Original Article

Physicochemical and Antioxidant Properties of Malaysian Tualang Honey on Long Term Storage

R. Krishna Kishore [1, 3], S. Abdul Nasar [1], P.T.Viji [1], Chin Keong Lim *[,2,3,4], Ahmad Sukari Halim [3]

[1]*Department of Biochemistry, CMR Institute of Management Studies, Kalyana Nagar, Bangalore, 560043, Karnataka, India.*
[2]*Centre of Studies for Preclinical Sciences, Faculty of Dentistry, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.*
[3]*Reconstructive Sciences Unit, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.*
[4]*Skin Research and Innovation Centre, Research & Development Division, Wipro Manufacturing Services Sdn Bhd, Persiaran Subang Permai, Taman Perindustrian Subang, 47610 Subang Jaya, Selangor.*

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Abstract Honey is a rich source of natural nutrients. Its production is a slow, natural process with the pace of which varies seasonally. However, based on recent reports, we hypothesize that the long-term storage of processed honey, even under the most appropriate storage conditions, results in a deterioration of its quality. To test our hypothesis, we collected Tualang honey samples harvested during the years 2005, 2008, 2009 and 2010 and tested various parameters including physicochemical properties and also performed comparative analyses of antioxidant capacities to assess its medicinal values. Our results indicate that, upon long-term storage, the quality of honey samples deteriorates, as observed in our TH 2008 and TH 2005 year honey samples, which showed unacceptable quality based on the recommended criteria of free acidity (71.34±1.31 meq/kg), moisture (27.72%), diastase activity (3.38±0.34 Goth scale) and hydroxymethylfurfural (HMF) (449.89±3.23 mg/kg) by Codex and European Commission Regulation. A significant ($p<0.05$) decrease in antioxidant properties were also observed. In the present study, we show that, even after appropriate processing, most of the quality parameters of honey decrease, which suggest that these parameters could otherwise be used as markers to assess the age of the honey.

Keywords: Honey, Physicochemical properties, HMF, Antioxidant capacity

Introduction

Oxidative stress occurs in a system when free radicals and reactive oxygen species (ROS) overwhelm its endogenous and exogenous antioxidant defenses beyond the ability of the system to neutralize and eliminate them (Ortial et al., 2006). However, increases in exogenous antioxidants are believed to alleviate any oxidative damage and help balance cellular free radicals and ROS.

Honey has been studied extensively because of its physicochemical, antimicrobial, antioxidant, antiviral, antitumor and anti-inflammatory properties (Viuda-Martos et al., 2008; Yolanda et al., 2011). Recent investigations of several natural compounds have revealed that honey is one of the most nutritious products that exists in the form of a traditional remedy containing monosaccharides, amino acids, proteins (including enzymes), organic acids, vitamins, minerals, various phytochemicals and many biologically active entities (Ahmed et al., 2007).

Honey is obtained from a variety of sources all

*Corresponding to: Lim Chin Keong, Skin Research and Innovation Centre, Research & Development Division, Wipro Manufacturing Services Sdn Bhd, Persiaran Subang Permai, Taman Perindustrian Subang, 47610 Subang Jaya, Selangor.
Email:lim125@hotmail.com
Tel: 03-56315588 ext:397 (Office),
+6012-2512255 (mobile)

across the world, and each variety varies in its composition and hence, in its beneficial properties. Recently, we performed comparative analyses using honey collected from various sources and have successfully shown that Malaysian tualang honey is among the best honey available based on its high antioxidant and free radical-scavenging ability (Kishore et al., 2011). However, in addition to the source, the physicochemical and the antioxidant capacities of honey samples are highly dependent on the storage conditions during climatic changes (Bath & Singh, 2000). Therefore, a lack of knowledge about proper storage conditions may ultimately reduce the physicochemical properties of honey and subsequently reduce the medicinal properties and the quality of this food product. Apparently, although Malaysian Tualang honey is among the best honey, the extreme temperatures in Malaysia (temperatures of up to 40°C) may have drastic effects on the quality of honey during storage prior to consumption.

