

Comparison of Antioxidant Contents of Thai honeys to Manuka Honey

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ABSTRACT

Introduction: Natural honey has been valued in traditional medicine having demonstrated many antioxidant properties. The aim of this study was to compare the antioxidant activities of 12 types of honey from Thailand and manuka honey from New Zealand. **Methods:** Antioxidant contents of phenolic content, ferric reducing antioxidant power (FRAP), ascorbic acid content, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and Trolox Equivalent Antioxidant Capacity (TEAC) were determined in Thai and manuka honey samples. **Results:** All of the 12 types of Thai (n=54) and manuka (n=3) honey varied in range for phenolic content (210-1,519, 563-785 mg GAE/kg), FRAP value (600-9,183, 3,866-4,933 μ M Fe(II)/kg), ascorbic acid content (103-386, 913-1,212 mg/kg), DPPH radical scavenging activity (25-528, 259-310 mg Trolox/kg) and TEAC content (96-636, 328-421 mg Trolox/kg). Mangosteen honey had the highest content of phenolics (1,495 \pm 20 mg GAE/kg), FRAP value (9,083 \pm 100 μ M Fe(II)/kg), DPPH radical scavenging activity (522 \pm 6 mg Trolox/kg) and TEAC content (632 \pm 3 mgTrolox/kg). **Conclusion:** Antioxidant activities in mangosteen and rambutan honey presented significantly higher values than manuka honey in terms of phenolic content, DPPH and TEAC. Manuka honey contained the highest vitamin C content (1,194 \pm 18 mg/kg). However, the values for antioxidant properties were dependent on source of honey. Some honeys from Thailand were found to be a better source of antioxidant properties compared to manuka honey.

Key words: Antioxidants, free radicals, honey, Thailand

INTRODUCTION

Honey is a natural product obtained from the nectar of flowers. It has several properties including those of being a food, supplementary diet and traditional medicine. Honey imparts pharmaceutical

properties since it has antibacterial and antioxidant activities. Antioxidants are substances that protect the cells of the body from damage caused by unstable molecules known as free radicals. Natural antioxidants such as vitamin C and

phenolic compounds in many plants depend on a floral source and are important to prevent human diseases (Ferreira *et al.*, 2009; Gheldof, Wang & Engeseth, 2002; Lachman *et al.*, 2010). In recent years, the identification and quantification of the antioxidant components of honey have been reported worldwide (Gheldof *et al.*, 2002; Omotayo, Siti & Mohd, 2012).

Longan honey is mainly produced in the northern part of Thailand, but other varieties (macadamia, sunflower, rambutan, sesame, bitter bush, coconut, korlan, bittervine, cashew nut and mangosteen) are produced in different geographical locations in Thailand. The price of Thai honey range from 2.5 to 12 USD/kg. In contrast, manuka honey can be sold up to 10-30 times the retail price of Thai honey. Manuka honey is produced from the manuka bush (*Leptospermum scoparium*), indigenous to New Zealand and Australia. It is well known for its pharmaceutical properties and functional food value (Kwakman & Zaat, 2012; Majtan *et al.*, 2012; Stephens *et al.*, 2010). Currently, there is an increasing trend in the use of manuka honey in the cosmetics and food industry in Thailand. Though several studies have reported on the composition and quantity of antioxidant activities in honey (Srisayam & Chantawannakul, 2010; Moniruzzaman *et al.*, 2013), there is limited data comparing antioxidant content of various honeys from Thailand with manuka honey. Therefore, the aim of this study is to compare antioxidant content of manuka honey from New Zealand with various Thai honeys.

METHODS

Honey samples

In this study, reserchers investigated 12 types of 54 honey samples from different geographical regions and provinces between January 2013 to April 2014 provided by the Department of

Agricultural Extension in the Ministry of Agriculture and Cooperatives of Thailand and the Thai Beekeepers Association.

