Indian Journal of Natural Products and Resources
Vol. 5 (1), March 2014, pp. 9-19

An overview on antioxidative potential of honey from different flora and geographical origins

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Received 14 February 2013; Accepted 15 January 2014

Honey serves as a good source of natural antioxidants and hence it is free radical scavengers that either reduces the formation of or neutralize free radicals. Honey is a healthy foodstuff for better human health and nutrition. The composition and source of honey greatly indicates about its biochemical properties. The present paper is a review of studies on the antioxidant/radical scavenging capacity of various honeys of different flora and geographical origin using spectrophotometric tests: Folin-Ciocalteu assay for phenol content, ferric reducing antioxidant assay (FRAP assay) for total antioxidant activity, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay for antiradical activity and florimetric method namely ORAC, oxygen reactive antioxidant capacity for the anti-lipoperoxidant activity. The phenolic and other compounds in honey are responsible for free-radical scavenging and antioxidant activity that produce beneficial effects in human health.

Keywords: Honey, Antioxidants, Phenolics, Trolox Equivalent Antioxidant Activity (TEAC) assay, Ferric Reducing Antioxidant (FRAP) assay, Oxygen Reactive Antioxidant Capacity (ORAC) assay.

IPC code; Int. cl. (2013.01)–A61K 36/00.

Introduction

There is growing demand for natural antioxidants in human diet, both due to possible negative effects of synthetic food additives on human health and to the increased perception of this problem in recent years. Numerous studies demonstrate that a great number of medicinal and aromatic herbs, as well as fruits and leaves of some berry plants, biosynthesize phytochemicals possessing antioxidant activity and may be used as a natural source of free radical scavenging compounds[1-6]. A majority of these plants are used by bees to collect honey nectar, consequently plant origin bioactive components can be transferred to honey. Honey is known to be rich in both enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoids derivatives, organic acids, Maillard reaction products, amino acids and proteins[7-11]. Moreover, it has also been demonstrated by various researchers that honey has similar antioxidative potential to many fruits and vegetables on a fresh weight basis[12]. It can also prevent deteriorated oxidative reaction in foods[13,14] and enzymatic browning of fruits and vegetables[15,16].

There are different methods for assessing the appropriate antioxidant activity of a substance and in most cases it is necessary to use several tests to obtain good reliability[17-19]. There is no official method for honey antioxidant activity determination. Various tests are in use, each based on different principles and experimental conditions; the FRAP assay (ferric reducing antioxidant power), the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, ORAC (oxygen radical absorbance capacity), superoxide radical-scavenging activity, TEAC (Trolox equivalent antioxidant activity). Even when investigators use the same method, different modifications are often included. Thus, the results of different studies are hard to compare. A step forward regarding this problem was made by some researchers[20] where a practical analytical approach for standardization of the antioxidant properties of honey was set. Their finding includes the use of combination of antioxidant tests, comparative analyses and statistical evaluation to determine the antioxidant behaviour of honey.

The purpose of this review is to survey the antioxidant capacity and the total phenolic content of different types of honey by various methods from

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different origin around the world and to evaluate potential sources of natural antioxidants which provide basic information to the phytochemist for identification and characterisation of antioxidant components of honey.

Various assays used for estimation of antioxidant contents of honey

Although many methods are available to determine antioxidant activity, it is important to employ a consistent and rapid method. While each method has its own merits and demerits, it has been found that the most common and reliable methods are the ABTS and DPPH methods; these have been modified and improved in recent years. However, various methods used by researchers to assess anti-oxidative activity of honey are discussed below:

Ferric Reducing Antioxidant (FRAP) assay

Antioxidant activity can be measured by ferric reducing antioxidants power assay[21, 22]. The assay uses antioxidants as reductants in a redox linked colorimetric method. In this assay, reduction of ferric tripyridyl triazine complex into ferrous tripyridyl triazine. This can be monitored by measuring the change in absorption at 593 nm. Standard aqueous solution of ferrous sulphate were used for the calibration curve and results may be expressed by the FRAP value. The measured reducing capacity does not necessarily reflect antioxidant activity, instead provides a very useful 'total' antioxidant concentration, without measurement and summation of the concentration of all antioxidants involved. The disadvantage is that, it does not measure thiols because their reduction potentials are generally below that of the Fe (III)/Fe (II) half reaction. However, since only a small amount of these compounds is expected in honey, their contribution to the total antioxidant capacity can be considered negligible. However, in contrast to other tests of total antioxidant power, the FRAP assay is simple, speedy, inexpensive, robust and does not require specialized equipment. This assay can be performed using automated, semi-automatic or manual methods.

Total Phenolic Content assay

The phenolic content can be measured by Folin-ciocalteu method[23] which is sensitive to phenol and polyphenol entities and other electron donating antioxidants. The FCR actually measures a sample's reducing capacity, but this is not reflected in the name "total phenolic assay". Numerous publications applied to the total phenols assay by FCR and an ET-based antioxidant capacity assay (eg., FRAP, TEAC, etc.) are often found excellent and linear correlations between the "total phenolic profiles" and "the antioxidant activity" are observed. The exact chemical nature of the FC reagent is not known, but it is believed to contain hetero-polyphosphotungstates-molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^4$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI). Obviously, the FC reagent is nonspecific to phenolic compounds as it can be reduced by many nonphenolic compounds [e.g., vitamin C, Cu (I), etc.]. Phenolic compounds react with FCR only under basic conditions (adjusted by a sodium carbonate solution to pH -10). Dissociation of a phenolic proton leads to a phenolate anion, which is capable of reducing FCR. This supports that the reaction occurs through electron transfer mechanism. The blue compounds formed between phenolate and FCR are independent of the structure of phenolic compounds, therefore ruling out the possibility of co-ordination complexes formed between the metal center and the phenolic compounds. Despite the undefined chemical nature of FCR, the total phenols assay by FCR is convenient, simple, and reproducible. As a result, a large number of data has been accumulated and reported in the given Table 1.

