An antioxidant- and hydroxymethylfurfural-based index for health impact grading of honey

Article

Accepted Version

An antioxidant- and hydroxymethylfurfural-based index for health impact grading of honey

Shu Khang Yap[1], Nyuk Ling Chin[1,*], Keshavan Niranjan[2], Nor Nadiah Abdul Karim Shah[1], and Leslie Thian Lung Than[3]

[1]*Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

[2]*Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading RG6 6DZ, United Kingdom.*

[3]*Department of Medical Microbiology & Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

**(Tel.: +603 9769 6353; Fax: +603 9769 4440; Email: chinnl@upm.edu.my)*

Abstract

Honey quality is conventionally controlled by checking its compliance with the regulatory standards mainly through physical properties. A new index of honey quality known at the Honey Health Impact Metric (HHIM) which considers two main parameters related to health was developed for use as a more market-relatable criteria in the honey industry. The HHIM incorporates the balancing of the positive health-beneficial 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with the negative health-detrimental hydroxymethylfurfural (HMF) concentration present in honey. It is defined as the average score between fractional DPPH and the normalised HMF. The HHIM was tested using 30 randomly selected honey samples from different origins which were then graded by the different categories of quality. As both parameters are also affected by honey storage period, the HHIM was also used to monitor changes in two specific honey samples, the *Kelulut Itama* and *Tualang Dorsata*. With quality of honey deteriorating over storage time following a zero-order kinetic model, the indications by HHIM gave a storage recommendations of 10 to 12 months for both these honey samples. This idea of HHIM as an alternative honey quality indicator to gauge health potential of honey will support the genuine and real products in the honey industry.

Keywords: Honey grading; hydroxymethylfurfural; DPPH radical scavenging activity; health impact

Statements and Declarations

Authors declare there is no conflicts of interest in this research.

Introduction

Honey Properties and Quality

Honey is a premier natural food product which quality profile varies significantly with geographical origins, botanical sources, bee types and processing/handling procedures. In order to assure the quality of a given honey, its composition must comply with the standards issued by international and regional regulatory agencies, *e.g.* Codex Stan 12-1981 by Codex Alimentarius Commission [1], Directive 2001/110/EC by the council of European Union [2], *Kelulut* (Stingless bee) honey specification MS2683:2017 by Department of Standards Malaysia [3] *etc*.

According to the most commonly referred Codex Stan 12-1981, an acceptable quality of honey should have moisture content below 20%, reducing sugar concentration greater than 60 g/100g, sucrose content of no more than 5 g/100g, water insoluble solid content no more than 0.1 g/100g, free acidity no more than 50 meq/kg honey, diastase activity greater than 8 schade unit, electrical conductivity no more than 0.8 ms/cm and hydroxymethylfurfural (HMF) less than 80 mg/kg honey [1]. These parameters govern the authenticity and safety of honey and also serve to grade honey in some countries. According to Thrasyvoulou et al. [4], Canada has designated 'Canada No. 1' grade for honey with moisture content less than 17.8%, 'Canada No. 2' grade for honey with moisture content less than 18.6% and 'Canada No. 3' for honey with less than 20% moisture content. Indian Standards have also stipulated "special grade" when moisture content of honey <20%, grade A when <22%, and "standard grade" when <25%. [4]. In the United States, however, honey is graded by a scoring system that includes soluble solid content, flavour, aroma and clarity of honey [5]. From these studies, it appears that most honey regulations and grading systems do not consider any parameter that impacts on health beyond basic nutrition.

Following the pro-health trend in food market, health impact factor is gaining interest in the eyes of honey consumers. Market survey studies from different countries have consistently shown that majority of the consumers purchase honey for its health benefits, with 84% of the consumers from Croatia [6], 96.5% from Nigeria [7], 71.4% from Malaysia [8] and 66% from Italy [9]. Despite being a strong marketing point, the lack of a proper guidelines and regulations on the health impact labelling of honey has obscured its application in food market.

Manuka honey from New Zealand is an exception and its quality is rated using the Unique Manuka Factor (UMF) [10] which is based on methylgloxal (MGO) concentration that reflects its antibacterial potential, dihydroxyacetone (DHA) concentration that is indicative of its shelf life, leptosperin content that confirms its authenticity and hydroxymethylfurfural (HMF) concentration that indicates its freshness [11]. Manuka honey with UMF rating between 5

and 10 is considered to be low grade honey which is safe for consumption; mid-grade honey with UMF between 10 and 15 is considered to possess significant therapeutic potential; and honey with UMF rating of 15+ is considered to be medical grade honey with superior antibacterial potential. The UMF rating delivers a simple yet impactful message to consumers on the quality and grade of Manuka honey [10]. However, this unique manuka factor has also obscured the health potential of other types of honey, perhaps inadvertently, since none of the others honey types can even be attributed with UMF for comparison purposes.