The samples were Longan (n=21) from Chiang Mai and Lamphun, sunflower (n=3) from Lop Buri and Saraburi, lychee (n=3) from Lop Buri and Chiang Mai, korlan (n=3) from Uttaradit, sesame (n=3) from Lop Buri, rambutan (n=3) from Surat Thani and Nakhon Si Thammarat, para rubber (n=3) from Nakhon Si Thammarat, eucalyptus (n=3) from Khon Kaen, coconut (n=3) from Nakhon Si Thammarat, mangosteen (n=3) from Trat, macadamia (n=3) from Chiang Rai, and bittervine (n=3) from Chiang Mai. The Manuka honey samples (n=3) were from New Zealand. All honey samples were collected fresh in sterile containers and stored in darkness at 5°C before the test.

Determination of phenolic compounds content

Thirty percent honey solution in distilled water (0.2 ml) was added to 0.2 ml of Folin-Ciocalteu reagent. The mixture was allowed to react at room temperature for 3 min, before adding 0.2 ml of 10% sodium carbonate and 1.4 ml of distilled water. The mixture was incubated in the dark at room temperature for 180 min. Absorbance was measured at a wavelength of 725 nm. Gallic acid was used as a standard (20-200 µg/ml; $Y=15.542x-16.196$; $r^2=0.9727$) to prepare a calibration curve in order to determine the total amount of phenols in each honey sample (mg of gallic acid equivalents (mg GAE) /kg of honey).

Determination of ferric reducing antioxidant power (FRAP)

A quantum of 0.03 ml of diluted honey in distilled water (0.1 g/ml) was mixed with 0.9 ml of FRAP reagent and 0.09 ml distilled water. The reaction mixture was then incubated at 37°C for 4 min and its absorbance was read at 593 nm. Fresh

FRAP reagent was prepared by mixing 25 ml of 300 mM/L acetate buffer (pH 3.6) with 2.5 ml of 10 mM TPTZ solution in HCl and 2.5 ml of 20 mM ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$). The resulting mixture was then pre-warmed at 37°C for 15 min. A calibration curve was prepared using an aqueous solution of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) at 25-600 $\mu\text{M/L}$ ($Y=0.0006x$, $r^2=0.999$). FRAP values were expressed as micromoles of ferrous equivalent ($\mu\text{mole Fe(II)/kg}$ of honey).

Determination of ascorbic acid content

A quantum of 20 mg honey was mixed with 1 ml of 1% metaphosphoric acid, to which was added 0.1 ml of DTCS reagent and then incubated in a water bath at 37°C for 3 h and subsequently chilled for 10 min in an ice bath. Then 0.5 ml cold sulfuric acid (12 mol/L) was added slowly in solution. The ascorbic acid content was calculated based on a calibration curve of ascorbic acid (0.1-3.0 mg/ml; $Y=0.2610x$, $r^2=0.9982$) while absorbance was measured at 520 nm. The results were expressed as mg of ascorbic acid/kg of honey.

Determination of DPPH radical-scavenging activity

One part of methanol solution was mixed with 1 part of methanol solution containing DPPH (α , α -diphenyl- β -picrylhydrazyl) radicals (0.2mM/L) called DPPH solution. Then 10% honey solution in water (0.1 ml) was added to 0.2 ml of methanol and 1 ml of DPPH solution. The mixture was allowed to react at dark room temperature for 15 min. The reduction of DPPH radical was determined by measuring the absorption at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation (% RSA) then calibrated using a standard curve of Trolox (10-60 $\mu\text{g/ml}$; $Y=1.162x-5.546$; $r^2=0.998$). Results were expressed as mg of Trolox equivalents per 1 kg honey.

Determination of Trolox equivalent antioxidant capacity

One ml of 8 mM 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) solution was added to 3 mM potassium persulfate. The solution was stored in the dark at room temperature for 14 h and then diluted in distilled water (1:100) for a ABTS working solution. One ml of ABTS working solution was mixed with 0.1 ml of 10% honey aqueous solution in water. Absorbance was read at 734 nm and then adjusted at 0.7 with ABTS solution. Results were expressed as mg of Trolox equivalents/kg of honey, based on the standard calibration curve (10-60 $\mu\text{g/ml}$); $Y=0.749x+0.371$, $r^2=0.999$.

