Australian Journal of Basic and Applied Sciences, 3(3): 2477-2483, 2009
ISSN 1991-8178

Protein Structure, Physicochemical Properties and Mineral Composition Of *Apis Mellifera* Honey Samples of Different Floral Origin

[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 13314, Shambat, Sudan

Abstract: The protein structure, physicochemical properties and mineral composition of *Apis mellifera* honey of different floral origin, commercialized in several states of Sudan were studied. Gel electrophoresis was applied to study the protein structure in order to establish a correlation between botanical species and the protein structure. SDS-PAGE pattern of the samples showed that about 12 distinctive bands for each sample were detected. All samples shared four bands with different molecular weight. Physicochemical properties of the samples showed that *Acacia nilotica* honey contained higher moisture content compared to other types while *Acacia seyal* was rich in sugar. The pH, refractive index and color varied between the samples. *Azadirachta indica* honey had electrical conductivity significantly ($p \geq 0.05$) differed from other samples which could be a reliable and distinctive parameter to differentiate *Azadirachta indica* honey from other types. For all honey samples both major and trace minerals varied between them. The heavy metals also were found to be varied between the samples. Cr was found to be <0.1mg/kg and Cd ranged from 0.05 to 0.1mg/kg which is below the risk level set by the WHO while Pb was found to be <0.45 mg/kg which exceeded the maximum amount allowed by WHO.

Key words: Hoeny, Apsi mellifera, protein, physicochemical properties, minerals

INTRODUCTION

Honey has a wide range of applications in the food industry. It can be processed for direct consumption or be used as ingredient of various processed food products. Honey is a nectar collected from many plants and processed by honey bees (*Apis mellifera*). It has been reported to contain about 200 substances and is considered as an important part of traditional medicine (White, 1979). The various components of honey include carbohydrates as a major portion (Hak-Gil *et al*., 1988) with proteins that include a number of enzymes and eighteen free amino acids (White 1979). Proteins and other solids make up 0.26% of honey and total nitrogen average is 0.043%. The number and nature of the protein content is very complex with at least 19 proteins in addition to albumin (Bergner and Diemer, 1975). Hermosin *et al.* (2003) stated that the proteins and amino acids in honey attribute both to animal and vegetable sources, the major of these being pollen. The identification of authenticity of honey based on honey proteins is a new tool for evaluation of honey as physiologically active food that may promote honey consumption. Although Baroni *et al*. (2002) suggest that the proteins that come from the bees are much higher than that of plant origin. Honey as a food is rich in sugars, making it a natural source of energy. Sugars account for 95 to 99% of honey dry matter. The majority of these are the simple sugars fructose and glucose which represent 85-95% of total sugars (Hak-Gil *et al*., 1988). The moisture, or conversely the soluble solids in honey is determined by measuring the refractive index of honey, a special moisture chart must be used (AOAC, 1990). The control of moisture content is an important requirement for the established Codex Alimintarius (1993) standards for honey. Honey is color graded into categories and the difference in color is due to botanical origin and also the amount of suspended particles such as pollen (Atrouse *et al*., 2004). The measurement of electrical conductivity is the most useful quality parameter for the classification of unifloral honeys. The parameter was introduced recently as a new international standard for honey (Bogdanov *et al.* 2004). It has been reported that characterization of uniforal commercial honeys is a hard task (Terrab *et al*. 2004). Attempts have been made to use the information provided by honey proteins to detect their floral origin (Babacan and Rand, 2005; Pontoh and Low, 2002; and

Corresponding Author: Elfadil E. Babiker, Faculty of Agriculture, University of Khartoum, Department of Food and Technology, Khartoum North 13314, Shambat, Sudan
E-mail: elfadilbabiker@yahoo.com