To the best of our knowledge, there has only been one study on the effect of storage on the physicochemical and antioxidant properties of honey samples (Nombre et al., 2010). The current study specifically addresses the effect of long-term storage on processed honey by evaluating medicinal properties of Malaysian tualang honey that was harvested over several years (collected during 2005, 2008, 2009 and 2010). The findings of the present study will help to provide appropriate guidelines for processing and storage of Malaysian tualang honey and for identifying markers to assess the age of honey.

Methods and materials

Chemicals

All chemicals, reagents and solvents used were of analytical grade and were purchased from Merck (Germany). Standards used in the antioxidant assays were L-ascorbic acid, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu's phenol reagent (Sigma; St. Louis, MO, USA).

Honey samples

Malaysian Tualang honey samples harvested from the nectar of Apis dorsata bee's nectar during 2005, 2008, 2009 and 2010 from Tualang trees in the rain forest of Kedah (peninsular Malaysia) were included in this study. The honey was initially filtered to remove solid particles by Federal Agricultural Marketing Authority (FAMA) of Malaysia and was subjected to gamma irradiation at 25 kGy at Sterilgamma (M) Sdn. Bhd. prior to being submitted to us for analyses.

Physicochemical analyses

Moisture, color, pH, free acidity, electrical conductivity, ash content, reducing sugars and sucrose content were analyzed in all honey samples using respective methods *mentioned by Association of Analytical Communities* (AOAC) (AOAC, 2000).

- Diastase activity
  Diastase activity, which is a measure of the hydrolytic activity of the enzymes in honey, was determined using methods described by Horwitz et al. (1980).
- Hydroxymethylfurfural
  HPLC method
  The HPLC method used was a modification of the basic method published by the International Honey Commission in 1999, as described by Khalil et al. (2010). The hydroxymethylfurfural (HMF) content of a sample was calculated by comparing the corresponding peak areas of the sample with those of the standard solutions of HMF (Sigma–Aldrich, USA) after correcting for the honey dilution. There was a linear relationship (R^2 = 0.9998; Fig. 1) between the concentration and the area of the HMF peak, and the results are expressed in mg/kg.

Total phenolic and biochemical analyses

- Determination of total phenolic content
  The total phenolic content of honey samples was determined using the Folin-Ciocalteau method (Singleton et al., 1999).

The total phenolic content is expressed in mg of gallic acid equivalents (GAE)/100 g of honey.

- Determination of total flavonoid content
  The total flavonoid content of the honey sampl was determined using the method described by Woisky and Salatino (1998). Total flavonoids were calculated as mg quercetin equivalents (QE)/100 g of honey.
- Evaluation of total antioxidant capacity using the phosphomolybdenum method
  The total antioxidant activity of tualang honey samples was evaluated using the green phosphomolybdenum complex method (Prieto et al., 1999). The total antioxidant capacity was calculated as ascorbic acid equivalents (mg AAE/ g honey).
- Determination of ascorbic acid content
  The standard 2, 4-dinitrophenylhidrazine method was used to determine the ascorbic acid content of the honey samples (Omaye et al., 1979).
- Determination of protein content
  The protein content of honey samples was measured using the Bradford assay (Bradford, 1976).

Analyses of antioxidant activities

- Free Radical scavenging activity of DPPH
  The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging effect of honey samples was measured using the method described by Hatano et al. (1988) and the radical-scavenging activity was expressed as IC_{50} (mg/mL).
- Ferric reducing/antioxidant power assay (FRAP)
  The antioxidant capacity of tualang honey samples was estimated according to the procedure described by Benzie and Strain (1996). Ferrous sulfate was used for the calibration curve (0-500 µM), and the results, which were obtained from triplicate analyses, are expressed in µM of ferrous sulfate per 100 g of honey (µmol Fe (II)/100 g of honey).
- Superoxide anion radical-scavenging activity
  Superoxide radicals generated by the xanthine-xanthine oxidase system were determined spectrophotometrically by monitoring the product of the reaction with nitroblue tetrazolium salt (NBT) (Robak and Gryglewski, 1988). The results are expressed as the concentration of the test sample required for a 50% reduction in the absorbance of the control in mg/mL (IC_{50}).
- Peroxynitrite radical-scavenging activity
  Peroxynitrite-scavenging activity was determined according to methods described by Koppenol et al. (1996). The IC_{50} value was determined and expressed as mg/mL.