Statistic analysis

The significant differences represented by Kruskal-Wallis Statistics, H test and Tamhane's T_2 method was used for multiple comparison tests when equal variances were not assumed. Values were presented as mean \pm SD, maximum and represented a minimum of three determinations. Data were analysed using IBM SPSS software Version 16.

RESULTS AND DISCUSSION

Phenolic compounds are some of the most important groups of antioxidant substances present in honey. The assay measured the phenol and polyphenol derivatives, and other electron-donating antioxidants. All of the different types of Thai and manuka honey had a varied range of phenolic content of 210-1,519 and 563-785 mg GAE/kg, respectively.

The highest phenolic content was found in mangosteen honey followed by rambutan (Table 1), both of which were higher than the phenolic content in manuka honey. However, the range and average values of total phenolic content observed for the Thai honey samples used in this study (lychee, longan, korlan, sunflower,

Table 1. Antioxidant properties of Thai and manuka honey

Type	Phenolic (mg GAE/kg)	FRAP (μ M Fe(II)/kg)	Vit C (mg/kg)	DPPH (mg Trolox/kg)	TEAC (mg Trolox /kg)
Korlan	\bar{x} = 465.49 ^b s = 14.90	\bar{x} = 2,755.56 ^d s = 141.75	\bar{x} = 200.19 ^a s = 0.96	\bar{x} = 203.49 ^e s = 0.01	\bar{x} = 363.79 ^e s = 1.05
Macadamia	\bar{x} = 485.10 ^b s = 7.65	\bar{x} = 2,150.00 ^c s = 33.33	\bar{x} = 323.75 ^b s = 1.92	\bar{x} = 144.59 ^d s = 2.81	\bar{x} = 277.94 ^d s = 2.09
Sunflower	\bar{x} = 432.55 ^b s = 8.91	\bar{x} = 2,755.56 ^d s = 91.79	\bar{x} = 219.35 ^a s = 0.96	\bar{x} = 178.72 ^e s = 4.93	\bar{x} = 360.30 ^e s = 15.18
Coconut	\bar{x} = 576.47 ^c s = 8.15	\bar{x} = 3,305.55 ^e s = 125.09	\bar{x} = 165.71 ^a s = 2.87	\bar{x} = 194.61 ^e s = 2.14	\bar{x} = 343.55 ^e s = 6.81
Bittervine	\bar{x} = 253.28 ^a s = 6.95	\bar{x} = 938.89 ^a s = 108.44	\bar{x} = 106.32 ^a s = 2.88	\bar{x} = 57.16 ^b s = 0.81	\bar{x} = 124.73 ^a s = 1.60
Rambutan	\bar{x} = 1,361.68 ^e s = 1.32	\bar{x} = 4,016.67 ^f s = 83.34	\bar{x} = 164.75 ^a s = 1.92	\bar{x} = 306.35 ^g s = 4.92	\bar{x} = 467.44 ^f s = 1.05
Lychee	\bar{x} = 216.79 ^a s = 8.04	\bar{x} = 605.55 ^a s = 9.62	\bar{x} = 120.69 ^a s = 7.66	\bar{x} = 26.90 ^a s = 0.81	\bar{x} = 107.98 ^a s = 10.53
Pararubber	\bar{x} = 478.43 ^b s = 8.26	\bar{x} = 2,622.22 ^d s = 58.53	\bar{x} = 185.82 ^a s = 3.84	\bar{x} = 182.31 ^e s = 2.16	\bar{x} = 293.29 ^d s = 5.77
Mangosteen	\bar{x} = 1,495.79 ^f s = 20.60	\bar{x} = 9,083.33 ^g s = 100.00	\bar{x} = 379.31 ^b s = 7.66	\bar{x} = 522.79 ^h s = 6.37	\bar{x} = 632.51 ^g s = 3.68
Eucalytus	\bar{x} = 354.60 ^a s = 25.13	\bar{x} = 2,050.00 ^c s = 83.33	\bar{x} = 147.51 ^a s = 1.92	\bar{x} = 91.89 ^c s = 9.08	\bar{x} = 168.01 ^b s = 2.10
Sesame	\bar{x} = 314.18 ^a s = 5.79	\bar{x} = 1,372.22 ^b s = 108.44	\bar{x} = 134.10 ^a s = 1.92	\bar{x} = 91.35 ^c s = 2.15	\bar{x} = 170.10 ^b s = 3.14
Longan	\bar{x} = 425.67 ^b s = 14.64	\bar{x} = 2,588.89 ^d s = 175.07	\bar{x} = 190.61 ^a s = 0.96	\bar{x} = 157.51 ^d s = 3.54	\bar{x} = 235.01 ^c s = 8.38
Manuka	\bar{x} = 657.91 ^d s = 83.16	\bar{x} = 4,342.59 ^f s = 397.05	\bar{x} = 1,067.37 ^c s = 121.52	\bar{x} = 284.07 ^f s = 17.17	\bar{x} = 374.72 ^e s = 38.30
Test of homogeneity of variances	Levene Statistic = 4.87 p = 0.000***	Levene Statistic = 3.74 p = 0.001**	Levene Statistic = 6.45 p = 0.000***	Levene Statistic = 2.83 p = 0.009**	Levene Statistic = 4.49 p = 0.000***
Test statistic	H = 42.55	H = 42.45	H = 43.74	H = 43.67	H = 41.42
p-value	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***