A plethora of studies has shown that every type of honey, regardless of provenance, possesses significant health-benefits [12–14]. For example, Malaysian *Tualang* honey has been shown to be comparable to Manuka in terms of its antibacterial performance against certain gram-negative bacteria [12]. Similarly, Zae et al. [13] have reported a better overall nutritional value in *Kelulut* honey in relation to *Gelam*, *Tualang* and Pineapple honey. Additionally, Malaysian Sourwood honey, Longan honey [14] and wild honey [13] were also previously reported to possess better antioxidant potential than Manuka honey. Thus, from functional points of views, some local less-recognised Malaysian honey may have comparable interest with the famous Manuka honey. A key purpose of this paper is to establish an effective functional parameter that will highlight the health impact of honey beyond basic nutrition. This will facilitate comparison between different honey samples and help develop fair grading scales.

DPPH Radical Scavenging Activity and HMF of Honey

Antioxidant activity is a well-recognised functional property all types of honey [15]. Many researchers have shown the existence of a pool of antioxidants in honey, which include phenolic compounds [16], amino acids [17] and enzymes [18]. These compounds act synergistically and impart honey the ability to quench free radicals. Thus, the antioxidant capacity of honey is often quantified by its radical scavenging power, examined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [19]. The DPPH test is widely applied for measuring the antioxidant levels in honey due to its DPPH radical stability, operational simplicity, good reproducibility and general applicability to all types of honey [16, 20]. DPPH reading of honey has also been shown to correlate with other antioxidant parameters, *i.e.* total phenolic content ($r = 0.789$), flavonoid content ($r = 0.607$), ferric ion reducing antioxidant power assay (FRAP) ($r = 0.671$) and ascorbic acid content ($r = 0.542$) [14]. DPPH test is also known to be more reliable and is not affected by certain side reactions, *e.g.* enzyme inhibition and metal ion chelation [20].

Although honey is well-known health-promoting food, its hydroxymethylfurfural (HMF) content can be potentially hazardous. HMF is a cyclic aldehyde compound naturally present at low concentration in high quality freshly harvested honey. It is formed by sugar degradation or non-enzymatic browning reactions and can be accelerated by adulteration or

inappropriate processing and storage of honey [21]. HMF is the most consistent indicator for honey freshness and has been used in honey quality control for decades [22]. Regulatory agencies stipulate legal limits for HMF and Codex standard states that its concentration in honey must not exceed 80 mg/kg honey [1]. The recently reported carcinogenic and mutagenic potential of high HMF intake further justifies the need to include HMF content in honey quality assessment [21], especially since honey labels tend to specify a very long "sell by" or "use by" dates of 3-4 years [23].

Based on a review of literature, the HMF content and DPPH radical scavenging activity are two significant parameters which can be used to indicate honey quality from its health perspective [16, 20, 21, 24]. By incorporating the health-beneficial DPPH parameter and health-detrimental HMF parameter of honey, this study proposes a Honey Health Impact Metric (HHIM) to assess honey quality and quantify its potential health benefit level. This development of HHIM has some comparable concepts to the Body Mass Index (BMI) where human health status is guided by the measure of obesity level calculated from the ratio of weight and height [25]. Similarly, the LDL/HDL cholesterol ratio calculated from low-density lipoproteins (LDL) and high-density lipoproteins (HDL) cholesterol levels was also widely used as a valuable indicator for estimating the risk of cardiovascular disease [26].

Materials and Method

A total of 30 randomly selected honey samples from different bee and floral sources were used in this study. The honey samples were from the stingless bee, *i.e. Heterotrigona itama* and *Geniotrigona thoracica* species as well as other honey bee varieties *i.e. Apis dorsata*, *Apis mellifera*, and *Apis cerana* species. After purchasing the honey directly from bee farms and local markets, samples were stored in a dark room at temperatures of 23-26^{o}C. The Manuka honey used in this work was a commercial product purchased from a healthcare product store.

Two common types of Malaysian honey, *i.e. Kelulut Itama* and *Tualang Dorsata* honey, were selected to investigate the changes in HHIM occurring during storage. These honey samples were collected directly from the farm in order to guarantee freshness at the point of purchase. These samples were also stored in a dark room at temperature of 23-26^{o}C for a period of 1 year with properties measured on a monthly basis.