Weirich *et al*., 2002). Baroni *et al.*, (2002) used SDS-PAGE immunoblot techniques as a novel alternative method for assessment of floral origin in honey. Honey contains minerals such as calcium, iron, zinc, potassium, phosphorus, magnesium, selenium, chromium and manganese (Alkathiri & Khanbash, 1996, Murray *et al.,* 2001). Mineral/ash content contributes 0.17% by weight, darker honeys have been shown to be substantially richer in minerals than lighter honeys, particularly potassium, chlorine, sulfur, sodium, iron, manganese and magnesium (Hermosin *et al.*, 2003). Emma and Susana (2006) reported that heavy metals in honey are of interest not only for quality control, but can be used as an environmental indicator. Honey can characterize the level of soil, plant, and air pollution. Post Chernobyl radiocaesium distribution in honeys enhahances the use of honey as an indicator of environmental radioactivity monitoring (Boselli *et al*., 2003; Atrouse *et al*., 2004). The aim of the present work is to study the protein structure, physicochemical properties and mineral composition of *Apis mellifera* honey of different floral origin, commercialized in several states of Sudan.

MATERIALS AND METHODS

Materials:

Fifteen pure samples from five sources (*Helianthus annuus*, *Azadirachta indica*, *ziziphus spina-christi*, *Acacia seyal* and *Acacia nilotica*) certified and labeled were purchased from Mamlakat Alnahl Company, Khartoum, Sudan during the season 2006/2007. The samples were packed in plastic containers of 500g. The botanical origin of each sample was verified according to the standard acetolysis method of Erdtman (1969). For the determination of the protein structure, the samples were dialyzed to remove sugar and other extraneous materials. Unless otherwise stated, all reagents used in this study were reagent grade.

SDS–polyacrylamide Gel Electrophoresis:

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out using the method of Laemmli (1970) with a 15% acrylamide separating gel and 3% acrylamide staking gel containing 0.1% SDS. Samples (15 ml, 0.2%) were prepared in a Tris–glycine buffer at pH 8.8 containing 1% SDS. Electrophoresis was done at a current of 10 mA for 5 h in electrophoretic Tris–Glycine buffer containing 0.1% SDS. After electrophoresis, the gel sheets were stained for proteins and carbohydrate with 0.2% Coomassie brilliant blue-R250 and 0.5% periodate-funchsin solution (Zacharius, Zell, Morrison, & Woodlock, 1969), respectively. Protein stain was destained with 10% acetic acid containing 20% methanol. The molecular weight (kDa) of the bands was determined by using relative distance of the band from the standard marker.

Jacard Index:

Jaccard index (J) was calculated according to Ludarig and Reynolds (1988) method using the following equation:

$$J = a/a + b + c$$

Where a = bands shared between two types; b = total number of bands in type 1; c = total number of bands in type 2.

Physicochemical Properties:

The refractive index of the samples was measured using Abbe refractometer {Hilger, M 64.315/56304, England}. Electrical conductivity of the samples (20%) in carbon dioxide-free deionized distilled water was measured at 20 ºC using CM18 conductivity meter (032/ SN 3622/AE/11, Elico ltd) and the results were expressed in µ S/cm. The pH was determined with a pH meter (SN. 478524, Hanna instruments, Portugal) in a solution containing 75 ml carbon dioxide-free distilled water. The color of the samples was determined using the Lovibond comparator {PFX880, Salisbury, UK}. Moisture content of the samples was determined using Abbe refractometer (Hilger, M 64.315/56304, England) at 20 ºC and the corresponding moisture content (%) was calculated using the Wedmore Table (AOAC, 1990). Sugar content was determined using a refractometer (Kross optronic D-22976, Hamburg, Germany} and the results were expressed in ºBrix. Nitrogen and crude protein (%) were determined using micro-Kjeldahl method (AOAC, 1990).

Total Minerals Determination:

Minerals were extracted from the samples by the dry ashing method that described by Chapman & Pratt (1982). About 2.0 g of sample was acid-digested with diacid mixture (HNO3:HClO4, 5:1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was made to 50 mL with double-distilled water and was used for determination of minerals. Calcium

was determined by a titration method. Phosphorus was determined spectrophotometerically by using molybdovanadate method. All other minerals were determined using atomic absorption spectrophotometer (Perkin–Elmer 2380, England).