Statistical Analyses

Three different harvests for each year were analyzed for each parameter, and the data are expressed as the mean ± standard deviation (SD) (n=6). Each sample was analyzed in duplicate, and the statistical differences represented by letters were obtained through a one-way analysis of variance (ANOVA). Differences between the means at the $p<0.05$ confidence level were considered statistically significant. Correlations were obtained using Pearson's correlation coefficient (r) in bivariate linear correlations using the SPSS statistical program.

Results and Discussion

Physicochemical properties

Physicochemical parameters that have been previously studied were divided based on origin and their involvement with conductivity, coloration, sugar and quality control, which include moisture, HMF and diastase activity. The obtained data are presented in Table 1.

Physicochemical parameters based on origin

- Honey pH
  Regardless of geographical origin, honey is acidic in nature, and its acidity influences its texture and stability as well as its storage conditions (Williams et al., 2009; Saxena et

al., 2010). The pH of honey is also a useful indicator of any possible microbial contamination. As shown in Table 1, the pH was statistically different ($p<0.05$) for samples collected during different years and for samples that were stored for different periods of time, even though the pH range obtained remained within the limits specified by the EU Council (2002) and Codex Alimentarius (2001). The pH of Tualang honey samples varied from 3.02 to 4.14 with gradual differences observed over extended periods of storage.

- Color intensity
  The color intensities of Tualang honey significantly ($p<0.05$) increased during storage. The most recent honey samples, which were harvested in 2010 and 2009, had an intensity varying from 214.17±3.05 to 281.50±4.23 mAU. For the 2008 and 2005 samples, the extremely dark intensities (412±2.97 and 679.83 mAU) from the Millard reaction were perhaps due to the formation of colored pigments and higher colloidal content. Similar observations have been made in previous studies involving Italian, Slovenian and Malaysian honeys (Beretta et al., 2005; Bertoncelj et al., 2007).

- Electrical conductivity
  An electrical conductivity of <0.8 mS/cm is a good indicator of the botanical origin of honey as well as its mineral content and increases upon storage. Accordingly, in the present study, the electrical conductivity (mS/cm) values increased significantly from 0.30 for the freshest harvest (2010) to 1.13 for the oldest harvest (2005). The high conductivity was perhaps due to an increase in the organic ionizable fraction of the honey. This is further explained by a high acidity (Persano et al., 2008). Previous studies have indicated that electrical conductivity does not depend exclusively on mineral content but increases with an increase in ash, organic acids, proteins, and some complex sugars content as well (Terraberint et al., 2003).

- Free Acidity
  The maximum free acidity permitted by Codex Alimentarius honey standards is 50 meq/kg. In the present study, honey harvests from 2009 and 2010 yielded free acidity values between 17.82 and 23.30 meq/kg that are well within international regulations, thus indicating the absence of an undesirable fermentation. However, the other two older harvests from 2008 and 2005 had a free acid content of 56.76 and 71.34 meq/kg, respectively, and thus, they exceed the acceptable range proposed by Codex Alimentarius (2001). Higher levels may results from a possible microbial alteration as a consequence of higher temperatures during prolonged storage (Kaur-Bath and Narpinder, 2000). In the present study, the values indicate that the sugars present in the samples have undergone fermentation in the presence of yeasts, and it is well known that during fermentation, glucose and fructose are converted into carbon dioxide and alcohol. In the presence of oxygen, the alcohol is further hydrolyzed and converted into acetic acid, which contributes to the free acid content of the honey (Ouchemoukh et al., 2007). These findings, along with the results from the current study, clearly indicate that the moisture content in Tualang honey samples is influenced by the botanical source, geography, climatic conditions, season and storage.

