.40)[a]	65.54 (±2.80)[a]	75.42 (±2.50)[a]	56.70(±2.90)[a]

Values are means (± SD) of triplicate samples. Means in the same row having the same letter(s) are not significantly different (P ≥ 0.05).

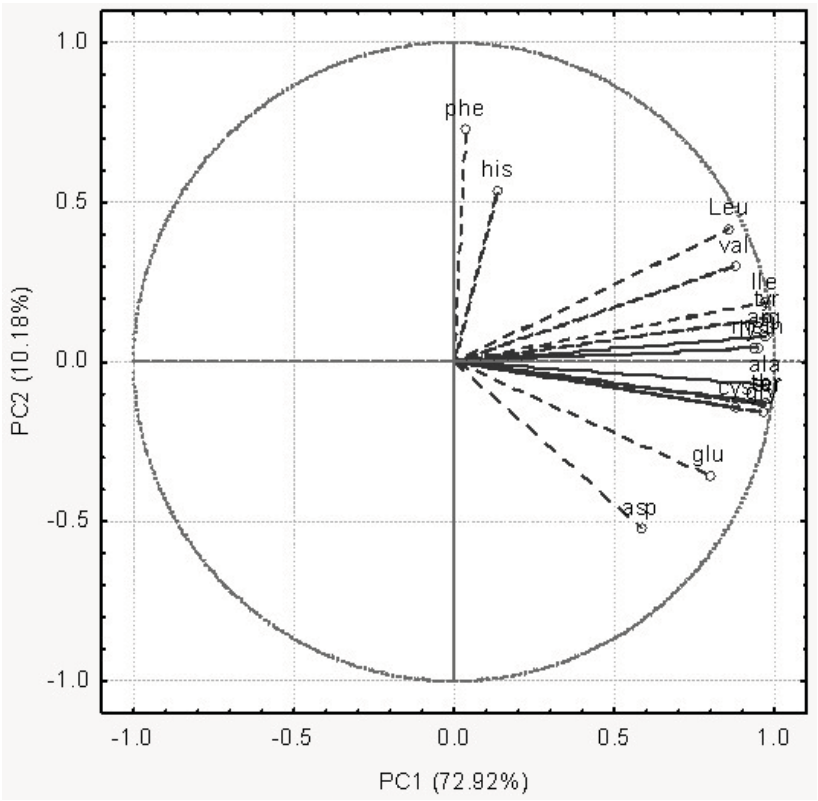

Fig. 1: 2 D Loadings of variables (16 amino acids) in the first two principle components for honey samples.

Table 2: Classification Matrix of the discriminant function analysis.

Group	% correct	*Ziziphus*	*Helianthus*	*Acacia nilotica*	*Acacia seyal*	*Azadirachta*
Ziziphus	100.00	3	0	0	0	0
Helianthus	100.00	0	3	0	0	0
Acacia nilotica	66.67	0		2	1	0
Acacia seyal	100.00	0	0	0	3	0
Azadirachta	100.00	0	0	0	0	3
total	93.33	3	3	2	4	3

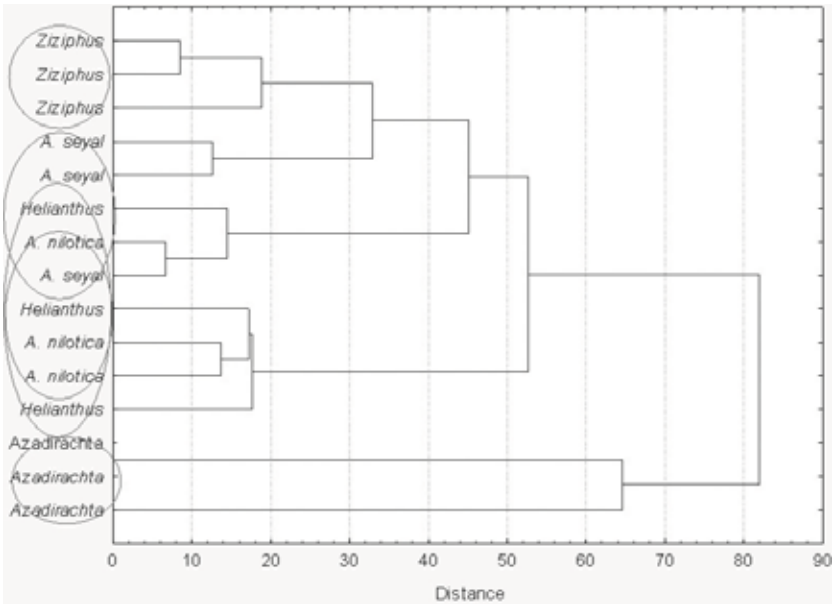

Fig. 2: Dendrogram from cluster analysis (Ward's method) in Euclidean distance for five group of honey samples from five floral origins.

Conclusion:

Statistical models used in this study proven efficient and true categorization and sorting of some single floral honey types. The results revealed that PCA pinpoints the active variables for sorting, discriminant function analysis distinguishes the floral origin of honey and cluster analysis in Euclidean distance was able to sort honey groups with some worth overlapping. The results show that it possible to apply these models to amino acids composition to identify honey source.

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