Honey Property Analyses

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of honey was measured by slightly modifying method of Chong et al. [27]. Honey (0.5 g) was diluted with 10 ml of methanol. The honey solution was centrifuged at 5700g for 15 mins (Universal 320, Hettich, USA). The supernatant (2 ml) was mixed with an equal volume of 0.1 mM DPPH solution and incubated in dark for 30 mins. The absorbance of the mixture at 517 nm was measured against methanol with

a UV/Visible spectrophotometer (Ultrospec 3100 pro, Amersham Biosciences, USA). The DPPH radical scavenging activity of honey was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \left[1\text{-}\frac{A_s}{A_c}\right] \text{x } 100\% \quad (1)$$

where A_s and A_c are the absorbance of sample and control, respectively.

Hydroxymethylfurfural (HMF) content of honey was determined using White's spectrophotometric method [28]. Diluted honey solution was mixed with Carrez I, Carrez II, and ethanol. The absorbances of the solution at 284 nm and 336 nm were measured with UV/Visible spectrophotometer (Ultrospec 3100 pro, Amersham Biosciences, USA) and compared against a reference solution. The HMF content was calculated as follows:

$$\text{HMF (mg/kg honey)} = (A_{284} - A_{336}) \times 149.7 \times 5 \times \frac{D}{W} \quad (2)$$

where A_{284} and A_{336} are the absorbances at 284 nm and 336 nm, respectively. D is the dilution factor and W is the weight of honey sample [28].

Statistical Analysis

The statistical analysis of data was performed using Minitab statistical software (Version 18, Minitab Inc., USA). One-way ANOVA was performed. Tukey's test was used to examine for any significant differences among the mean values at confidence level of 0.05. Mean values were averaged from triplicate measurements.

Formulation and Development of Honey Health Impact Metric (HHIM)

In the formulation of HHIM, the DPPH and HMF readings were normalised to a dimensionless index following Eqns (3) and (4). Both normalised indices will have an upper limit of 1 and a higher fractional DPPH and normalised HMF values signify a better honey quality.

$$\text{Fractional DPPH} = \frac{\text{DPPH}}{100} \quad (3)$$

$$\text{Normalised HMF} = \frac{(60 - \text{HMF})}{60} \quad (4)$$

where 60 is calculated according to 25% deficit of the maximum permissible limit of HMF (80 mg/kg honey for tropical honey) on the account of lower permissible limit (40 mg/kg honey) for other honey [1].

The HHIM mathematical model is modified based on food quality metric equation (Eqn. 5) previously developed by Molnár [29]. The equation was previously developed to define the quality of food from its weighted sum of individual quality parameters.

$$\text{Food Quality} = \sum_{j=1}^{h} w_j \text{ x } z_j \quad (5)$$

where *j* and *h* are the number quality parameters, *z* is the normalised values of the quality parameter and *w* is the weighting factor.

The weighting factors for both DPPH and HMF parameters were assumed to be 0.5 which signify the equally weight of both parameters in the formulation of HHIM model. Thus, the dimensionless Honey Health Impact Metric (HHIM) is simplified and defined as the average score between fractional DPPH radical scavenging activity and normalised HMF (Eqn. 6).

$$\text{Honey Health Impact Metric (HHIM)} = \left[\frac{\left(\frac{\text{DPPH}}{100}\right)+\left(\frac{60-\text{HMF}}{60}\right)}{2}\right] \quad (6)$$

Based on Eqn. (6), HHIM has an upper limiting value of 1. A higher HHIM value indicates honey with better health impact. HHIM has no lower limit and can also assume negative values. A negative value of HHIM suggests HMF value is greater than 60 and at best approaches the limit of compliance with regulatory standards or at worst does not comply with regulatory standards when HMF> 80 mg/kg honey. It should be noted that negative values of HHIM does not imply that the honey has "gone off" or "expired". It only means that the health impact of the honey can either be minimal or potentially adverse. It is also important to note that both DPPH and HMF are functions of time. Therefore, HHIM is an indicator of the condition of the honey sample at any given time; it is not a property of the honey variety or brand *per se*.

Results and Discussion

Honey Quality Evaluation with Honey Health Impact Metric (HHIM)

Table 1 lists the Honey Health Impact Metric (HHIM) values calculated with Eqn. (6) for all 30 samples tested, alongside with their respective variation of fractional 2,2-diphenyl-1-picrylhy-drazyl (DPPH) radical scavenging activities and hydroxymethylfurfural (HMF) contents. The overall average fractional DPPH of all samples considered in Table 1 is 0.559. Remarkable variation of fractional DPPH between different types of honey were recorded with values ranging from 0.066 for *Kelulut Itama* honey (No. 5) to 0.900 for Manuka honey (No. 28). Similarly, a study by Khalil et al. [30] has reported an average fractional DPPH of 0.570 for 9 honey samples with values markedly ranged from 0.270 to 0.820.