RESULTS AND DISCUSSION

Protein Structure of Honey Samples:

Figure 1 shows the SDS-PAGE pattern of the protein of honey samples of different floral origin. The pattern of the gel showed that about 12 distinctive bands were detected in the separating gel. The appearance of such bands on both seaparating and stacking gels indicated that the protein concentration of the samples is relatively high. The molecular weight of the proteins was found to be between 14 and 110.6 kDa with some bands above the stacking gel of higher molecular weight. It was observed that the samples shared only four bands with molecular weight of 104.6, 60.3, 45.6, and 14 kDa. *Ziziphus spina-chiristi* and *Azadirachta indica* honey were characterized by the presence of a band with a molecular weight of 92 kDa for the former and 64.1 for the latter. *Ziziphus spina-chriti* honey might contain lysophospholipase and lysozyme, since bands with molecular weight of 20 and 14 kDa were detected. The molecular weights detected indicated that all honey samples contained catalase, ovalbumin, phosphatase and lysozyme. Six bands with a molecular weight ranging from 14 to 106.4 kDa were found in *Acacia nilotica* honey. About seven bands of molecular weight ranging from 14 to110.6 kDa were detected in *Acacia seyal* honey with a protein band of molecular weight of 97 kDa was found to be a characteristic of this type and of *Helianthus annuus* honey. For *helianthus annuus* the protein pattern is similar to that of *Ziziphus spina-christi*. *Azadirachta indica* honey had seven bands with a characteristic band (64.1 kDa.) which could be used to distinguish this honey from other types. This band could be regarded as protein marker for *Azadirachta indica* honey and was found to correspond to phosphomonoesterase. Marshal and Williams (1987) detected at least 19 bands in a variety of Australian honeys following SDS-PAGE with a molecular weight ranging from 10.5 to 500 kDa. A Study carried out by Iglesias *et al*. (2006) applied fast protein liquid chromatography to differentiate floral and honeydew honeys. Seven peaks have been separated; four of which were shared in all honey types. The molecular weight was ranging from 13.1 to 94 kDa. Higher protein bands were detected in this study in the protein and carbohydrate (data not shown) stained gel. This finding is similar to that reported by Sano *et al.* (2004) who attributed the occurrence of substances with higher molecular weight in major royal jelly proteins to the attachment of sugars with the protein. Jaccard similarity index is shown in Table 1 and used to compare between the protein bands of the honey samples. It was found that the highest similarity (0.3) occurs between *Acacia seyal* and *Helianthus annuus* honeys. Generally result obtained for the separated protein and the bands detected agreed with those reported by Iglesias *et al*. (2006), Abd Al aal *et al*. (2002) and Marshal and Williams (1987).

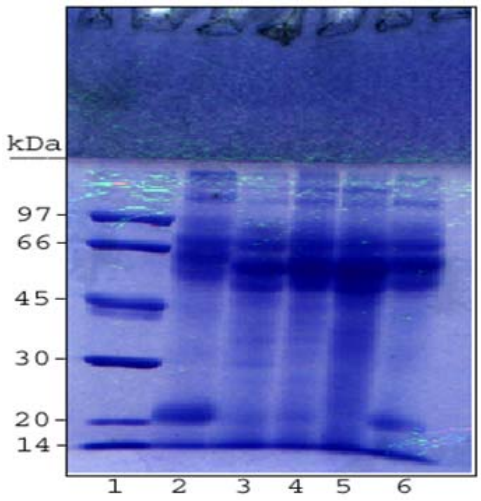

Fig. 1: SDS-PAGE patterns of honey protein of different floral origin. Lane 1, Molecular marker; lane2, *Zizyphus spina-christi* honey; lane 3, *Helianthus annuus* honey; lane 4, *Acacia nilotica* honey; lane 5, *Acacia seyal* honey; lane 6, *Azadirachta indica* honey.

