A Study of Ibn Hayan Honey Antioxidant Power

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ABSTRACT

Reaching for a spoon of honey may do more than simply satisfy a sweet tooth; this paper looks to compare the ferric reducing abilities of plasma (FRAP), for a 5 honey sampled from around the world. FRAP was found to be as follows Australian Manulka+15 > Ibn Hayan II > Ibn Hayan I > As-Sombola (KSA) > Ibn Hayan III (Iraq) > Essex-Bx (UK). The main indicator of anti-oxidant power was the total polyphenol contents, and hydroxymethylfulfural (HMF) to test the freshness of each sample sourced. All of the honey studied had an additive impact of the levels of anti-oxidants in blood plasma. This plasma was collected from 25 randomly selected healthy male volunteers from Baghdad, Iraq. FRAP values were determined using different concentrations of honey samples and found to have a positive correlation for all the honey included in this study. Interestingly, Ibn Hayan (II) demonstrated a significant difference between experimentally determined and calculated FRAP values. This may be attributed to the possibility of excess metallic or herbal contaminants; this seems plausible given the relative purity of its Australian counterpart, Manulka+15.

دراسة القوى المضادة للأكسدة لعسل ابن حيان

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الخلاصة

البحث هو جزء من محاولة تقييم بعض خصاص العسل العراقي المعرف تجاريا بعسل ابن حيان ببعض الانواع العالمية والمختلفة جغرافيا. فقد تم دراسة قدرة هذه الانواع كمضادات للاكسدة بطريقة فراب ووجد ان عسل ابن حيان يقع ضمن هذة الانواع باعتماد هذه الخاصية. وقد تأكد ذلك ايضا من خلال تعيين كمية متعدد متعدد الفينولات والمعتمد كمؤشر لخاصية مضادات الاكسدة وكالتالي: عسل منوكا + 15 الاسترالي > ابن حيان 2 > ابن حيان 1 > عسل السنبلة السعودي. > ابن حيان 3 > عسل اسكس- بي اكس

وعند دراسة مدى تأثر العسل بالخزن من خلال مؤشراتش ام اف وجد ان عسل ابن حيان يحتل موقعا جيدا بالرغم من حرارة جو الخزن نسبة الى الاوربي لكن قصر فترة الخزن عوض عن ذلك. اتمدت الدراسة على اخذ عينات بلازما لثلاثين شاب بصحة جيدة من اهالي بغداد اختيروا بالطريقة العشوائية. اضهرت الدراسة ان مضادات الاكسدة الكلية تمثل ناتج جمع حسابي من العسل ومضادات الاكسدة الموجودة في البلازما.

1. INTRODUCTION

Antioxidants are part of the defense mechanism involved in the prevention of degenerative diseases caused by oxidative stress such as stroke, cardiovascular diseases, and cancer[1][3]. One of the main sources of antioxidants in food are those with high polyphenolic levels. Reactive oxygen species (ROS) result in the oxidative damage to lipids, proteins, nucleic acids and carbohydrates. Previous studies show that ROS may be removed by antioxidants including those found in honey [4].

The main components of Honey are a complex mixture of sugars (65-71%) and water (21%). Polyphenols are the main antioxidants found in honey. In order to maintain the standard of honey, there are set criteria in various European Countries.5The content and

composition of honey varies with different floral sources as well as environmental conditions. The polyphenolic content, color and minerals are the main sources of honey's antioxidant capacity[6], [7].

The HMF contents of honey are a measure of freshness of honey. HMF results from the thermal decomposition of fructose. The latter is a main constituent of honey [5]. It varies with processing conditions and prolonged storage conditions such as high temperatures, the lower the HMF the better the honey will be [8].

The antioxidant capacity of buckwheat honey has shown to increase the serum antioxidant capacity in humans.9The total antioxidant capacity was studied in vitro and in vivo [10], [11].

Various methods have been used for the evaluation of antioxidants including, spectrometric and electrochemical, chromatographic techniques and biosensors [12], [14].

The ferric reducing antioxidant power, FRAP, model is one of the main specrophotometric methods of measuring the total antioxidant capacity of a sample. It is based on the color change associated with the reduction of the iron (III) complex with TPTZ (2,4,6-tri(2-pyridyl)- 1,3,5-triazine) to its Fe(II) complex measured λ = 593nm.Trolox or ascorbic acids are used as references [11].