Oxygen Reactive Antioxidant Capacity (ORAC) assay

The ORAC assay is based upon the early work of Ghiselli *et al*[24] and Glazer[25] as developed further by Cao *et al*[26]. ORAC measures antioxidant inhibition of peroxyl radical induced oxidations and thus reflects classical radical chain breaking antioxidant activity by H atom transfer[27]. The ORAC assay provides a controllable source of peroxyl radicals that model reactions of antioxidants with lipids in both food and physiological systems, and it can be adapted to detect both hydrophilic and hydrophobic antioxidants by altering the radical source and solvent.

Trolox Equivalent Antioxidant Activity (TEAC) assay

TEAC assay was first reported by Miller *et al*[28] in 1993 which was later improved by Re and co-workers[29]. In the improved version, ABTS-, the

Table 1—A comparative analysis of antioxidative parameters of honey from flora of different countries and geographical origin (*contd.*)

Origin	Types of honey	Phenolic content (mg gallic acid/kg	FRAPμMFe(II)	ORAC (TE/g)	DPPH (IC50)	ABTS	References
Malaysian	Tulang honey	251.7±7.9	322.1±9.7	_	41.3±0.78	_	37
Honey		83.96±4.53	121.89±3.87	_	5.80±0.12	_	42
Slovenian	Acacia	44.8±14.8	71.0±10.2	_	_	_	35
Honey	Multifloral	157.3±20.9	224.8±24.7	_	_	_	
	Forest	233.9±21.7	426.4±41.5	_	_	_	
	Lime	83.7±14.3	118.8±20.3	_	_	_	
	Chestnut	199.9±34.1	360.1±66.5	_	_	_	
	Fir	241.4±39.5	478.5±95.5	_	_	_	
Africa	Buckwheat	482.2±2.4	800.7±23.8	11.60±0.027	4.00±0.44	_	20
	Chestnut	211.2±5.5	388.6±8.2	8.90±0.45	7.93±0.04	_	
	Multiflora	170.4±1.7	361.9±10.8	8.22±0.42	5.32±0.03	_	
	Dandelion I	52.5±1.5	212.2±2.2	2.00±0.02	47.62±0.39	_	
	Acacia	55.2±2.8	79.5±3.7	2.12±0.01	45.45±0.04	_	
	Clover	67.1±5.6	72.8±3.0	2.15±0.02	25.00±0.01	_	
	Sulla	106.6±4.6	155.2±6.6	5.66±0.13	16.90±0.11	_	
	Chicory	158.5±3.8	209.5±2.8	6.72±0.33	5.81±0.04	_	
	Straberry Tree	789.6±13.8	1501.4±60.2	21.07±0.34	1.63±0.17	_	
	Africa I	567.3±1.2	808.1±18.3	11.07±0.43	3.61±0.13	_	
	Honey dew	255.6±7.5	772.0±215	6.30±0.22	8.48±0.24	_	
	Africa II	595.2±13.1	448.1±4.7	18.23±0.33	5.13±0.13		
	Dandelion II	102.1±10.0	224.4±6.0	6.59±6.60	24.39±0.07		
Poland	Blackchokeberry	_	_	_	67	_	46
	Chamomile	_	_	_	25	_	
	Mint	_	_	_	34	_	
	Nettle	_	_	_	36	_	
	Raspberry	_	_	_	81	_	
	Thyme	_	_	_	80	_	
	Pine	_	_	_	41	_	
	Aloe	_	_	_	31	_	
	Marigold	_	_	_	42	_	
	Hawthorn	_	_	_	85	_	
Lithuania	Multifloral	_	_	_	80.9±3.8	79.6±1.7	41
	Unifloral Willow honey	_	_	_	82.6±0.2	94.0±0.8	
	Unifloral Spring Rape honey	_	_	_	75.7±0.6	72.4±1.6	
	Unifloral Linden honey	_	_	_	31.1±4.5	54.8±2.4	
Czech Republic	Floral	_	295.35±48.73	_	141.52±30.34	489.44±47.49	34
	Lime	_	415.59±31.2	_	150.5±141.65	596.87±14.75	
	Raspberry	_	443.37±5.97	_	206.11±6.3	658.73±4.77	
	Rape	_	370.25±27.07	_	166.57±17.95	543.97±32.56	
	Mixture	_	565.48±63.53	_	284.72±42.67	814.77±64.12	
	Honeydew	_	776.05±68.19	_	407.08±22.56	982.93±32.18	
Croatia Burkina	Acacia	_	72.87±15.44	_	111.05±45.10	_	47
Faso	Multifloral	83.80±3,35	_	_	9.60±1.40	_	40
	Combretaceae-(64.9%)	59.67±1.35	_	_	10.40±0.50	_	
	Acacia	93.43±0.87	_	_	10.53±0.65	_	
	Multifloral	56.47±1.61	_	_	6.90±0.53	_	
	Honeydew	113.05±1.10	_	_	4.93±0.23	_	
	Multifloral	61.49±1.87	_	_	6.00±0.52	_	

(*contd.*)

Table 1—A comparative analysis of antioxidative parameters of honey from flora of different countries and geographical origin (*contd.*)