Physicochemical Properties of Honey Samples:

The results of the physicochemical properties of honey samples are shown in Table 2. There was a significant ($p \geq 0.05$) difference between honey of different floral origin in moisture content. *Acacia nilotica* honey contained high amount of moisture (20.6%) compared to *Acacia seyal* honey which contained about 16.5%. On the other hand, there was no significant ($p \geq 0.05$) difference in moisture content between *Helianthus annuus* and *Azadirachta indica* honeys. The results obtained indicated that the floral source generally affected moisture content of honey type, and they are comparable with those obtained by Bath and Singh (1999), Adebiyi *et al.* (2004) and Mohammed (2005). Bogdanov *et al*. (2004) stated that moisture content has a minor importance as an index to differentiate between honey samples. The protein content obtained in this study for all samples was relatively high compared to previous findings of Bath and Singh (1999) who reported that the protein content of *Ziziphus* was varied between 0.048% and 0.42% but agreed with the data reported by Azeredo *et al.* (2003). Sugar content was found to be ranged from 78 to 80.3% (Table 2). There was a significant ($p \geq 0.05$) difference in ºBrix between *Acacia nilotica*, *Acacia seyal, Azadarichta indica*, and *Ziziphus spina-christi* honeys. The values obtained are similar to those reported by Terrab and Heredia (2004) and those of *Helianthus* honey reported by Bath and Singh (1999). The results obtained for the refractive index showed that there was a slight difference between the samples which agree with the values reported by Adebiyi *et al*. (2004) for Nigerian honey. As shown in Table 2 a significant ($p \geq 0.05$) variation in electrical conductivity between samples was observed. *Azardirachta indica* honey had the lowest mean of electrical conductivity (51.6 μ S/cm). It has been reported that the electrical conductivity is the most useful quality parameter for the classification of unifloral honeys (Bogdanov et al. 2004). Therefore, the electrical conductivity could be a reliable and distinctive parameter to differentiate *Azadirachta indica* honey from other honey types used in the study. The results obtained for electrical conductivity are agreed with results of Paramas *et al*. (2000), Terrab and Heredia (2004) and Adebiyi *et al*. (2004). Analysis of honey samples showed that the color was significantly ($p \geq 0.05$) different and it was found to be light amber to dark brown. It is important to note that the pH of honey does not directly reflect the total acid content, but rather reflects the buffering action of the inorganic cation constituents on the organic acids present. The pH also plays an important role in antimicrobial activity of honey. In this study a significant variation in pH value was observed between the samples. *Acacia nilotica* and *Helianthus annuus* honey had similar pH value. The values of pH obtained in this study agreed with the values obtained by Terrab and Heredia (2004), Atrouse *et al.* (2004), Adebiyi *et al*. (2004), Bath and Singh (1999), Paramas *et al.* (2000) and Terrab *et al.* (2004).

Mineral Composition of Honey Samples:

