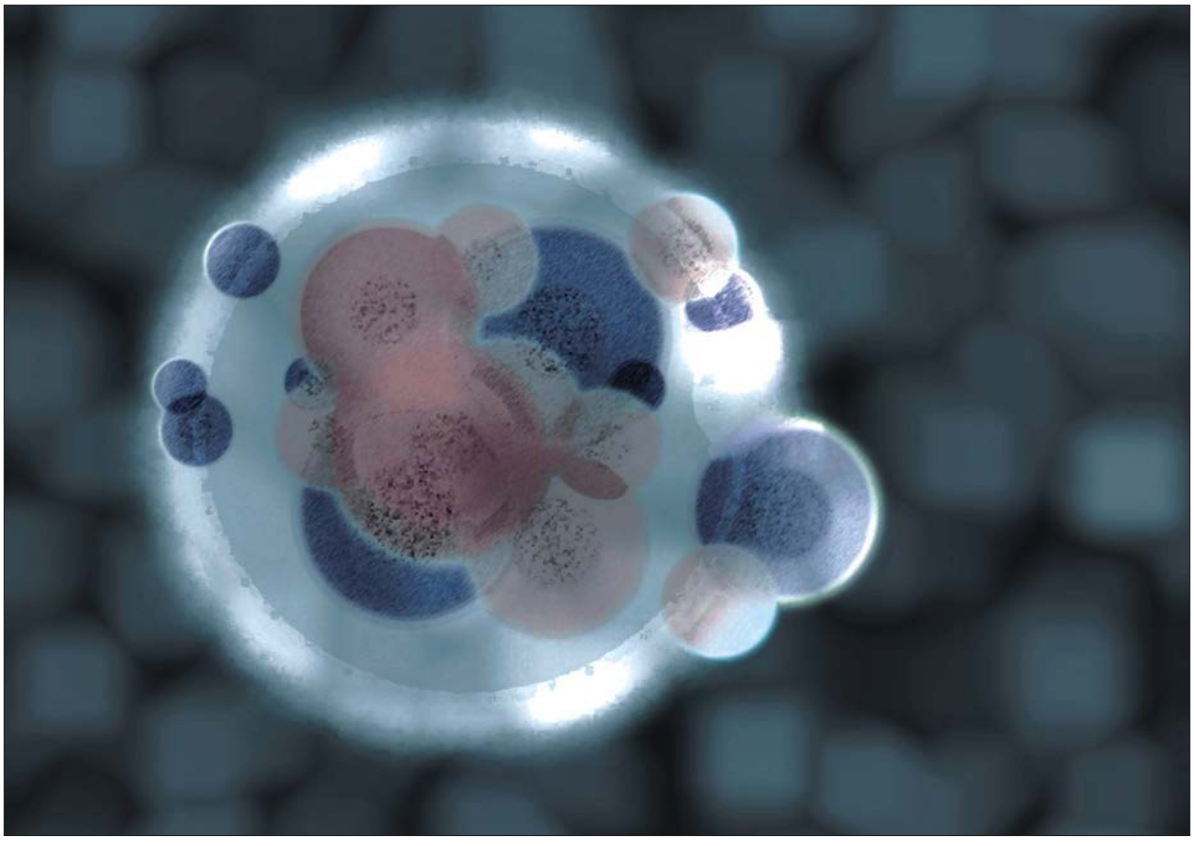

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Studies on Physical and Chemical Analysis of Various Honey Samples and Their Antioxidant Activities

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Abstract: Results of this study intended to determine the different properties of various honey samples collected from different regions in Jordan. These results proved that there were differences in some properties of honey samples and were found to meet the major national and international honey specifications. The thermal behavior of honey samples showed differences in the onset of degradation temperature (124-130°C) and in the degradation temperature (137-148°C). The levels of flavonoids were found to be in the range of 7.4-106 μg g^{-1}, this difference depends on the origin where honey sample collected. Results of antioxidant activities were also evaluated by determining the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging ability and they were found to be significant (21.5-21.8 mg g^{-1}).

Key words: Honey, flavonoids, antioxidant, DPPH, thermal degradation test

INTRODUCTION

Honey is produced by honeybees from nectars extracted from the nectarines of flowers (Adebiyi *et al*., 2004) or from the secretion of living parts of plants or from excretion of plant sucking insects when live on plants (White and Landis, 1980).