- Ash content
  Ash represents the direct measure of the inorganic residues after honey carbonization and indicates the possible botanical origin of honey (Malika et al., 2005). The ash content in all Tualang honey samples studied varied widely between the 2010, 2009, 2008 and 2005 samples and measured 0.35, 0.38, 0.51 and 0.68 g%, respectively. The 2008 and 2005 samples had the highest ash contents, where as 2005 sample is above the 0.6% limit (Codex Alimen-

tarius Commission, 2001). Honey normally has low ash content and, in the current study, the high variability observed in the honey's ash content may indicate that the harvest processes and/or the beekeeping techniques used by the producers were non-uniform. Nevertheless, it has also been proposed that the ash content of honey depends on the floral origin of the material collected by the bees during foraging on the flora (Ojeda De Rodriguez et al., 2004).

- Sugar content
  Monosaccharides, such as glucose and fructose form the major carbohydrate content (60%) of honey (Mendes et al., 1998). Although the ratio of the two monosaccharides mainly depends on the source of the nectar, it varies from 0.9 to 1.4 (fructose/glucose) and is a good indicator for explaining the structure and crystallization of honey. The concentration of sucrose should be less than 5%. In the present study, this ratio fell within the Codex Alimentarius (2001) limits for all honey samples, which suggested no variation over the period of storage. Any observed changes in the concentrations are due to the hydrolysis of sucrose by the enzyme invertase or sucrase, which release the monosaccharides fructose and glucose. Similar results were observed by Cantarelli et al. (2008).

Physicochemical parameters based on quality control

- Moisture content
  The moisture content of honey significantly affects its quality during storage as it slows fermentation and granulation to provide a longer shelf life. However, moisture content also depends on various factors, such as the harvesting season, degree of maturity reached in the hive, and climatic factors (Finola et al., 2007). Two of the four Tualang honey samples, especially from 2010 and 2009, yielded moisture contents between 10.29% and 16.25%, which indicate a proper degree of maturity and that the beekeepers had used the optimal time for extraction. The values were within the allowed limit of 21% moisture content permitted by the EU Council (2002) and Codex (2001). Nevertheless, two of the samples, from 2008 and 2005, had moisture contents of 22.77 and 27.72%, respectively, which are slightly above 20% and were probably due to a prolonged storage period and prior extraction of honey from hives.

- Diastase activity
  Diastase activity has been found to be mainly influenced by honey storage and heat. It facilitates the conversion of starch to maltose and is added by bees during honey production. Although there is a large natural discrepancy of this parameter in honey, a minimum value of 8 on the Gothe scale is expected of a high quality honey (Bogdanov et al., 1999). In the current study, diastase activities were determined to be 19.53, 14.80, 5.78 and 3.38 (Goethe unit) for the 2010, 2009, 2008 and 2005 honey samples, respectively ($p<0.05$). The diastase activity decreased after storage for at least 24 months with respect to fresh samples, which has been observed in previous studies (Sahinler, 2007). This further substantiates that a measure of diastase activity in honey can be an effective indicator of the storage time/freshness of the honey.

- HMF Content
  The HMF content in fresh honey is generally very low; however, honey with a HMF content up to 80 mg/kg is still considered to be fresh (Codex Alimentarius, 2001; EU council, 2002). Higher HMF values suggest probable exposure to heat and/or prolonged storage periods, which result in a caramelization of carbohydrates, termed the Maillard reaction, as well as fructose decomposition (Ruoff et al., 2007). Based on the HPLC method (Table 1), the HMF concentrations in the tualang honey samples stored for 1 and 2 years (the 2010 and 2009 tualang honey samples) exhibited initial HMF contents of 6.92±0.02 and

13.20±1.60 mg/kg, respectively. However, Tualang honey samples stored for more than 2 years (2008 and 2005) had the highest HMF values, 109.61±0.45 and 449.89±3.23 mg/kg, respectively, ($p<0.05$). It is obvious that heating is not the only factor influencing HMF formation in honey, i.e., the honey's composition, pH and floral source also contribute. Our results are consistent with Khalil et al. (2010), who found that the amount of HMF in Tualang honey stored for more than one year was significantly higher compared to newly harvested honey.