The antioxidant capacity of honey is contributed by its bioactive compounds, *i.e.* phenolics, flavonoids, vitamins, peptides, enzymes, Maillard reaction products, organic acids and other minor components [18]. The composition of these compounds vary greatly with the source of the bees, geographical origins, floral sources, processing methods and storage conditions [19]. Thus, the significant variation of DPPH values between different honey samples is expected.

From Table 1, Manuka (No. 28) and *Tualang* honey (No. 14-19) seem to possess good antioxidant potential based on their consistently higher fractional DPPH values of 0.900 and 0.569-0.848 respectively. The *thorasica* honey samples (No. 11-13), meanwhile, have recorded significantly lower ($p<0.05$) below-average DPPH values of 0.150-0.368. In agreement with our results, the good antioxidant level of Manuka [31] and *Tualang* honey [32–34] were also previously reported. The *in-vivo* animal studies on health impact of Manuka honey showed that its good antioxidant level helps in decreasing oxidative DNA damages in liver [35], alleviating breast cancer [36], and reducing severity of gastric lesion [37]. Similarly, the antioxidant capacity of *Tualang* honey was also shown to exert protective effect on diabetic pancreas [38] and reduce the growth rate of breast tumour in *in-vivo* animal models [36]. Thus, the collective outcomes from these studies highlighted the significance of antioxidant activities of honey on its health impact since a higher antioxidant level of honey always confer to its better health impact. This supports that DPPH antioxidant parameter is appropriately selected as a parameter indicating health related quality despite no literatures have related this parameter in establishing honey standards of quality control in honey [1–3, 5].

Contrarily, the HMF has been used as a common parameter in honey quality control. Generally, honey is regarded as acceptable quality if its HMF concentration is within the defined limit, *i.e.* 80 mg/kg following the Codex Standards [1]. From Table 1, the HMF contents of the 30 honey samples ranged between 0.00 and 470.49 mg/kg honey. The results are consistent with earlier work which report values ranging from 0.00 to 136.00 mg/kg honey for 481 honey samples [39], 0.36 to 74.90 mg/kg honey for Australian honey [40] and 2.80 to 1131.76 mg/kg honey for Malaysian honey [41]. HMF is normally found only in trace amount in fresh honey and will increase with storage time. Table 1 shows that honey stored for over a year shows exceptionally high HMF content, well in excess of the defined limit of 80 mg/kg honey. It is also evident from Table 1 that *Tualang* honey (No. 16) and multifloral honey (No. 21) possess relatively high HMF concentrations of 52.89 and 21.94 mg/kg honey respectively despite being in storage for only one month. This finding shows lack of awareness in checking and controlling HMF concentration in the present honey market. HMF concentration is a critical quality parameter of honey because consumption of honey with high HMF values are known to cause irritation of mucous membranes and in serious cases, induce DNA damage, cause gene mutation and even facilitate the growth of tumour cells [42]. HMF concentration has been connected to negative health effects.

Fig. 1 shows the relationship between HMF and fractional DPPH radical scavenging activity values of honey where a lower HMF concentration and higher fractional DPPH value result in a higher Honey Health Impact Metric (HHIM). The higher HHIM signify the better health impact and quality of honey. The positive HHIM values are arbitrarily divided into four grades: Honey samples with HHIM values between 0.75-1.00 are termed "excellent"; those with values between 0.50-0.75 are termed "good"; those in the range 0.25-0.50 are termed "moderate"; whereas those with values below 0.25 are termed "low" grade.