Table 3 shows the major and trace elements of honey of different floral origin. A significant variation between the samples in both major and trace elements was observed. *Acacia seyal* honey contained higher concentration of K (74.66 mg/kg) and lower level of Mn (0.761 mg/kg). Phosphorus (204.6 mg/kg) and sulphur (131.5 mg/kg) are found to be abundant in *Acacia nilotica* honey. *Azadirachta indica* honey contained lower amount of phosphorus (28.1 mg/kg) while *Helianthus annuus* contained higher amount of Ca (82.92 mg/kg) but lower amount of S (46.1 mg/kg). In our study values reported for Ca were lower than those reported by Terrab and Heredia (2004) and Adebiyi *et al*. (2004). *Acacia nilotica* contained higher level of Mg (177.15 mg/kg) and Na (28.2 mg/kg). *Azadirachta indica* honey contained lower amount of Mg (23.76 mg/kg) and Mn (0.12 mg/kg). *Helianthus annuus* honey contained lower amount of K (17.6 mg/kg) and Na (14.1 mg/kg). It has been reported that K is the abundant and predominant element in honey (Rodrigues-Otero *et al*., 1994). According to our study, K was predominant only in *Acacia Seyal* and *Azadirachta indica* honeys; while Mg was abundant in *Acacia nilotica*; *Ziziphus spina-christi*; and *Helianthus annuus* honey. Thyme honey from Spain was found to contain elements as K (679 mg/kg), Na (389 mg/kg) and Mg (77 mg/kg) as reported by Terrab *et al*. (2004) were higher than the results obtained in this study. Parmas *et al*. (2000) determined mineral composition of honey from Western Spain by flame photometric method and they found that it contained K (183.3 mg/kg), Na (55.8 mg/kg) and Mg (23.9 mg/kg). Trace element analysis (Table 3) showed a significant difference between honey samples. *Helianthus annuus* honey contained lower amount of Cu, Zn, and Ni (2.94, 4.86, 1.96 mg/kg, respectively) compared to other samples. Both *Ziziphus spina-chiristi* and *Helianthus annuus* honeys contained similar amount of Fe but *Azadirachta indica* honey contained lower amount. No significant difference was observed in Cu level between *Acacia nilotica*, *Helianthus annuus* and *Azadirachta indica* honey. *Azadirachta indica* honey contained low Co (0.005 mg/kg) compared to *Acacia seyal* (1.26 mg/kg), *Ziziphus spina-chiristi* (1.7 mg/kg), *Acacia nilotica* (1.004 mg/kg) and *Helianthus annuus* (0.528 mg/kg). The contents of Zn and Ni were 9.61 and 3.33 for *Acacia nilotica* honey, 7.96 and 3.99 for *Ziziphus spina-chiristi* honey, 5.18 and 4.06 for *Acacia seyal* honey and 4.86 and 1.96 mg/kg for *Helianthus*

annuus honey. Ni was not detected in *Azadirachta indica* honey but Zn level was 6.98 mg/kg. Cr was present in amount less than 0.1mg/kg for all honey types. Fe concentration was higher than the values reported by Merin *et al*., (1998) except in *Azadirachta* honey. Rashed and Soltan (2004) and Adebiyi *et al.*, (2004) reported higher values of Fe concentrations than those reported in this study. However, the result of Fe in our study is inconsistent with those reported by Roriguez- Otero *et al*. (1994). The level of Cu was similar to that found by Adebiyi *et al*. (2004) in Nigerian honey. The results of Zn agreed with the results of Merin *et al*. (1998) and Rashed, and Soltan (2004). The concentrations of Co and Cr are similar to those reported by Merin *et al*., (1998). Lead (Pb) was less than 0.45 mg/kg for all honey samples. Cadmium (Cd) was not detected in *Azadirachta indica* honey but in *Helianthus annuus* honey was 0.45 mg/kg and less than 0.1 mg/kg in *Ziziphus spina-chiristi, Acacia nilotica*, and *Acacia seyal* honeys. Several workers have studied the trace and toxic elements in honey. The investigation of honey contamination by heavy metals (Cd and Pb) revealed the presence of such metals in minute concentrations similar to that reported by Munoz and Pamero (2006) and Dragun *et al.* (2003). For toxic metals Pb and Cd, no toxicological problems can be expected from the consumption of honey, since the concentration of such metal are below the risk levels set by WHO (1982). Dragun *et al.* (2003) stated that the potential carcinogenic effect in the human body for cadmiun and possibly for lead, are of high importance in chronic exposure to these substances. The results obtained for Cd showed that it occurred in all samples but fortunately below the maximum amount allowed by WHO (1982). Lead concentration in all honey samples exceeded the maximum allowed amount. However, it has been declared by Dragun *et al.* (2003) that the maximum allowed amount of Cd and Pb are, respectively 0.10 mg/kg and 0.4 mg/kg in honey based products.

Table 1: Jacard index of honey samples of different floral origin.