Freshly collected honey is a viscous liquid, has a greater density than water, a strong hygroscopic character, relatively low heat conductivity, a low surface tension and various colors that are basically all nuances of yellow amber (Krell, 1996; Schmidt, 1996; Adriana *et al*., 1999). It has been also claimed to have therapeutic properties in the treatment of digestive, respiratory, cardiac and rheumatic disorders (Schmidt, 1996; Pereira *et al*., 1998) and inhibits browning of fruits (Jan and Lee, 1990).

The largest portion of honey composition represents carbohydrates, where fructose and glucose are the most abundant sugars found (Cizmarik *et al*., 2004) and has a high invert sugar content (Hak-Gil *et al*., 1988). Honey also contains proteins that include a number of enzymes and eighteen free amino acids, of which the most abundant is proline 50-85% (Isidro *et al*., 2003; Dustmann, 1993). The minor components are more divers such as: flavonoids (which consider as the main group of antioxidant in honey) (Bogdanov, 1989), carotenoids, vitamins, minerals (Loschen and Ebeling, 1991; Bogdanov, 1989; Toporcak *et al*., 1992; Murray *et al*., 2001) and traces of pollens (Schmidt, 1996; Marie-Jose *et al*., 1987; Huidobra *et al*., 1993).

The change in the composition and properties of honey depends on the floral and honeydew sources collected by honeybees, as well as on regional and climatic conditions. Therefore, some of physicochemical parameters have been studied for their use in the identification of the botanical and geographical origin of honey (Diego *et al*., 2005; Cordella *et al*., 2002).

The aim of this study was to investigate some properties of various honey samples collected from different regions in Jordan by using different honey analysis tests as pH, ash content, electrical conducting, refractive index and the thermal degradation test. Flavonoids contents and antioxidant activities will also be determined in these honey samples. These determinations indicate the quality of honey, which is needed for medicinal treatments and international trade.

MATERIALS AND METHODS

Different honey samples were collected from May to September in 2005, from different regions in Jordan (Table 1). All samples were purchased from different honey farms upon our request.

Chemicals: Methanol (Scharlau, Barcelona, Spain); HCl (Surechem product LTD, England); Sodium carbonate (RIEDEL-Dehaen, Germany); Gallic acid, p-Dimethylaminocinnamaldehyde, Catechin and 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) (Sigma-Aldrich Chemie, Germany).

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Table 1: The honey samples, which collected from different regions in Jordan

Honey sample	Region
1	Gore (north)
2	Al-Walah
3	Karak valley
4	Al-Modwarah
5	Al-Yarmouk
6	Al-Rmaimini
7	Gore (south)
8	Shafa badran
9	Irbid (north)
10	Irbid (south)
11	Mu'tah

Determination of flavonoids: The determination of flavonoids was performed according to a colorimetric assay of Delcour and de Varebeke (Delcour and de Varebeke, 1985). One hundred milligrams of the honey were dissolved in 10 mL of methanol and 1 mL of this solution was pipetted into a test tube. Five milliliters of the chromogen reagent (1.00 g of 4-dimethylaminocinnamaldehyde dissolved in cooled mixture of 250 mL concentrated HCl and 750 mL methanol, made up to 1 L with methanol) were added to the extract solution and after 10 min the light absorbance was read at 640 nm by using spectrophotometer (Milton Roy Company, Spectronic 1201, USA) against a blank with water instead of extract solution. A calibration curve was prepared with (±) catechin and the results were expressed as micrograms (±) catechin equivalents per gram extract (Matthaus, 2002).

Determination of antioxidant activity with the DPPH radical scavenging method: Eighty milligrams of the honey were dissolved in 25 mL of methanol, sonicated for 20 min using ultrasonic bath (Clifton, England) and then 2 mL of this solution were added to 3 mL of DPPH solution (50 mg/100 mL) and the volume was brought with methanol to 25 mL. The mixture was shaken vigorously and allowed to stand for 45 min in the dark. The decrease in light absorbance was measured at 515 nm against a blank (without extract) using Spectronic 1201 Spectrophotometer. The antioxidant activity was calculated using gallic acid as standard and the results were expressed as milligram gallic acid per gram extract (Matthaus, 2002).