Origin	Types of honey	Phenolic content (mg gallic acid/kg	FRAPμMFe(II)	ORAC (TE/g)	DPPH (IC50)	ABTS	References
	Multifloral	62.04±0.53	_	_	13.43±1.12	_	
	Honeydew	114.75±1.30	_	_	4.37±0.10	_	
	Multifloral	69.43±1.24	_	_	12.38±1.53	_	
	Multifloral	74.39±0.90	_	_	7.00±0.50	_	
	Multifloral	63.37±0.90	_	_	10.43±1.31	_	
	Multifloral	43.41±0.00	_	_	29.13±1.50	_	
	Lannea	42.96±0.63	_	_	23.53±0.40	_	
	Multifloral	57.63±0.49	_	_	15.40±0.00	_	
	Combretaceae (82.8%)	52.08±0.31	_	_	17.07±1.44	_	
	Multifloral	32.59±0.48	_	_	28.00±0.50	_	
	Multifloral	79.99±0.11	_	_	6.55±0.61	_	
	Multifloral	81.44±0.29	_	_	6.52±0.30	_	
	Multifloral	90.84±0.54	_	_	5.03±0.06	_	
	Multifloral	93.66±0.44	_	_	6.42±0.28	_	
	Multifloral	86.07±2.98	_	_	6.97±0.45	_	
	Multifloral	65.69±0.19	_	_	11.80±0.36	_	
	Multifloral	84.82±0.58	_	_	9.60±1.40	_	
	Multifloral	85.07±0.41	_	_	10.40±0.50	_	
Polland	Buckwheat	201.6±16.8	_	_	_	_	38
	Heather	201.2±5.5	_	_	_	_	
	Lime	153.1±5.5	_	_	_	_	
	Rape	71.7±1.3	_	_	_	_	
Germany	Buckwheat	796±32	_	16.95±0.76	_	_	9
	Buckwheat	_	_	9.81±0.34	_	_	
	Buckwheat	456±55	_	9.75±0.48	_	_	
	Soy	_	_	9.49±0.29	_	_	
	Buckwheat	_	_	9.34±0.57	_	_	
	Buckwheat	_	_	9.17±0.63	_	_	
	Hawaiian Christmas berry	250±26	_	8.87±0.33	_	_	
	Soy	269±22	_	8.34±0.51	_	_	
	Buckwheat	_	_	7.47±0.27	_	_	
	Clover	_	_	6.53±0.70	_	_	
	Tupelo	183±9	_	6.48±0.37	_	_	
	Clover	128±11	_	6.05±1.00	_	_	
	Fireweed	62±6	_	3.09±0.27	_	_	
	Acacia	46±2	_	3.00±0.16	_	_	
Italy (Sardinia)	1.Traditional	1297.8±56.5	16.2±0.1	_	3.8±0.3	_	43
	2.Traditional	1995.8±5.5	19.4±0.2	_	5.7±0.1	_	
	3.Traditional	1377.6±54.1	13.3±0.1	_	3.8±0.2	_	
Italy (Sicilia)	Citrus spp.	20.4±0.5	_	_	15.1±0.4	_	39
Italy (Sicilia)	Citrus spp.	29.2±0.9	_	_	6.9±0.4	_	
Italy (Campania)	Citrus spp.	60±1	_	_	5.0±0.3	_	
Italy (Sardegna)	Citrus spp.	24.7±0.9	_	_	11.0±0.4	_	
Italy (Lombardia)	Rhododendron spp.	38±2	_	_	6.1±0.5	_	
Italy (Trentino)	Rhododendron spp.	31.2±0.9	_	_	7.1±0.5	_	

(*contd.*)

Table 1—A comparative analysis of antioxidative parameters of honey from flora of different countries and geographical origin (*contd.*)

Origin	Types of honey	Phenolic content (mg gallic acid/kg	FRAPμMFe(II)	ORAC (TE/g)	DPPH (IC50)	ABTS	References
Italy (Piemonte)	Rhododendron spp.	56±1	_	_	5.7±0.3	_	
Italy (Val d'aosta)	Rhododendron spp.	37±1	_	_	6.2±.3	_	
Italy (Toscana)	Rhododendron spp.	17.1±0.7	_	_	15.5±0.8	_	
Italy (Lombardia)	*Robinia pseudoacacia*	36.8±0.7	_	_	8±1	_	
Italy (Lombardia)	*Robinia pseudoacacia*	20.9±0.4	_	_	12.0±0.6	_	
Italy (Piemonte)	*Robinia pseudoacacia*	30.8±0.9	_	_	8.0±1.0		
Canada	Buckwheat	_	_	16.054±15.93	_	_	42
	Manuka	_	_	11.113±16.14	_	_	
	Dandelion	_	_	31.43±04.57	_	_	
Urbana	Buckwheat	_	_	9.75±0.48	_	_	48
	Hawaiian Christmas berry	_	_	8.87±0.33	_	_	
	Soy	_	_	8.34±0.51	_	_	
	Blueberry	_	_	6.89±0.20	_	_	
	Avocado	_	_	6.51±0.79	_	_	
	Tupelo	_	_	6.48±0.37	_	_	
	Blackberry	_	_	6.34±0.37	_	_	
	Saw Palmetto	_	_	6.07±1.27	_	_	
	Gallberry	_	_	5.38±0.04	_	_	
	Clover	_	_	4.41±0.78	_	_	
	Cabbage	_	_	3.95±0.42	_	_	
	Sourwood	_	_	3.80±0.47	_	_	
	Sage	_	_	3.63±0.47	_	_	
	Eucalyptus	_	_	3.65±0.26	_	_	
	Fireweed	_	_	3.09±0.27	_	_	
	Acacia	_	_	3.00±0.16	_	_	
	Blackberry	_	_	2.70±0.48	_	_	
	Orange Blossom	_	_	2.36±0.24	_	_	
	Star thistle	_	_	1.75±0.04	_	_	
Czech Republic (Brumovice)	Multifloral (fruit trees)	146.93±0.07	316.83±0.03	-	186.86±0.62	529.45±0.73	
	Multifloral (lime, ornament, wood)	83.60±0.20	339.37±0.20	_	159.17±0.32	431.3±1.25	
	Rape	96.79±0.03	332.58±0.10	_	141.72±0.73	514.42±0.89	
	Mixture (forest)	172.54±0.45	578.99±0.11	-	358.33±0.14	840.45±0.55	
	Multifloral (rape, fruit trees)	94.23±0.02	271.60±0.26	-	134.10±0.26	459.89±0.23	
Czech Republic (Pochen)	Mixture (rape, forest)	158.37±0.11	531.11±0.55	-	165.65±0.65	786.35±0.75	
	Mixture (lime, forest)	164.09±0.04	550.07±0.42	-	288.82±0.58	734.20±0.64	
	Rape	95.30±0.06	394.96±1.00	-	183.50±1.05	589.32±0.86	
	Mixture (rape, forest)	162.10±0.08	596.72±1.12	-	321.41±0.86	804.01±1.23	
	Mixture (lime, forest)	138.20±0.12	465.39±0.89	-	271.07±0.54	710.92±0.42	