Azadirachta	*Helianthus*	*Acacia nilotica*	*Acacia seyal*	*Ziziphus*	*Honey type*
0.25	0.21	0.25	0.22	-	*Ziziphus*
0.27	0.27	0.27	-	0.22	*Acacia seyal*
0.2	0.3	-	0.27	0.25	*Acacia nilotica*
0.2	-	0.3	0.27	0.21	*Helianthus*
-	0.2	0.2	0.27	0.25	*Azadirachta*

Table 2: Physicochemical characteristics of honey samples of different floral origin.

parameter	Floral origin				
	Ziziphus spina-	*Acacia nilotica*	*Acacia seyal*	*Helianthus annus*	*Azadirachta indica*
Moisture (%)	16.60 (± 0.50) [b]	20.60 (± 0.25)[a]	16.50 (± 0.36)[a]	18.60 (± 1.40) [c]	17.90 (± 0.80) [c]
Nitrogen (%)	0.107 (± 0.5) [a]	0.135 (± 0.49) [a]	0.140 (± 0.42) [a]	0.112 (± 0.48) [a]	0.121 (± 0.32) [a]
Protein (%)	0.685 (± 0.35) [a]	0.862 (± 0.31) [a]	0.893 (± 0.26) [a]	0.714 (± 0.31) [a]	0.774 (± 0.21) [a]
Sugar (ºBrix)	80.00 (± 0) [b]	78.00 (± 0.57) [a]	81.30 (± 0.57) [c]	80.60 (± 0.57) [a c]	80.30 (± 0.28) [b]
Refractive index	1.495 (± .002) [b]	1.493 (± 0) [a]	1.501 (±0.005) [a]	1.499 (± 0) [c]	1.499 (± 0) [c]
EC (µ S/cm)	167.7 (± 1.15) [b]	185.7 (± 30.4) [b]	151.0 (± 51.97) [b]	164.7 (± 47.1) [b]	51.6 (± 0.5) [a]
pH	5.4 (± 0.05)[d]	4.6 (±0.04) [b]	5.1 (± 0.06) [c]	4.6 (± 0.05) [b]	4.1 (± 0.2) [a]
Color (degree)	23.3 (± 0) [b]	23.4 (± 0) [c]	21.3 (± 0) [a]	25.5 (± 0) [d]	27.2 (± 0) [c]

Values are means of triplicate samples (± SD). Means in the same row having the same letter are not significantly different ($p \geq 0.05$).

Table 3: Major and trace elements content (mg/kg) of honey samples of different floral origin.

	Floral origin				
Mineral	*Ziziphus spina-*	*Acacia nilotica*	*Acacia seyal*	*Helianthus annus*	*Azadirachta indica*
Ca	42.37 (± 0.5) [b]	56.83 (± 0.04) [c]	35.63 (± 0.02) [a]	82.92(± 0.02) [e]	58.02 (± 0.07) [d]
P	49.6 (±0.75)	204.6 (±1.5) [a]	109.0 (±1.1)[c]	110.9 (±0.61) [b]	28.1(±0.56) [e]
K	41.66 (±1.5)	63.33 (± 1.2) [d]	74.66(± 0.57) [e]	17.60 (± 0.57)[a]	29.66 (±1.5) [b]
Na	16.78 (± 0.08)	28.24 (± 0.03)	22.18 (± 0.02)[ab]	14.11 (±.10.3)	22.9 (± 0.04) [ab]
Mg	48.28 (± 0.02) [d\]	177.15 (± 0.05) [e]	64.06 (± 0.05) [c]	73.31(± 0.01)	23.67 (± 0.01) [a]
S	59.4 (±0.7) [d]	131.5 (±0.52) [a]	66.4 (±0.41) [c]	46.1 (±) [e]	123.3 (±0.21) [b]
Fe	31.74 (± 0.4) [c]	18.97 (± 0.1) [b]	33.65 (± 0.01) [c]	32.33 (± 0.005) [c]	2.05 (± 0.03) [a]
Mn	0.818 (± 0.003) [b]	1.019 (± 0.005) [c]	0.761 (± 0.002) [a]	0.670 (± 0.001) [e]	0.121 (± 0.005) [d]
Cu	26.96 (± 0.03) [b]	11.45 (± 0.01) [a]	58.12 (± 0.01) [c]	2.94 (± 0.02) [a]	5.06 (± 0.05) [a]
Co	1.172 (± 0.001) [d]	1.004 (± 0.001) [c]	1.265 (± 0.001) [e]	0.528 (± 0.001) [b]	0.005 (± 0.001) [a]
Zn	7.96 (± 0.15) [d]	9.61 (± 0.02) [e]	5.18 (± 0.005) [b]	4.86 (± 0.01) [a]	6.98 (± 0.002) [c]
Ni	3.99 (± 0.003) [c]	3.33 (± 0.02) [d]	4.06 (± 0.003) [b]	1.96 (± 0.01) [a]	0.00
Cr	<0.1	<0.1	<0.1	<0.1	<0.1
Cd	0.1	0.1	0.1	0.05	0.00
Pb	<0.45	<0.45	<0.45	<0.45	<0.45