The aim of this study was to rank the antioxidant capacity of a selection of honey namely; Manulka+15 (Australian), As-Sombula (KSA), Essex-Bx (UK), Ibn Hayan types (I), (II) and (III) (Iraq). The ferric antioxidant reducing power, FRAP, assay using ascorbic acid as a control will be used throughout this investigation. The total polyphenolic and HMF contents of the types of honey understudy will be investigated using Folin's method.

The in-vitro interaction of Ibn Hayan Honey's antioxidants with plasma antioxidants as compared with that reported for the AustralianMaunika+15. This forms the basis of an attempt to establish the therapeutic benefits.

2. MATERIALS AND METHODS

All chemicals used in this work are of analytical grade from SIGMA Chemical Company or otherwise stated. Iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$), Sodium acetate buffer, pH 3.6, 10mM 2,4,6- tri(2-pyridyl)-s-triazine (TPTZ), Ferric chloride hexahydrate ($FeCl_3.6H_2O$) Honey samples, Ibn Hayan I, II and III, KSA, Essex Bx, and Australian Manuka +15. All types of honey are spring 2012 produce and a mix of randomly selected 10supplies of the same type. Human plasma, from 25 males age 20-25years volunteers from Baghdad Province were collected by trained medical staff. Ascorbic acid was used in this study as a control because it has been proven to have positive antioxidant capacity using the FRAP assay.All the honey samples had been previously filtered to remove solid particles. All the chemicals and solvents used were of analytical grade. FRAP method used was as described by Benzie and Strain (1996) [11], with minor modification. This method is based on the reduction of a ferric 2,4,6- tri(2-pyridyl)-s-triazine (TPTZ) complex to its ferrous, colored form, in the presence of antioxidants. Standard solutions of ferrous sulphate, $FeSO_4.7H_2O$, solution were used to produce calibration curves. Aqueous solutions of Fe^{2+} in the range of 0.25, 0.5, 1, 2 and 4 mM were used for calibration with the FRAP reagent. Results were expressed as ferrous reducing ability of plasma (FRAP) mMFe(II). Fresh working FRAP reagent was prepared at the beginning of every laboratory session. FRAP working reagent was prepared by mixing 0.30M acetate buffer pH= 3.6, 0.010M TPTZ and 0.020M $FeCl_3.6H_2O$ solution in the ratio of 10:1:1 respectively. The FRAP reagent was warmed to 37^{o}C in an incubator for 10 minutes then a reagent blank absorbance reading was measured using a spectrophotometer at 593 nm, using Shimatzu 1800 UV instrument. Honey samples were obtained from various geographical regions. Aliquots of 25µl of each of the diluted

honey solutions were mixed with 1.0ml of FRAP reagent and 15µl deionized water in spectrophotometer cuvettes. Plasma samples were taken from randomly chosen male, age 20-25years, Iraqi volunteers, from Baghdad, and stored frozen. Prior to the sampling procedure the frozen plasma samples were left to thaw. The honey selected for testing with plasma were Manulka+15 (Australian), As-Sombula (KSA), Essex-Bx (UK), Ibn Hayan types (I), (II) and (III) (Iraq). The honey concentration range used was 2, 4, 6, 8 and 10 (% w/v). When testing the samples, 25µl of sample used was this time made up in the cuvettes in the following way:

- Plasma 12.5µl and water 12.5µl
- Plasma 12.5µl and honey 12.5µl
- Honey 12.5µl and water 12.5µl
- Honey 25µl

Samples were mixed with 1ml of FRAP reagent and 15µl deionized water in spectrophotometer cuvettes. Absorbance was measured at 593nm after incubation at 37ºC for 10 minutes. This meant that the final plasma concentration used was 50% and the final honey concentrations used were 1.0-10.0 (% w/v).The mean of three measurements is reported.

Total polyphenol contents

The Golob method was used in this study [7]. A series of gallic acid solutions in the range 10-250µg/ml in water: methanol (1:1) was used to prepare the calibration curve. The method is based on Follin's test for the total polyphenol contents expressed in mg gallic acid equivalent (GAE) per kilogram of honey. Honey samples were vortex mixed with warm distilled water (1.0g / 10.0ml) at 50oC for 10 minutes. 0.10ml of honey solution was vortex mixed for 2 minutes with 1.0 ml of 0.20N Folin reagent, 4.0ml of 1.5N sodium carbonate was added. The mixture was mixed thoroughly for another minute and incubated in a water bath at 45oC for 10 minutes. The absorbance was measured at λ = 593 nm using Shimatzu 1800 UV instrument.