(*contd.*)

Table 1—A comparative analysis of antioxidative parameters of honey from flora of different countries and geographical origin

Origin	Types of honey	Phenolic content (mg gallic acid/kg	FRAPμMFe(II)	ORAC (TE/g)	DPPH (IC50)	ABTS	References
	Mixture (rape, forest)	145.09±0.03	520.27±0.66	-	309.35±0.68	801.62±0.11	
	Mixture (forest)	167.90±0.18	624.57±0.70	-	356.90±0.72	893.19±0.28	
	Lime	98.42±0.24	464.11±0.40	-	146.62±0.35	594.54±0.29	
Czech Republic (Lichnow)	Mixture (forest)	182.84±0.25	534.26±0.12	-	254.09±0.50	770.71±0.35	
	Lime	92.01±0.30	417.89±0.34	-	150.60±0.36	606.53±0.10	
Czech Republic (Bykov)	Rape	89.60±0.70	383.22±0.15	-	174.50±0.13	528.16±0.70	
	Raspberry	95.62±0.43	437.39±0.22	-	199.81±0.63	653.73±0.64	
	Lime	86.96±0.29	401.19±0.28	-	163.12±0.39	612.50±0.48	
Czech Republic (Stroka Niva)	Mixture (forest, fruit trees)	148.88±0.15	548.49±0.27	-	249.46±0.60	854.35±0.64	
	Mixture (forest)	174.04±0.28	607.78±0.30	-	270.18±0.39	827.72±0.88	
Czech Republic (Budisov)	Mixture	140.04±0.13	540.73±0.60	-	283.73±1.00	872.59±1.30	
Czech Republic (Podvihov)	Mixture	115.50±0.44	374.58±0.80	-	162.86±0.60	560.01±0.90	
Czech Republic (Zimrovice)	Mixture	189.50±0.38	647.99±0.33	-	345.87±0.21	894.31±0.56	
Czech Republic (Kruzberk)	Floral (fruit trees)	101.27±0.36	301.05±0.89	-	181.99±0.68	498.48±.78	
	Raspberry	102.10±0.05	449.34±0.79	-	212.41±0.44	663.28±0.83	
Czech Republic (Uvalno)	Multifloral (rape, raspberry)	106.12±0.08	222.98±1.22	-	98.73±0.84	433.26±0.77	
	Mixture (rape, raspberry, spruce)	143.31±0.23	339.26±0.78	-	120.66±0.90	551.29±0.59	
	Multifloral (rape, raspberry)	100.01±0.16	338.90±0.84	-	120.20±1.05	441.46±0.21	
	Mixture (raspberry, forest)	171.20±0.03	492.76±1.34	-	133.14±0.20	720.38±0.13	
	Mixture (lime, forest)	176.10±0.12	470.40±1.15	-	209.57±0.28	738.32±1.42	
Czech Republic (Nasavrky)	Mixture (forest)	208.94±0.67	678.20±0.92	-	291.88±0.36	888.02±0.66	
Czech Republic (Rymice)	Mixture (forest)	199.01±0.53	659.98±0.88	-	246.05±0.77	899.22±0.34	
	Lime	85.52±0.34	379.16±0.74	-	141.65±0.64	573.92±0.28	
Czech Republic (Ricany u prahy)	Honeydew	192.68±0.36	699.10±0.88	-	376.25±0.55	956.11±0.52	
Czech Republic (Tehov)	Honeydew	208.52±0.07	740.56±1.02	-	398.76±0.83	968.56±0.35	
Czech Republic (Kamenice nad Lipou)	Honeydew	219.23±0.24	758.23±0.42	-	408.12±0.79	998.86±0.64	
Czech Republic (Kasava)	Honeydew	198.98±0.38	722.88±0.63	-	388.56±0.48	935.23±0.70	
Czech Republic (Zbiroh)	Honeydew	229.11±0.11	848.43±0.12	-	428.78±0.23	1012.45±0.31	
Czech Republic (Nova Vcelnice)	Honeydew	242.52±0.06	887.12±0.22	-	411.98±0.36	1026.38±0.92	

oxidant, was generated by persulfate oxidation of 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$). The assay is based on the scavenging ability of antioxidants to the long-life radical anion $ABTS^{-+}$. In this assay, $ABTS^{-+}$ is oxidized by peroxyl radicals or other oxidants to its radical cation, which is intensely coloured, and is measured as the ability of test compounds to decrease the colour reacting directly with the $ABTS^{+}$ radical. Results of test compounds are expressed relative to Trolox.