Conclusion:

The results obtained in this study indicated that the protein structure and some physicochemical properties can be used as indices to differentiate between different types. Moreover, determination of heavy metals in honey samples could be an indicator of soil and environment pollution with such metals.

REFERENCES

Abd El Aal, M., S. Zalat, A. Abouzeid and A. Ibrahim, 2002. Protein pattern of the honeybee venoms of Egypt. Egyptian Journal of Biology, 4: 42-46.

Adebiyi, I.A., E.I. Obiajunwa and H.B. Olaniyi, 2004. Chemical /physical characterization of Nigerian honey. Pakistan Journal of Nutrition, 3: 278-281.

Alkathiri, M.A. and M.S. Khanbash, 1996. Organo leptic characteristics for some local and imported honeys. Agricultural Science, 23: 599-614.

AOAC., 1990. Official Methods of analysis, 15th edn. Association of Official Analytical Chemists, Arlington, VA.

Atrouse, O.M., S.A. Oran and S.Y. Al Abbadi, 2004. Chemical analysis and identification of pollen grains from different Jordanian honey samples. International J. Food Sci. and Tech., 39: 413-417.

Azeredo, L. dac., M.A.A. Azeredo, S.R. de Souza and V.M.L. Dutra, 2003. Protein contents and physicochemical properties in honey samples of Apis mellifera of different floral origins Food Chem., 80: 249-254.

Babacan, S. and A.G. Rand, 2005. Purification of amylase form honey. J. Food. Sci., 70 : 413-418.

Baroni, M.V., G.A. Chiabrando, C. Costa, and D.A. Wunderlin, 2002. Assessment of the floral origin of honey by SDS-page immunoblot techniques. J. Agric. Food Chem., 50: 1362-1367.

Bath, P.K. and N. Singh, 1999. A comparison between Helianthus annuus and Eucalyptus honey. J. Food Chem., 67: 389-397.

Bergner, K.G. and S. Diemair, 1975. Proteins in honey II. Gel. Chromatography enzymatic activity and origin of honey proteins. Z labensm. Unters Forsch, 157: 7-13.

Bogdanov, S., K. Ruoff and L.P. Oddo, 2004. Physico-chemical methods for the characterization of unifloral honey: a review. Apidologie, 35: 54-517.

Boselli, E., M.F. Caboni, A.G. Sabatini, G.L. Marcazzan and L. Giovanni, 2003. Determination and changes of free amino acids in royal jelly during storage. Apidologie, 34: 129-137.

Chapman, H.D. and F.P. Pratt, 1982. Determination of minerals by titration method. In: Methods of Analysis for Soils, Plants and Water, 2nd edn. Oakland, CA: Agriculture Division, California University, Pp: 169–170.

Codex Alimintarius, 1993. Standards for honey, Ref. No. Cl 1993/14, SH, Codex Alimintarius Commission FAO/WHO, Rome.

Dragun, Z., D. Puntaric, D. Prpic-Majic, J. Bosnir, R. Gmajanic, M. Klaric, 2003. Toxic metals and Metaloids in Dietetic Products. Croat Med J, 44: 214-218.

Emma, M. and P. Susana, 2006. Determination of heavy metals in honey by potentiometeric stripping analysis and using a continuous flow methodology, Food Chem., 94: 478-483.

Erdtman, G., 1969. Hand book of palynology. Munskgaard. Copenhagen, Denmark. pp: 234-256.