Hydroxymethylfurfural (HMF)

A 5.0 ml of each honey sample was dissolved in 10.0ml distilled water and thereafter transferred to 25ml volumetric flask and made up to 25.0ml with distilled water [8]. And 2.0ml of each sample was placed into two test tubes and 5.0ml solution of p-toludine was added to each tube. 1.0ml of babituric acid was added to each tube and 1.0ml of distilled water was added to the other tube. The absorbance was recorded at 550nm using Shimatzu 1800 UV instrument.

Results and Discussion

FRAP values of the effect of different concentrations of Manuka-15 honey together with Ibn Hayan honey types I, II, and III using 50% v/v plasma are shown in in Table 1 and plotted in Figure 1.

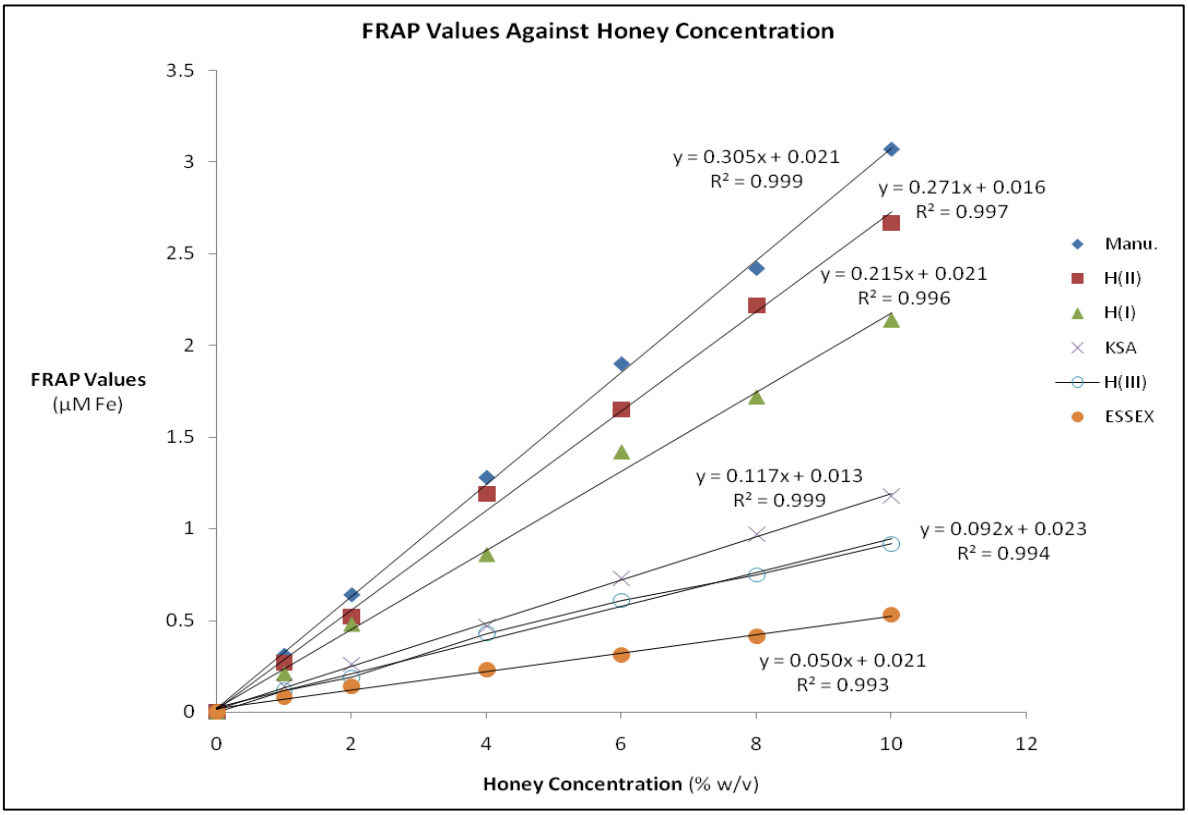

Figure 1: FRAP values.