$ABTS^{+}$ is highly soluble and has high chemical stability. Its absorption is max. at 342 nm. It is a peroxidise substrate which, when oxidized in the presence of H_2O_2, forms a metastable radical cation[28, 30]. It shows characteristic absorption spectrum and high molar absorptive at 414 nm. It also has secondary absorption maxima in the wavelength regions of 645, 734 and 815 nm. It forms ferrylmyoglobin radical, from reaction with metamyoglobin and hydrogen peroxide. Ferrylmyoglobin radical is free to react with ABTS to produce the $ABTS^{+}$ cation. The accumulation of cation $ABTS^{+}$ can be inhibited by the presence of an antioxidant in the reaction medium .The relative ability of hydrogen- donating antioxidants to remove $ABTS^{+}$ generated in the aqueous phase, can be measured spectro-photometrically, in the near-infrared region at 734 nm, which minimised interference from other absorbing components and from sample turbidity.

Due to its operational simplicity, the TEAC assay has been used in many research laboratories for studying antioxidant capacity and TEAC values of many compounds and food samples are reported. It does not show a clear correlation between TEAC values and the number of electrons that an antioxidant can give away for pure antioxidant compound.

2, 2- Diphenyl-1-Picrylhydrazyl (DPPH) assay

The DPPH radical scavenging assay is one of the shortest assays available to investigate the overall hydrogen/ electro donating activity of single antioxidants and health-promoting dietary antioxidant supplements. DPPH is stands for 2,2 diphenyl-1-picryl hydrazyl. It is a dark coloured crystalline powder composed of stable free-radical molecules. DPPH has two major applications both in laboratory research one is the monitor of chemical reaction involving radicals and other is a standard of the position and intensity of electron paramagnetic resonance signals[31-33]. The DPPH radical absorbs at 517 nm and in second substrate-free system, antioxidant activity can be determined by monitoring the decrease in this accordance, to consider the effect of both parameters on antiradical capacity, a new parameter, antiradical efficiency, which combined both factors, was defined.

The DPPH assay is technically simple, but some disadvantages limit its applications. Besides the mechanistic difference from the HAT reaction that normally occurs between antioxidants and peroxyl radicals. It is long-lived nitrogen radical, which bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH.

Discussion

In the recent years, there has been an increasing interest in determination of the antioxidant activity of honey. Many studies indicated that the antioxidant activity of honey varies widely, depending on the floral source. A review of the literature of antioxidant powers of different types of honey from different floral sources are listed in the given Table 1. Lachman and his co-workers[34] determined antioxidant activity by three different assays-DPPH, $ABTS^{\cdot+}$ and FRAP which revealed floral honeys had lesser activity (in average 141.5 mg AA kg^{-1}honey) in DPPH assay as compared to honeydew honeys (in average 407.1 mg AA kg^{-1} honey). Analogous to honeydew honeys, mixture honeys also had high antioxidant activity (284.7 mg AA kg^{-1} honey) and their increasing order was: floral honey < lime honey < rape < raspberry< mixture< honeydew. Average antioxidant values were determined by $ABTS^{\cdot+}$ assay which was two to three times higher as compared to values determined by DPPH and FRAP assays (98.73-441.98 mg AA eq kg^{-1}) honey. In DPPH assay, 431.4- 1026.6 mg AA eq kg^{-1} honey in $ABTS^{\cdot+}$ assay or 223-295.4mg AA eq kg^{-1} honey in FRAP assay. Moreover, FRAP assay showed that floral honey had lowest average antioxidant activity (295.44 mg AA eq kg^{-1}) where as honeydew honey showed highest values (776.14 mg AA eq kg^{-1} honey) which was 2.5 times higher in comparison to floral honeys. However, antioxidant values determined by FRAP assay ranged 223 to 887.1 mg AA eq kg^{-1} honey and the increase of antioxidant activity was similar with DPPH and $ABTS^{\cdot+}$ assays. Flora honey < rape honey< lime honey< raspberry honey <

mixture honey and honeydew honey. Lesser antioxidants activity of floral honey was in agreement with the results of Al-Mamary *et al*[8] and which was indicative if the presence of different phenolics with different antioxidant activity. These results were in agreement with Bertoncelj *et al*[35] who measured antioxidant activity of Solvenian honey and found that least active for unifloral honey (Acacia + lime honey) where as the most active was honeydew honey. Raspberry honeys had showed relatively high antioxidant activity and this result is confirmed by the result of Buricova and Reblova[36]. Mohamed and his co-workers[37] worked on total antioxidant activity of gamma-irradiated Tulang honey and reported same as Solvenian honey[35].

Qualitative and quantitative determination of phenolic compounds was done by Kaskoniene and co-workers[38] and reported that the total content of phenolic compounds in the honey samples varied from 71.7 to 202.6 µg/g. Darker honeys such as buckwheat and heather had highest while the lowest amount of phenolic compounds had been found in rape honey. According to Buratti *et al*[39] the antioxidant power of honey samples varies from 14 mg/g to 43 mg/g. Except for a Citrus sample, Rhododendron had exhibited the highest antioxidant power while Robinia the lowest. Total phenolic content ranges from 17.1 mg/g to 60.0 mg/g caffeic acid equivalents for honey.