Analysis of honey samples

pH: Five grams of each honey samples were diluted with 20 mL of distilled water. The pH values for these samples were measured using Digital pH Meter (pH-525 WTW Germany TUV product service).

Ash and water contents: For each test, 5 g of each sample were put in a flat clean dish and dried in an oven at 110°C for 3 h. For ash content test: after cooling, the material was ashed in an electrical furnace at 600°C overnight, then was cooled and weighed. For water content test: after cooling and weighing, the samples re-dried for one hour in the oven, cooled and reweighed. This process was repeated at one hour drying intervals until a constant weight was obtained.

Electrical conductivity: The conductivity for the honey samples were measured using WTW conductivity meter (LF 320-Germany).

Refractive index: The refractive indices were measured at wave length (λ) = 589.3 nm, at temperature 20°C and at atmospheric pressure of 1007 m bar using an Abbe refractometer. The temperature of Abbe refractometer is controlled by a circulating constant temperature bath and was calibrated and verified through measurement of refractive indices of ethanol and cyclohexane.

Thermal Degradation Test (TDT): Differential scanning calorimeter model (FP85) was used, connected with a computer reinforced with thermal analysis software (FP99) from Mettler Company. The sample mass was measured by using a microbalance (Mettler AE 240) with a sensitivity of 10 μg. Samples were crumbled inside aluminum crucibles from Mettler Company to fit the differential scanning calorimetry (DSC) cell. The samples were heated at rate of 10°C min^{-1} to 200°C.

Statistical analysis: All analysis was carried out in triplicate. Analysis of variance ANOVA was performed. For all analysis, $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The content of flavonoids were determined by a colorimetric assay based upon the reaction of the A rings with the chromogen p-dimethylaminocinnamaldehyde (Table 2), the reaction was specific for compounds with a single bond at 2,3 position and meta-oriented di- or tri-hydroxy substituted benzene rings. It was observed that methanolic extraction was efficient in extraction of flavonoid compounds from honey samples. The flavonoid contents were found to be varied from one sample to another and these differences were significant and the concentration order as follows (according to the number of sample):

6>7>5>8>4>9>3>2>11>1 = 10

These varied quantities depend on the botanical origin of honey that reflects variations in the quality of plant distributions in Jordan.

Table 2: The concentration of flavonoids compounds by the colorimetric assay method. The results were expressed as micrograms equivalents per gram extract (μg g^{-1})

Sample No.	Concentration (μg g^{-1})
1	7.4
2	18.6
3	20.9
4	30.0
5	86.0
6	106.0
7	100.0
8	68.0
9	23.0
10	7.4
11	14.0
Control (Catechin)	423.0

Table 3: The concentration of the antioxidant activities of different honey samples by DPPH method. The results were expressed as milligrams gallic acid equivalents per gram extract (mg g^{-1})

Sample No.	Concentration (mg g^{-1})
1	21.5
2	21.5
3	21.5
4	21.6
5	21.6
6	21.5
7	21.6
8	21.7
9	21.7
10	21.7
11	21.8
Control (Catechin)	22.7

For the determination of the Reactive Scavenging Capacity (RSC) in the honey extracts, there are many methods used to evaluate the antioxidant activity. DPPH spectrophotometric assay is a very effective method used for scavenging different free radicals. It was found that (Table 3) the antioxidant activities of different honey extracts were effective and the reduction in the initial concentrations of DPPH were high with a range of 21.5-21.8 mg g^{-1}, which illustrate that they posses a good reactive scavenging capacity. There are small differences between the different extracts.

Present finding has confirmed the strong antioxidant activity of honey samples due to the presence of flavonoid compounds; this effect may be attributed to flavonoid ability to donate a hydrogen atom to the peroxy radical. As shown by results obtained in these tests (Table 2, 3), there is a clear relationship between the antioxidant activity and flavonoid content.

Presence of flavonoids in honey, have been shown to possess several biological properties (including hepatoprotective, antithrombotic, anti-inflammatory and antiviral activities) many of which may be related, partially at least, to their antioxidant and free-radical-scavenging ability (Schmidt, 1996; Pereira *et al*., 1998).