Table 1: Summary of FRAP values, total polyphenols contents and HMF contents for the six types of honey

Type of Honey	FRAP (mM)	Total Polyphenolic Contents GAE (mg/kg)	HMF contents (mg/kg)
Manuka +15 (Australia)	0.141 ± 0.01	312 ± 12.6	33.9 ± 6.9
As-Sombola (KSA)	0.125 ± 0.02	188 ± 14.2	71.5 ± 5.3
Essex-Bx (UK)	0.074 ± 0.02	157 ± 11.9	12.7 ± 3.8
Ibn Hayan (I) (Iraq)	0.106 ± 0.05	166 ± 16.3	32.7 ± 4.6
Ibn Hayan (II) (Iraq)	0.133 ± 0.05	205 ± 11.4	28.2 ± 3.1
Ibn Hayan (III) (Iraq)	0.109 ± 0. 07	120 ± 18.0	34.6 ± 7.9

The results show that the two indicators of the antioxidant power of honey viz. FRAP values, total polyphenolic contents for the six types of honey give the same conclusion and can be summarized as follows:

As HMF values are indicators of the freshness of honey the sequence of these honey were found to be within literature values. The high level of HMF in KSA honey is mainly

due to thelong term storage at high temperatures. On the other hand the low HMF value of Essex honey reflects its freshness.

All three types of Ibn Hayan honey fall within the internationally accepted level of less than 40mg HMF/kg of honey. The sequence of freshness may be as follows:

Essex < Ibn HIII ≈ Ibn II ≈ Ibn I ≈ Aust M << KSA as-Sombola

This means that there is no clear correlation between freshness and the antioxidant power of these types of honey. Our results for the polyphenol contents are within the range reported by other researchers [15], [17]. The main factor for such difference is due to the nectar contents of honey. This is related to the environment in which bees live, with the main element being the food available from flowers and other sources. The polyphenol contents of honey were generally accepted as the main factor affecting the antioxidant ability of honey. This was attributed to the dissipation of the negative charge on free radicals when they become attached to the delocalized system of polyphenols, hence reducing the activity of the free radicals.

REFERENCES

[1] llison R.C. et al (1995) Protective of fruit and vegetables on development of stroke in men, J Am Med Assoc, Vol. 273 pp1113-1117.

[2] Stampher M.J et al (1996) Vegetable, fruit and cereal fibre intake and risk of coronary heart disease in men. J Am Med Assoc. Vol. 275 pp 447-451.

[3] and Stamford JL (2000) Fruits and vegetable intakes and prostate cancer risk. J Natl Cancer Ins Vol 92 pp 61-68.

[4] Cohen J.H., Kristal A.R. Xu T et al (2011) Phenolic content and antioxidant capacity of Chinease raisins produced in Xinjiang Province, Food Res Int.

[5] Ball W.D. (2007) The Chemical Composition of Honey, J. Chem, Ed. Vol: 84: 10 pp1643-1646.

[6] Bertoncelj J and Golob T. (2007) Evaluation of Phenolic content, antioxidant activity and colour of Slovenian Honey. Food Chemistry. Vol 105 pp822-828.

[7] Singleton V, Orthofer R, Lamuela R. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Foiln-Ciocalteu reagent. Methods Enzymol. Vol. 299pp152-178.

[8] Yilmaz, H and Kufrevioglu I (2000) Composition of Honey collected from eastern and south eastern Anatolia and effect of storage on hydroxymethyfulfural content and diastase activity. Turk J. Agric. Vol 25(2001) 347-349.

[9] Gheldof N. and Engeseth N.J. (2003) Bukwheat honey increases serum antioxidant capacity in humans. J Agric Food chem. Vol. 51 pp1500 -1505.

[10] Niki E (2010) Assessment of antioxidant capacity in vitro and in vivo, Free Radical Biology and Medicine Vol 49 pp 503-515.

[11] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical biochemistry. 1996; 239(1) pp 70-76.

[12] Psoschi AM, CheregiMC and Danet AF (2009) Total Antioxidant capacity of some commercial fruit juices: Electrochemical and Spectrophotometrical Approaches, Molecules Vol 14 pp 480-493.

[13] Lopez M et al (2001) Analysis of phenolic constituents of biological interest in red wines by HPLC, J. Chromatography A 922: 359-363.

[14] Mello L.D., Kubota L.T. (2007) Biosensors as a tool for the antioxidant status evaluation. Talanta 72: 335-348.