Moreover, the work reported by Meda *et al*[40] showed Vitlania honey as most active radical scavenger followed by honeydew, *Acacia, Lannea* honey and honey from family *Combretaceae* family. Moreover, honeydew honey had the total amount of phenolic compounds and possessed good radical scavenger. According to Baltrusaityte and co-workers[41] the radical scavenging activity for natural honey ranged from 31.1±4.5 to 86.9±0.9 % in DPPH system and from 50.4±1.0 to 96.8±0.7 % in ABTS·+ system, while that of honey with plant extracts from 80.4±1.6 to 93.0±1.0 % and from 89.5±2.7 to 98.3±0.7 %, respectively. They also reported that ABTS·+ decolourisation assay another widely used antioxidant activity screening method which is applicable both for lipophilic antioxidants in general radical scavenging activity of honey samples in ABTS·+ system was slightly higher comparing to DPPH radical. The antioxidant activity of multifloral honey samples varied from 64.2%- 80.9 % in DPPH radical scavenging activity and from 76.5 to 81.9 % in ABTS·+ radical cation decolorisation assay. Bertoncelj and co-workers[35] worked on different honey samples which were obtained directly from beekeepers from different locations across Slovenian and had reported significant differences among the different types of honey. Their antioxidant activities had been reported in the following order: *Acacia*< lime<multifloral<chestnut< spruce< forest< fir honey. Acacia honey had an average FRAP value of only 71.0 µM. Fe(II) while the higher FRAP value were reached by Salvenian fir and forest honey. These results were similar to those obtained by Beretta *et al*[20] the least active honeys are those of monofloral origin, Acacia, Sulla, Dandelion and floral. A positive linear co-relation between the total antioxidant activity determined by the FRAP method and phenolic content was observed. They had reported statistically significant co-relation which was in agreement with other authors[1,20] who had also found a strong relationship between antioxidants capacity determined by the FRAP assay and phenolic content of honey. Gheldof and co-workers[10] had reported that the phenolic compound contributes significantly to the antioxidant activity of honey but in spite of this it seems that it appears to be a result of the combined activity of honey phenolics, peptides, organic acids, enzymes and millard reaction products.

However, highly statistically significant co-relation between the free radical scavenging and total phenolic content has been reported. The relation between FRAP and DPPH had also been found significant. According to Beretta *et al*[20] the phenol content was low in pale honeys of monofloral origin, Clover, Acacia, Dandelian I, higher in Sulla and Dandelian II rising further in Chicory and mountain multiflora Strawberry tree honey had highest content approaching 0.1% (789.57± 13.79 mg gallic acid kg^{-1}). These results had been found good agreement with that reported in literature for the same kinds of honey in particular the value for Maxican Buckwheat honey was same as reported in literature for the Californian I one (482.17± 2.40 versus 456 ± 55 $mg_{gallic\ acid}kg^{-1}$). In addition, their FRAP assays shows large difference in antioxidant profile of various honey. The least active being those of monofloral origin Clover, Sulla, Acacia and Dandelion. The scavenging ability reported by Berrata *et al*[20] had showed marked difference between honeys the least active were those of monofloral origin Clover, Acacia, Sulla, Dandelion. Most active strawberry tree has antiradical potency which was

30 times that of Dandelion I. The ORAC value for strawberry tree free honey of exceptionally high and highest observed for any honey till date. Similar ORAC values have been reported by Gheldof *et al*[9] for American Buckwheat, Acacia and Clover honey. In 2007, they had also reported ORAC activities of various honey and showed a relationship between concentration of other honey samples and ORAC activity. Krishna Kishore *et al*[42] had reported that Tulang honey had the highest total phenolic content followed by Gelam, Indian forest and pineapple honeys. The average phenolic content obtained from the Tulang honey sample is similar to previous reports of the total phenolic contents of other honeys from the various floral sources. The highest DPPH scavenging activity reported from Tulang honey suggesting that it may contain the most effective free radical scavenging compounds. Significant difference in antioxidant activity as assessed by FRAP, had been found between honey samples with Tulang honey having the highest activity. Several studies have shown that antioxidant activity is strongly correlated with the content of total phenolics[1,8,9,20,40]. Beside, this strong correlation was found between antioxidant activity and the colour of honey. Many researchers found that honeys with dark colour have a higher total phenolic content and consequently a higher antioxidant capacity[12, 20]. Baltrusaityte *et al*[41] had worked on 35 honey samples of different floral origin, the results obtained had shown that all tested samples are active antioxidants. The radical scavenging activity of natural honey extracts was formed 31.1±4.5 to 86.9±0.9 % in reaction system and formed 50.4±1.0 to 96.8± 0.7 % in ABTS·+ reaction system. According to them the radical scavenging activity of honey samples in ABTS·+ reaction system was slightly higher comparing to DPPH reaction. The antioxidant activity of multifloral honey samples varied from 64.2 % to 80.9 % in DPPH radical scavenging assays and from 76.5 % to 81.9 % in ABTS·+ cation decolorisation assay. Moreover, Brudzynski and Miotto[42] worked on the antioxidant activity and ORAC values of honey and had made a comparative study on unheated and heat treated honey samples and reported the changes in total phenolic content of melanoidins in heated versus unheated honeys which were strongly co-related with changes in antioxidants activity. It suggested that phenolics in may be components of melanoidians structure and had a direct interaction between polyphenols and melanoidins. That results in a loss or a gain of function of melanoidians. Jerkovic *et al*[43] had reported the total antioxidant activity which was measured with the FRAP assay ranged from 13.3 to 17.2 mmol Fe (II) Kg^{-1} while antiradical activity measured with the DPPH assay ranged from 3.8 to 23.3 mmol TEAC/kg. Total phenolic amount ranged from 1297.8 to 4469.5 mg GAE/kg and it is linearly co-related with antioxidant and antiradical activities. The reported values had been found very high and had been compared to those published although a direct comparison is very hard due to different types of antioxidant assay and way of quantification[44]. However, dark and honeydew honeys that are known to have the highest levels of total phenolic compounds usually had not been exceeded[45-48] from the level of 1250 mg GAE/kg. FRAP value for honey rich in phenolic compounds such as Chestnut, *Satureja hortensis* and honeydew honeys ranged between 3.7 and 4.4 mmol Fe (II)/kg.