Analysis of honey samples: The results of pH, ash content, water content and refractive index (RI) of honey samples were found to be in the normal range

Table 4: The results of some parameters of different honey samples

Sample No.	pH	Water content (%)	Refractive index	Conductivity (μS cm^{-1})	Ash content (%)
1	4.620	19.00	1.486	24.0	0.59
2	5.140	19.00	1.486	23.5	0.76
3	5.350	19.40	1.483	27.5	0.90
4	5.290	18.20	1.488	19.0	0.89
5	4.880	19.20	1.186	25.5	0.71
6	4.780	19.20	1.484	25.0	0.61
7	4.770	18.40	1.487	20.0	0.53
8	5.030	18.20	1.489	19.0	0.84
9	4.820	17.80	1.490	17.0	0.62
10	4.530	19.10	1.485	24.5	0.43
11	4.550	17.60	1.491	16.0	0.39
Mean	4.890	18.65	1.459	21.9	0.66
SD	0.283	5.68	0.488	3.8	0.18
V_{min}	4.530	17.60	1.186	16.0	0.39
V_{max}	5.350	19.40	1.491	27.5	0.90

μS cm^{-1}: micro siemens per cm; SD: Standard Deviation; V_{min}: Minimum value; V_{max}: Maximum value

Table 5: The results of DSC experiments on honey samples in temperature range 20-200°C

Sample No.	T_0 (°C)	T_D (°C)	dH (J g^{-1})
1	124	143	-79.2
2	130	144	-67.7
3	127	143	-52.2
4	126	143	-65.3
5	130	139	-21.9
6	125	142	-100.5
7	129	141	-110.7
8	125	145	-82.5
9	128	144	-64.4
10	127	137	-46.3
11	128	148	-99.8

T_0: Onset of degradation; T_D: Degradation temperature; dH: Degradation heat (J g^{-1})

(Table 4) and their values are: 4.53-5.35 with a mean of 4.89; 0.39-0.90%; 17.6-19.4% with a mean of 18.65% and 1.186-1.491 with a mean of 1.459, for pH, ash content, water content and refractive index (RI), respectively. These values were found to be within the values of US honey range (White, 1975).

Electrical conductivity: According to the results of electrical conductivity, the range was between 16 and 27.5 μS cm^{-1} with a mean of 21.9 μS cm^{-1} and it was correlated with the ash contents (Table 4). Conductivity is considered as a good criterion of the botanical origin of honey. The measurement of electrical conductivity depends on the ash and acid content of honey, which reflects the presence of various ions and organic acids; the higher their content, the higher the resulting conductivity.

Thermal Degradation Test (TDT): Differential analysis is one of the most useful techniques in evaluation of temperature effect on the physico-chemical properties of the organic materials, which are represented as exothermic or endothermic peaks at elevated temperatures. In general, exothermic peaks are due to the chemical decomposition and oxidation which are accompanied with exothermic

processes, while endothermic peaks are due to the physical processes which are the melting points, the boiling points and the transition period.

TDT was conducted to investigate the effects of physico-chemical properties on thermal, by using a Differential Scanning Calorimeter (DSC) over a temperature range from 20 to 200°C. Table 5 shows the peaks for all samples with onset of degradation temperatures in the range of 124-130°C and with degradation temperatures in the range of 137-148°C. These range differences in the thermal activation energy may be due to variation in the transition and chemical decomposition of honey components that leads to a wide range of differences in the degradation heat between -21.9 and -110.7 J g^{-1}.

The results show that these honey samples contain different compounds such as polyphenols (flavonoids), organic acids, minerals, various ions and others. These compounds affect the physicochemical properties that enhance their characteristics. It was found that these properties pH, moisture, refractive index, electrical conductivity and ash contents values were comparable to equivalent values obtained for US honey (White, 1975).

CONCLUSION

The obtained results of different properties of the studied honey samples proved that there were differences in some honey analysis tests, flavonoid's contents and in the thermal degradation. The variations in the properties of honey samples which were collected from different regions in Jordan illustrated the differences in the botanical origin where honeybees feed. Results of the thermal behavior of honey samples showed significant differences in thermal phenomena, as well as in their amplitude and position on the temperature, which indicated the variation in the transition and chemical decomposition.