Hak-Gil, C., H. Myung-Kyoo and K. Jae-Gil, 1988. The Chemical Composition of Korean honey. Korean J. Food Sci. and Tech., 20: 631-636.

Hermosin, I., R.M. Chicon and M.D. Cabezudo, 2003. Free amino acid composition and botanical origin of honey, J., Food chem., 83: 263-268.

Iglesias, M.T., P.J. Martin-Alvarez, M.C. Polo, C.D. Lorenzo and E. Pueyo, 2006. Protein analysis of honey by fast protein liquid chromatography: Application to differentiate floral and honeydew honeys. J. Agric Food Chem., 54: 8322-8327.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Ludarig, J.A. and J. F. Reynold, 1988. Statistical ecology. A primer on methods and computed. John Willy and Sun, New York, pp: 85-103.

Marshall, T. and K. M. Williams, 1987. Electrophoresis of honey: characterization of trace protein from a complex biological matrix by silver staining. J. Analytical biochemistry, 167: 301-3003.

Merin, U., S. Bernstein and I. Rosenthal, 1998. A parameter for honey quality, Food Chem., 63: 241-242.

Mohammed. S.A., 2005. Effect of the processing heat on the hydroxmethyl furfural formation in stored *Apis mellifera* and *Apis florae* honeys. The 2nd National Conference of integrated pest management in the Sudan (NCIPM). Gezira University. Wad Medani, 6-8/Novembner/2005).

Munoz, E. and S. Palmero, 2006. Determination of heavy metals in honey by potentiometer stripping analysis and using a continuous flow methodology, Food Chem., 94: 478-483.

Murray, S.S., J.M. Schoeninger, H.T. Bunn, T.R. Pickering, and J.A. Marlett, 2001. Nutritional composition of some wild food and honey used by hadzaforagers of Tanzania. J. Food Composition and Analysis, 13: 1-11.

Paramas, A.M.G., J.G. Barez, R.G. Villanova, T.R. Pala, R.A. Albajar and J.S. Sanchez, 2000. Geographical discrimination of honeys by using mineral composition and common chemical parameters J. Sci Food Agric., 80:157-165.

Pontoh, J. and N.H. Low, 2002. Purification and characterization of beta glucosidase form honey bee (*Apis mellifera*). Insect Biochem. Mol., 32: 679-690.

Rashed, M.N. and M.E. Soltan, 2004. Major and trace elements in different types of Egyptian mono. Floral and non –floral bee honeys. J. Food Composition and Analysis, 17: 725-735.

Rodriguez-Otero, J.L., P. Paseiro, J. Simal and A. Cepeda, 1994. Mineral content of the honeys produced in Galicia (North-West Spain). Food Chem., 49: 169-171.

Sano, O., T. Kunikata, K. Kohno, K. Iwaki, M. Ikeda and M. Kurimoto, 2004. Characterization of Royal Jelly proteins in both Africanized and European Honeybees (*Apis mellifera*) by two–dimensional Gel Electrophoresis, J. Agric. Food Chem., 52:15-20.

Terrab, A. and F.J. Heredia, 2004. Characterization of Avocado (*Persea American* mill) honeys by their physicochemical characteristics. J. Sci. Food Agric. 84: 1801-1805.

Terrab, A., A.F. Re camales, D. Hernanz, and F.J. Heredia, 2004. Characterization of Spanish thyme honeys by their physicochemical and mineral contents. Food Chem., 88: 537-542.

Weirich, G.F., A.M. Collins and V. P.Williams, 2002. Antioxidant enzymes in the honeybee, Apis mellifera. Apidologie, 33: 3-14.

White, J.W., 1979. Composition of honey. In E. Crane (Ed.), Honey: A comprehensive survey. London Heinemann., pp: 157-158.

WHO., 1982. Evaluation of certain food additives and contamination. Technical report series, No. 663, WHO, Geneva.

Zacharius, R.M., T.E. Zell, J.H. Morrison and J.J. Woodlock, 1969. Glycoprotein staining following electrophoresis on acrylamide gels. Annals of Biochemistry, 30: 148–152.