Total phenolic and biochemical activities

- Total phenolic and flavonoid content
  Phenolics and flavonoids are the most widely detected secondary metabolites distributed in honey samples, and it has been reported that the antioxidant activity of phenolics is mainly due to their redox properties and abilities to serve as hydrogen donors and singlet oxygen quenchers (Chuanphongpanich et al., 2006). According to the results shown in Table 2, the fresh honey samples, which had strong antioxidant activities, also had high total phenolic and flavonoid contents. However, in the older honey samples, these values deteriorated during storage and varied significantly ($p<0.05$) between samples. The average content of total phenolics and flavonoids obtained for our honey samples is similar to those of other honey samples from various floral sources, which have been reported in the literature (Meda et al., 2005).
- Total antioxidant capacity
  The influence of storage on total antioxidant capacity is shown in the table 2. The total antioxidant capacity assay is mainly based on the reduction of Mo (VI) to Mo (V) by the extraction and subsequent formation of a green phosphate/Mo (V) complex. A high absorbance value for a sample indicates a strong antioxidant activity. During the present study, the fresh Tualang honey samples (2010 & 2009) were observed to function more effectively as antioxidants and had significant ($p<0.05$) total antioxidant capacities. The results of the total antioxidant capacity assays indicate that during storage the antioxidant capacities of the samples decreased considerably with age. A good correlation between total phenolics and total antioxidant capacity was observed ($r = 0.987$) (Kishore et al., 2011), and this may be the reason for the greater total antioxidant capacity displayed by the fresh honey samples.
- Ascorbic acid and Total Protein content
  Honey also contains ascorbic acid, which is an important water-soluble antioxidant and a cofactor for several enzymes. The obtained values for ascorbic acid and total protein content levels for the Tualang honey samples are presented in Table 2. The ascorbic acid levels (mg/100 g of honey) of the Tualang honey samples varied from 37.04 (year 2010) to 14.75 (year 2005). A reduction in ascorbic acid content was observed in older honey samples, and this reduction was mainly due to the sensitive nature of ascorbic acid towards water, heat and oxygen content. In addition to these factors, different processing and storage techniques and tropical conditions may have also led to the higher losses because of a higher oxidation rate of ascorbic acid (Padayatty et al., 2003). In the current study, the protein content (mg BSA/100 g of honey) of the Tualang honey varied from 79.16 (year 2010) to 37.41 (year 2005), which was determined using bovine serum albumin (BSA) as a standard. The protein level of honey is dependent on the type of flora as well as the enzymes introduced by the honey bees themselves, in addition to enzymes in the nectar (Alvarez-Suarez et al., 2010).

Analyses of antioxidant activities

- DPPH radical-scavenging activity
  DPPH is a relatively stable nitrogen-centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule and has been widely used to evaluate the antioxidant activity of various natural products (Amarowicz et al., 2004). The IC_{50} (mg/mL) values of the honey samples (Table 2) varied from 6.16 (year 2010) to 15.02 (year 2005). The fresh honey samples showed a greater scavenging activity when compared to the older honey samples, and this greater activity was mainly due to the deterioration of the antioxidants, especially phenolics and flavonoids, present. A significant difference ($p<0.05$) in the radical-scavenging activities between the honey samples was also observed. The results obtained in this study are comparable to the DPPH radical-scavenging abilities of Tualang honey samples obtained from previous studies (Mohamed et al., 2010; Kishore et al., 2011).
- Ferric reducing antioxidant power assay
  The FRAP assay results for the Malaysian Tualang honey samples (µmol Fe (II)/100 g of honey) tested are displayed in Table 2, and the obtained results varied from 124.04 (year 2010) to 38.79 (year 2005). Storage had a significant ($p<0.05$) effect on ferric ion-reducing activities, a relatively higher absorbance value indicates a higher reduction rate of ferric ions to ferrous ions. In the current study, the observed range of FRAP values was comparable to the reducing capacity range of Slovenian honey samples (Bertoncelj et al., 2007) and raw Milleflori honey (Blasa et al., 2006). A positive linear correlation between the DPPH assay and the total polyphenol content ($r = 0.975$) to the FRAP method and the phenolic content ($r = 0.991$) was observed. This correlation indicates that the phenolics are the major antioxidant compounds responsible for the antioxidant activities of fresh honey samples.
- Superoxide anion radical and Peroxynitrite scavenging activity
  Superoxide anions are precursors of other reactive oxygen species, which include hydrogen peroxide, hydroxyl radicals, and singlet oxygen. The further reaction of these superoxide radicals with nitric oxide results in the formation of cytotoxic peroxynitrites, which oxidize cellular components, such as proteins, lipids, and nucleic acids (Balavoine and Geletti, 1999). Peroxynitrites are relatively stable but once protonated, they form highly reactive peroxynitrous acid. The superoxide anion and peroxynitrite scavenging activities of the Tualang honey samples were investigated, and the results were compared with those of reference antioxidants. A higher extract concentration required to scavenge radicals indicates a lower antioxidant activity. Based on the results (Fig. 2), it is evident that the fresh honey samples (years 2010 & 2009) showed significant superoxide anion and peroxynitrite scavenging activities when compared to the older stored honey samples.