Note : ** for $p < 0.01$, *** for $p < 0.001$

H is Kruskal- Wallis test 's statistic value

Tamhane's T2 method was used for multiple comparison tests when equal variances were not assumed.

plaunoi, sesame and sabsue) are lower (587-1,652 mg GAE/kg; Limpawattana, 2013) than the level of the Venezuelan honey (3811,821 mg GAE/kg; Vit *et al.*, 2009), Nigeria honey (362-1,028 mg GAE/kg; Buba, Gidado & Shugaba, 2013.)

However, the phenolic content range in Thai honey(wild flowers, sunflowers, longan and lychee) was higher (100-144 mg GAE/kg; Sangsrichan & Wanson, 2008) than that of honey from south-west Nigeria (7-28 mg GAE/kg; Adetuyi *et al.*, 2009),

Czech (39-167.1 mg GAE/kg; Lachman *et al.*, 2010), Poland (71.7- 201 mg GAE/kg; Kaskoniene *et al.*, 2009), Malaysia (144-580 mg GAE/kg; Moniruzzaman *et al.*, 2013) and monofloral Cuban honey (213- 595 mg GAE/kg of honey; Alvarez-suarez *et al.*, 2010).

Determination of antioxidant activity of honey by FRAP assay is based on the ability of the sample to reduce the ferric ions to ferrous ions. The FRAP activities for the different types of Thai and manuka honey ranged from 600-9,083 and 3866-4,933 $\mu\text{M Fe(II)}/\text{kg}$, respectively. Similar to phenolic content, the highest FRAP values were found in mangosteen honey followed by rambutan and manuka honey (Table 1). The FRAP values of Thai honey (lychee, longan, korlan, sunflower, plaunoi, sesame and sabsue) in the present study were higher (1,475-5,500 $\mu\text{M Fe(II)}/\text{kg}$; Limpawattana, 2013) than those of Croatian monofloral honey (250-1160 $\mu\text{M Fe(II)}/\text{kg}$; Piljac-Zegarac, Stipcevic & Belscak, 2009) and Algerian honey (3377 \pm 10 $\mu\text{M Fe (II)}/\text{kg}$; Khalil *et al.*, 2012).