Conclusion

In this review article it had been established that all types of honey contain phenolic compounds and possessed antioxidant property. The total phenolic content and antioxidant activity varied in different types of honey. The botanical origin of honey has the greatest influence on its antioxidant activity, while processing, handling and storage affect honey antioxidant activity only to a minor degree. The variation in the antioxidant; power among unifloral honeys with different geographical origin may be due to climate and environmental factors such as humidity, temperature and soil composition.

Acknowledgements

The authors are thankful to Head, Chemistry Department, Brahmanand PG College, Kanpur and Head, Chemistry Department, DDU Gorakhpur University, Gorakhpur for laboratory and library facilities. Thanks are also due to University Grants Commission, New Delhi for providing Emeritus Fellow to Gurdip Singh.

References

1 Blasa M, Candiracci M, Accorsi A, Piacentini M P and Piatti E, Honey flavonoids as protection agents against oxidative damage to human red blood cells, *Food Chem,* 2007, **104**, 1635-1640.

2 Javanmardi J, Khalighi A, Kashi A, Bais HP and Vibanco J M, Chemical characterisation of basil found in local

accessions and used in traditional medicines in Iran, *J Agric Food Chem*, 2002, **50**, 5878-5883.
3 Miliauskas G, Venskutonis PR and Van beek TA, Screening of radical scavenging activity of some medicinal and aromatic plant extracts, *Food Chem*, 2004, **85**, 231-237.
4 Pizzale L, Bbortolomeazzi R, Vichi S, Uberegger E and Conte LS, Antioxidant activity of sage (*Salvia officinale* and *S. fructicosa)* and *Oregano (Origanum onites* and *O. inderscedens)* extracts related to their phenolic compound content, *J Sci Food Agric,* 2002, **82**, 1645-1651.
5 Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M and Bruni R, Comparative evaluation of eleven essential oils of different origin as functional antioxidants antiradicals and antimicrobials in foods, *Food Chem,* 2005, **91**, 621-632.
6 Wang S Y and Lin HS, Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and development stage, *J Agric Food Chem*, 2000, **48**, 140-146.
7 Aljadi A M and Kamaruddin MY, Evaluation of the phenolic contents and antioxidant capacities of two Malasian floral honeys, *Food Chem*, 2004, **85**, 513-518.
8 Al-mamary M, Al-Meery A and Al-Habori M, Antioxidant activities of total phenolic of different types of honey, *Nutr Res*, 2002, **22**, 1041-1047.
9 Gheldof N and Engeseth N J, Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples, *J Agric Food Chem,* 2002, **50**, 3050-3055.
10 Gheldof N, Wang X H and Engeseth NJ, Identification and quantification of antioxidant components of honeys from various floral sources, *J Agric Food Chem*, 2002, **50**, 5870-5877.
11 Schramm D D, Karim M, Schrader H R, Holt R R, Cardetti M and Keen C L, Honey with high levels of antioxidants can provide protection to healthy human subjects, *J Agric Food Chem,* 2003, **51**, 1732-1735.
12 Frankel S, Robinson G E and Berenbaum M R, Antioxidant content and correlated characteristics of 14 monofloral honeys, *J Apic Res,* 1998, **37**, 27-31.
13 Mc Kibben J, Engeseth N J, Honey as a protective agent against lipid oxidation in ground Turkey, *J Agric Food Chem,* 2002, **50**, 592-595.
14 Antony S M, Rieck J, Dawson R and Hi P, Effect of dry honey on oxidation in turkey breast meat*, Poll Sci,* 2000, **79**, 1846-1850.
15 Mc Lellan M R, Kime RW, Lee CY and Long TM, Effect of honey as an anti-browning agent in light raisin processing, *J Food Proc Pres*, 1995, **19**, 1-8.
16 Chen L, Mehta A, Berenbaum M, Zangerl A R and Engeseth N J, Honey from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates, *J Agric Food Chem*, 2000, **48**, 4997-5000.
17 Fukumoto L R and Mazza G, Assessing antioxidant and prooxidant activities of phenolic compounds, *J Agric Food Chem,* 2000, **48**, 3597-3604.
18 Michel E, Becker Nissen L R and Skibsted L H, Antioxidant evaluation protocols: Food quality or Health effects, *Eur Food Res Tech*, 2004, **219**, 561-571.
19 Roginsky V and Lissi E, A review of methods to determine chain breaking antioxidant activity in Food, *Food Chem*, 2005, **92**, 235-254.
20 Beretta G, Granata P, Ferrero M, Orioli M and Facino RM, Standardisation of antioxidant properties of honey by combination of spectrophotometric/fluorimetric assays and chemometrics*, Anal Chim Acta,* 2005, **533**, 185-191.
21 Benzie I F F, An automated, specific, spectrophotometric method for measuring ascorbic acid in plasma (EFTSA*), Clinic Biochem,*1996, **29**, 111-116.
22 Benzie I FF and Strain J J, The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay, *Anal Biochem*, 1996, **239**, 70-76.
23 Folin O and Ciocalteu V, Tyrosine and tryptophan determinations proteins, *J Bio Chem*, 1972, **73**, 627.
24 Ghiselli, A, Serafini M, Maiani G, Azzini E and Ferro-luzzi A, A fluorescence-based method for measuring total plasma antioxidant capability*, Free Rad Bio Med,* 1995, **18**, 29-36.
25 Glazer A N, Phycoerythrin flurorescence based assay for reactive oxygen species, *Methods Enz*, 1990, **186**, 161-168.
26 Cao G, Alessio H M and Cutler R G, Oxygen-radical absorbance capacity assay for antioxidants, *Free Rad Bio Med,* 1993, **14**, 303-311.
27 Ou B, Hampsch-Woodill M and Prior RL, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, *J Agric Food Chem,* 2001, **49**, 4619-4626.
28 Miller N J, Diplock A T, Rice-evans C, Davies M J, Gopinathan V and Milner A, A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, *Clin Sci*, 1993, **84**, 407-412.
29 Re R, Pelligrini N, Proteggente A, Pannala A, Yang M and Rice-Evans CA, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Rad Bio Med*, 1999, **26**, 1231-1237.
30 Strube M, Haenen G R M M, Van den berg H and Bast A, Pitfalls in a method for assessment of total antioxidant capacity, *Free Rad Res,* 1997, **26**, 515-521.
31 Jime´nez-escrig A, Jime´nez-jime´nez I, Sa´nchez-moreno C and Saura-Calixto F, Evaluation of free radical scavenging of dietary carotenoids by the stable radical 2, 2-diphenyl-1-picrylhydrazyl, *J Sci Food Agric,* 2000, **80**, 1686-1690.
32 Bondet A, Brand-williams W and Berset WC, Kinetics and mechanisms of antioxidant activity using the DPPH free radical method, *Food Sci Tech Leb*, 1997, **30,** 609-615.
33 Foti M C, Daquin, C and Geraci C, Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH radical in alcoholic solutions, *J Org Chem*, 2004, **69**, 2309-2314.
34 Lachman J, Orsak M, Hejtmankova A and Kovarova A, Evaluation of antioxidant activity and total phenolic of selected Czech honeys, *Food Sci Tech,* 2010, **43**, 52-58.
35 Bertoncelj J, Dobersek U, Jamnik J and Golob T, Evolution of the phenolic content, antioxidant activity and colour of Slovenian honey, *Food Chem,* 2007, **105**, 822-828.
36 Buricova L and Reblova Z, Czech Medicinal plant possible sources of antioxidant, *Czech J Food Sci,* 2007, **26** (2), 132-138.