Conclusions

Both the physicochemical properties and the phenolic compound composition of honey are responsible for its quality and antioxidant activity, thus rendering it a healthy food and source of antioxidants. Our findings suggest that the long-term storage of honey, even under the appropriate temperatures and storage conditions, decreases the quality in a variety of aspects and parameters and ultimately leads to the loss of its major nutritive and medicinal values. Based on our findings, we also suggest that highly sensitive parameters, such as moisture, free acidity, diastase activity, HMF content, total phenolics and antioxidant activity, can be used as markers for approximating the shelf life and age of honey. However, further extensive studies need to be performed to determine

the most sensitive markers and to specify any guidelines for the assessment of the age and quality of the honey.

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Tables

Table 1: Physicochemical parameters studied according to the control of the origin and designation with conductivity, coloration, sugars and acidimetry and quality control in tualang honey samples.

According to the European Codex Honey Standards (Bogdanov et al., 1999), a well processed and ready to be consumed honey must contain the following characteristics: electrical conductivity < 0.8 mS/cm, reducing sugars content >65 g/100 g, sucrose content <5 g/100 g, free acidity <50 milliequivalents/kg, maximum moisture content of 20–21 g/100 g of honey, diastase number >8G, 5-HMF content < 80 mg/kg of honey. Data are expressed as means±SD. SD, standard deviation. Significant P values are presented ($p<0.05$). Significantly different values are represented by different letters.

Table 2: A compilation of data from tualang honey samples: Total phenolic, flavonoid, ascorbic acid content, antioxidant and free-radical scavenging activity of tualang honey samples.

Data are expressed as means±SD. GAE, gallic acid equivalents; QE, quercetin equivalents; AAE, ascorbic acid equivalents; BSA, bovine serum albumin equivalents; IC_{50} , 50% inhibitory concentration; FRAP, Ferric reducing/ antioxidant power; SD, standard deviation. Significant P values are presented ($p<0.05$). Significantly different values are represented by different letters.

Figure legends

Figure 1: Linear relationship between the concentration of HMF and peak area.

Figure 2: Superoxide anion radical and peroxy nitrite radical scavenging activity of tualang honey samples. Data are expressed as the means±SD; SD, standard deviation. IC_{50} represents the mg/mL concentration providing 50% inhibition of peroxy nitrite scavenging and superoxide anion radical formation or scavenging of the available free radicals. Ascorbic acid (IC_{50}: 2.3 mg/mL) was used as a reference antioxidant for comparison. Significant P values are presented ($p<0.05$).

Physico-chemical analysis	Tualang honey 2010	Tualang honey 2009	Tualang honey 2008	Tualang honey 2005
Moisture (%)	10.29	16.25	22.77	27.72
Colour (mAU)	214.17±2.05[a]	281.50±4.42[b]	412.00±3.45[c]	679.83±4.58[d]
pH	4.15±0.12	3.84±0.14	3.52±0.12	3.06±0.19
Electrical conductivity (mS/cm)	0.307±0.006	0.529±0.009	0.802±0.011	1.134±0.043
Free acidity (meq/kg)	17.82±0.98[a]	23.30±0.63[b]	56.76±0.83[c]	71.34±1.31[d]
Ash (%)	0.35±0.03	0.38±0.03	0.51±0.04	0.68±0.04
Fructose (%)	46.13	39.90	34.72	29.70
Glucose (%)	41.92	36.83	32.50	26.60
F+G	88.05	76.73	67.22	56.30
Sucrose (%)	3.68	3.10	1.45	0.22
Diastase activity (Gothe scale)	19.53±0.93[a]	14.80±0.49[b]	5.78±0.56[c]	3.38±0.34[d]
HMF (mg/kg)	6.92±0.02[a]	13.20±1.60[b]	109.61±0.45[c]	449.89±3.23[d]