Ascorbic acid content from Thai and manuka honey ranged from 103-379 and 913-1,212 mg/kg, respectively. The highest vitamin C content was found in manuka honey followed by mangosteen honey and macadamia honey (Table 1). Similar results were obtained from Nigeria honey which ranged from 138 to 273 mg/kg (Buba *et al.*, 2013) and the Venezuelan honey (128-370 mg/kg ; Vit *et al.*, 2009). Thai honey showed a higher content of ascorbic acid than Malaysian honey (129-132 mg/kg; Moniruzzaman *et al.*, 2013), Bangladesh honey (129-154 mg/kg; Islam *et al.*, 2012), Portuguese honey (140-145 mg/kg; Ferreira *et al.*, 2009) and Malaysian pineapple honey (146 mg/kg; Kishore *et al.*, 2011).

The range values for Vitamin C content observed in the honey samples used in this study are lower than the level of a various types of honey from Bosnia Herzegovina (372 to 3783 mg/kg; Kesic *et al.*, 2009) and

from Romania (2,260 to 2,960 mg/kg; Matei *et al.*, 2004).

The DPPH radical scavenging analysis was used to investigate the overall hydrogen or electron donating activity of single antioxidants. The values for all of the different types of Thai and manuka honey ranged from 25-528 and 259-310 mg Trolox/kg in DPPH radical scavenging activity with the average values being 179 \pm 127 and 284 \pm 17 mg Trolox/kg, respectively. The DPPH levels in Thai and Manuka honey were lower than those obtained in buckwheat honey (304 mg Trolox/kg, Cheng *et al.*, 2015), but higher than Mexican honey (81 to 255 $\mu\text{mol Trolox}/\text{kg}$; Beatriz *et al.*, 2012)

TEAC content from Thai and manuka honey were 96-636 and 328-421 mg Trolox/kg, respectively. The TEAC contents obtained for the honey samples used in this study were within the range reported by Buenocosta *et al.*, (2016) from Brazil (82 and 1,114 mg trolox/kg), but higher than the values reported for Mexican honey (910 to 2,927 $\mu\text{mol Trolox}/\text{kg}$; Beatriz *et al.*, 2012).

In this study, lychee and bittervine honey from the north of Thailand showed the weakest antioxidant properties (Table 1) as in the study by Srisayam and Chantawannakul (2010) who found lychee had the weakest antioxidant properties. Whereas mangosteen from the East and the rambutan from the southern of Thailand showed high antioxidant properties (Table 1) similar to that of the report of Srisayam and Chantawannakul (2010) who found rambutan to have the highest antioxidant property.

Each of the antioxidant assays of DPPH, FRAP, ascorbic acid antioxidant content, and TEAC have their own advantages and disadvantages. This study found at least one method to detect the difference in amount of antioxidant activity in honey ($p < 0.05$). Mangosteen honey was found to have the highest amount of antioxidants using FRAP, phenolic content, DPPH and

TEAC analyses ($p < 0.001$). Manuka was found to contain the highest ascorbic acid content ($p < 0.001$) (Table 1).

The antioxidant activities in honey originate from nectar, pollen or propolis and substances that contain organic acids, vitamins, and enzymes are known to occur in honeys (Gheldof, Wang & Engeseth, 2002).

The differences in the antioxidant activities of honey depend on the floral sources, the sources of collection (Jantakee & Tragoolpua, 2015; Al-mamary, Al-meeri & Al-habori 2002; Kaskoniene *et al.*, 2009; Kesic *et al.*, 2009), seasonal factors, and environmental factors (Estevinho *et al.*, 2008), processing, handling, and storage of honey (Al-mamary *et al.*, 2002).

CONCLUSION

Determination of antioxidant activity of 12 types of Thai honey samples from different plant origins, geographical regions and provinces showed differences in antioxidant activities. Antioxidant activity in mangosteen and rambutan honey presented significantly higher values than Manuka honey in terms of FRAP, phenolic, DPPH and TEAC analytical results. Mangosteen and rambutan honey which are rich in antioxidant activity may be recommended as a dietary supplement and natural nutraceutical.

Conflict of interest

The authors declare that they have no conflict of interest.

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