These results indicate a high quality of honey, which is needed for medicinal treatment and international trade.

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REFERENCES

Adebiyi, F.M., I. Akpan, E.I. Obiajunawa and H.B. Olaniyi, 2004. Chemical/Physical characterization of Nigerian honey. Pak. J. Nutr., 3: 278-281.

Adriana, P.G., B. Leila and P.B. Maria, 1999. Color changes during storage of honey in relation to their composition and initial color. Food Res. Int., 32: 185-191.

Bogdanov, S., 1989. Determination of pinocembrin in honey using HPLC. J. Apicultural Res., 28: 55-57.

Cizmarik, J., K. Hrobonova and J. Lehotay, 2004. Determination of monosaccharides and disaccharides in honey by ion-exchange high-performance chromatography. Acta Facultatis Pharmaceuticae Universitatis Comeniane, 51: 73-78.

Cordella, C., J.F. Antinelli, C. Aurieres, J.P. Faucon and D. Cabrol-Bass *et al*., 2002. Use of differential scanning calorimetry as a new technique for detection of adulteration in honey. J. Agric. Food Chem., 50: 203-208.

Delcour, J.A. and D.J.D. Verebeke, 1985. A new colourimetric assay for flavonoids in pilsner beers. J. Inst. Brewing, 91: 37-40.

Diego, G.D., M.N. Jose and C.Q. Lourdes, 2005. Effects of water content upon the galician honey viscosity. Elect. J. Environ. Agric. Food Chem., 4: 949-955.

Dustmann, J.H., 1993. Honey quality and its control. Am. Bee J., 133: 648-651.

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

Huidobra, J.F., M.E. Rea, P.C.B.D. Andrade, M.T. Sancho and S. Muniategui *et al*., 1993. Enzymatic determination of glycerol in honey. J. Agric. Food Chem., 41: 557-559.

Isidro, H., M. Rosa and M.C. Dolores, 2003. Free amino acid composition and botanical origin of honey. Food Chem., 83: 263-268.

Jan, O. and C.Y. Lee, 1990. Inhibition of polyphenol oxidase activity and browning by honey. J. Agric. Food Chem., 38: 1892-1895.

Krell, R., 1996. Value-Added products from beekeeping. Food Agric. Organization. FAO Agric. Services Bull., 124: 24-24.

Loschen, G. and L. Ebeling, 1991. Inhibition of the arachidonic acid metabolism by an extract from rye pollen. Arzneimttelforschung, 41: 162-167.

Marie-Jose, F., P. Johanne and A.S. Alexander, 1987. Microscopic analysis of honeys from alberta. Can. J. Apic. Res., 26: 123-132.

Matthaus, B., 2002. Antioxidant activity of extracts obtained from residues of different oilseeds. J. Agric. Food Chem., 50: 3444-3452.

Murray, S.S., J.M. Schoeninger, H.T. Bunn, T.R. Pickering and J.A. Marlett, 2001. Nutritional composition of some wild foods and honey used by hadza foragers of Tanzania. J. Food Compos. Anal., 14: 3-13.

Pereira, P.C.M., B. Barraviera, R.C. Burini, A.M.V.C. Soares and M.A. Bertani, 1998. Use of honey as nutritional and therapeutic supplement in the treatment of infectious diseases. J. Venomous Anim. Toxins, 1: 2-2.

Schmidt, J.O., 1996. Bee Products: Chemical Composition and Application. In: Published as a Chapter in Bee Products, Mizrahi, A. and Y. Lensky (Eds.). Plenum Press, New York, pp: 25-26.

Toporcak, J., J. Legath and J. Kulkova, 1992. Mercury levels in bee and honey samples from industrially contaminated and uncontaminated areas. Vet. Med., 37: 405-412.

White, J.W., 1975. Physical Characteristics of Honey. In: Honey a Comprehensive Survey, Crane, E. (Ed.). Heinemann, London, ISBN: 434902705, pp: 207-239.

White, J.W. and W.D. Landis, 1980. Honey Composition and Properties. 1st Edn., Beekeeping in the US Agriculture, USA.