37 Mohamad M, Sirajudeen K N S, Swamy M, Yaacob N S and Sulaiman S, A Studies on the antioxidant properties of Tulang honey of Malaysia, *African J Trad Comp Alter Med*, 2010, **7**, 59-63.

38 Kaskoniene N, Maruska A, Kornysova O, Charczun N, Ligor M and Buszewski B, Quantitative and qualitative determination of phenolic compounds in honey, *Chemin Tech*, 2009, **3**, 52.

39 Buratti S, Benedetti S and Cosio MS, Evaluation of the antioxidant power of honey, propolis and royal jelly by amperometric flow injection analysis, *Talanta*, 2007, **17**, 1387-1392.

40 Meda A, Lamien C E, Romito M, Millogo J and Nacoulma O G, Determination of the total phenolic, flavonoid and proline content in Burkina Fasan honey as well as their radical scavenging activity, *Food Chem*, 2005, **91**, 571-577.

41 Baltrusaityte V, Venskutonis P R and Ceksteryte V, Radical scavenging activity of different floral origin honey and beebread phenolic extracts, *Food Chem*, 2007, **101**, 502-514.

42 Brudzynski K and Miotto D, The recognition of high molecular weight melanoidins as the main components responsible for radical-scavenging capacity of unheated and heat-treated Canadian honeys, *Food Chem*, 2011, **125**, 570-575.

43 Jerkovic I, Kasum A, Marijanovic Z and Tuberoso CIG, Contribution to the Characterisation of honey-based Sardinian product abbamele: Volatile aroma composition, honey marker compounds and antioxidant activity, *Food Chem*, 2011 **124**, 401-410.

44 Alverez-Suarez J M, Tulipani S, Romandini S, Vidal A and Battino M, Methodilogical aspects about determination of phenolic compounds and in vitro evaluation of antioxidant capacity in the honey, A review, *Curr Anal Chem*, 2009, **5**, 293-302.

45 Ferreira I C F R, Aires E, Barreira J C M and Esterinn L M, Antioxidant activity of Potuguese honey samples different contributions of the entire honey and phenolic extract, *Food Chem*, 2009, **89**, 609-616.

46 Socha R, Juszcjk S and Pietrzyk Fortuna T, Antioxidant activity and phenolic composition of herb honey, *Food Chem*, 2009, **113**, 568-574.

47 Krpan M, Markovic K, Saric G, Skoko B, Hruskar M and Vahcic N, Antioxidant activity and total phenolics of Acacia honey, *Czech J Food Sci*, 2009, **27**, 245-247.

48 Rasmussen CN, Wang XH, Leung S, Andrae-Nightingale LM, Schmidt S J and Engeseth, JN, Selection and use of honey as an antioxidant in a French Salad Dressing System, *J Agric Food Chem*, 2008, **56**, 8650-8657.