Table 1: Comparative physico-chemical properties of Tualang honey samples on storage

Biochemical parameter	Tualang honey 2010	Tualang honey 2009	Tualang honey 2008	Tualang honey 2005
Total Phenolic content (mg GAE/100 g)	85.52±2.69[a]	76.72±1.28[b]	53.03±1.63[c]	23.13±0.66[d]
Total Flavonoid content (mg QE/100 g)	51.92±1.31[a]	43.91±1.97[b]	27.37±1.07[c]	12.52±0.89[d]
Total Antioxidant capacity (mg AAE/g honey)	54.27±1.24[a]	50.37±0.97[a]	33.32±0.85[b]	14.75±0.65[c]
Ascorbic acid content (mg/100 g of honey)	37.04±0.86[a]	31.15±0.70[b]	24.45±0.60[c]	10.02±1.12[d]
Protein content (mg BSA/100 g of honey)	79.16±0.99[a]	76.42±1.68[a]	50.57±0.82[b]	37.41±1.22[c]
DPPH radical-scavenging activity (IC_{50} values mg/mL)	6.16±0.43[a]	6.69±0.36[a]	9.71±0.36[b]	15.02±0.45[c]
FRAP (µmol Fe(II)/100 g of honey)	124.04±2.93[a]	112.17±2.49[b]	74.80±2.68[c]	38.79±1.42[c]

Table 2: A compilation of data from Tualang honey samples: Total phenolic, flavonoid, ascorbic acid content, antioxidant and free-radical scavenging activity of Tualang honey samples.

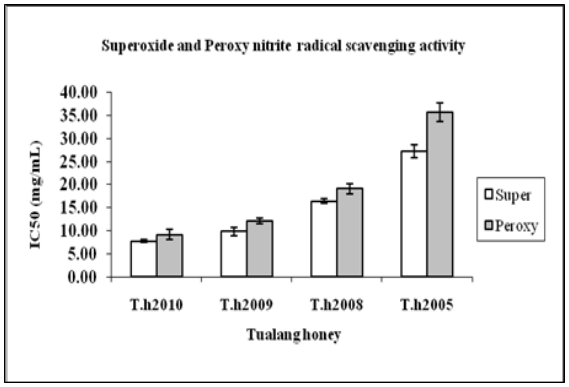

Figure 1: Linear relationship between the concentration of HMF and peak area

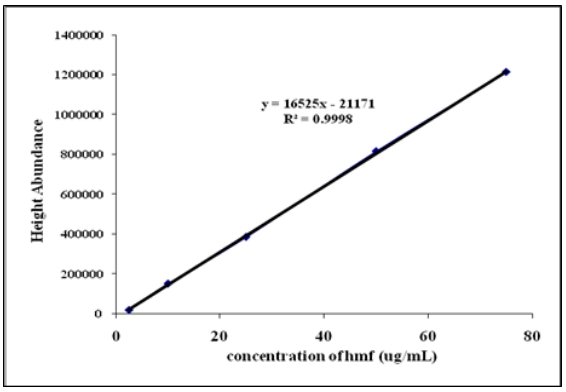

Figure 2: Superoxide anion radical and peroxy nitrite radical scavenging activity of tualang honey samples. Data are expressed as the means±SD; SD, standard deviation. IC_{50} represents the mg/mL concentration providing 50% inhibition of peroxy nitrite scavenging and superoxide anion radical formation or scavenging of the available free radicals. Ascorbic acid (IC_{50}: 2.3 mg/mL) was used as a reference antioxidant for comparison. Significant P values are presented ($p<0.05$).