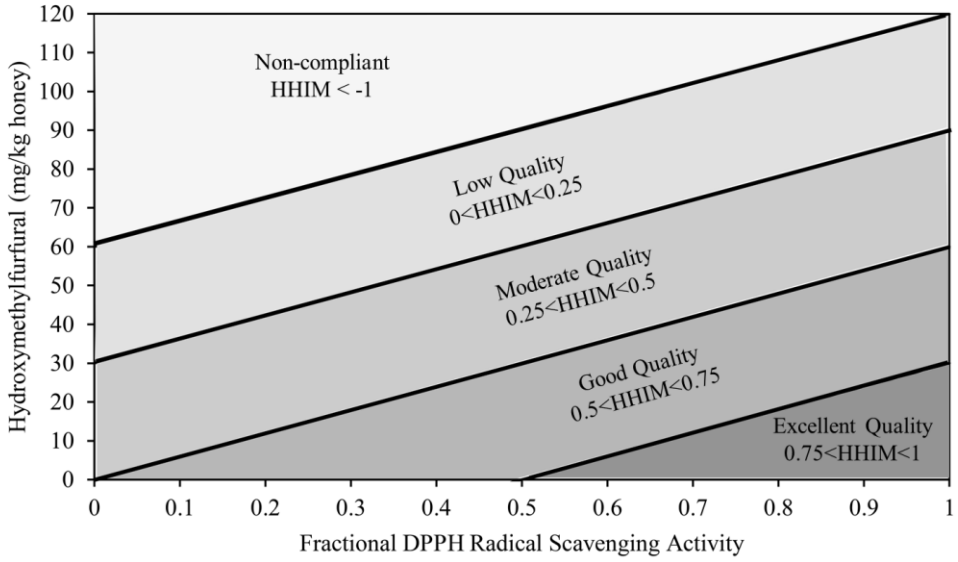

Fig.1 Proposed Honey Health Impact Metric (HHIM) for honey grading. The grading scale is based on the quartiles of positive HHIM values from 0 to 1

The values of HHIM ranged between -3.04 and 0.92 for the 30 honey samples (Table 1). *Tualang* honey samples (No. 14 and 15 as listed Table 1) seem to fall into the 'excellent' grade category due to their high HHIM scores of 0.92 and 0.76, resulting from their relatively lower HMF and higher fractional DPPH values. Although honey samples No. 5 and 13 possess low fractional DPPH values of 0.066 and 0.150, respectively, they are graded as 'good' on the basis of their borderline HHIM values of 0.53 and 0.58 which resulted from very low values of HMF. It is also possible that some honey samples may have a high value of fractional DPPH but may also contain a high concentration of HMF. The Manuka honey sample No. 28, for example despite having a very high fractional DPPH value of 0.900, has a moderate HHIM of 0.42 due to its relatively high HMF concentration of 63.29 mg/kg honey.

From the list of 30 honey samples covered in this work, *Tualang* honey sample tends to indicate a better health quality and grade. However, it must be emphasised that HHIM applies to the state of the honey sample under consideration and not to the type of honey *per se*. For instance, *Tualang* honey No. 16 with high HMF value (52.89 mg/kg honey) has scored

a significantly lower HHIM value of 0.40 than other 1-month old honey (No. 2, 3, 4, 12, 13, 20, 21 and 24) with HHIM ranging from 0.58-0.71. Similarly, fresh *Kelulut* honey No. 5 with low fractional DPPH value also scored a significantly lower HHIM of 0.53 than other zero-month fresh honey. This HHIM grading system is considered robust for evaluating honey quality from the perspective of its health impact by weighing the effects of the two important health related parameters in honey.

The metric proposed here is not intended to replace current tests used for specifying honey standards and authenticity, *e.g.* reducing sugar concentration, free acidity and diastase activity, but to suggest a health based parameter for honey quality evaluation. With the implementation of this new metric label, consumers can compare the health impact of different types of honey and thus a fairer trading in the honey market can be achieved.

Quality Changes of Honey during Storage

From Table 1, it was observed that fractional DPPH and HMF values in honey changed with storage time and caused reduction of HHIM value. This is especially true for honey stored for more than 12 months as HHIM values are negative. This is evident in honey samples No. 8, 9, 10, 17, 18, 19, 23, 25, 26, 27, 29 and 30 where HMF value which exceeds 80 mg/kg. Other researchers have reported unacceptably high HMF concentration in honey samples stored for 12, 24, and 48 months at 128.19, 1131.76 [41] and 1426.00 mg/kg honey [43] respectively. Thus HHIM scores of honey is also demonstrating the effects of honey deteriorate with storage time. The rate and trend of this deterioration were further detailed for better understanding of changes in honey quality over its shelf life period.

Fig. 2(a) shows that fractional DPPH value of *Kelulut Itama* honey slightly increases from 0.497 to 0.516 while *Tualang* honey decreases from 0.852 to 0.769 after 1 year of storage. The DPPH values fluctuated during storage and did not show a specific trend of change. Similarly, previous studies on honey storage have reported that antioxidant activies of Clover, Buckwheat [44], Acacia and multifloral honey [45] decreased while *Apis Mellifera* honey increased [46]. The changes of honey's antioxidant level is due to the degradation of existing antioxidants and formation of new bioactive compounds [46]. Studies have reported the reduction of phenolics and flavonoids [45], formation of Mailard reaction products [47], emergence of new compounds from hydrolysis of polysaccharides and amino acids [48] and accumulation of HMF [44, 49] occurred during storage of honey. The synergy effects of these complex reactions explain the fluctuations of antioxidant level of honey during storage.

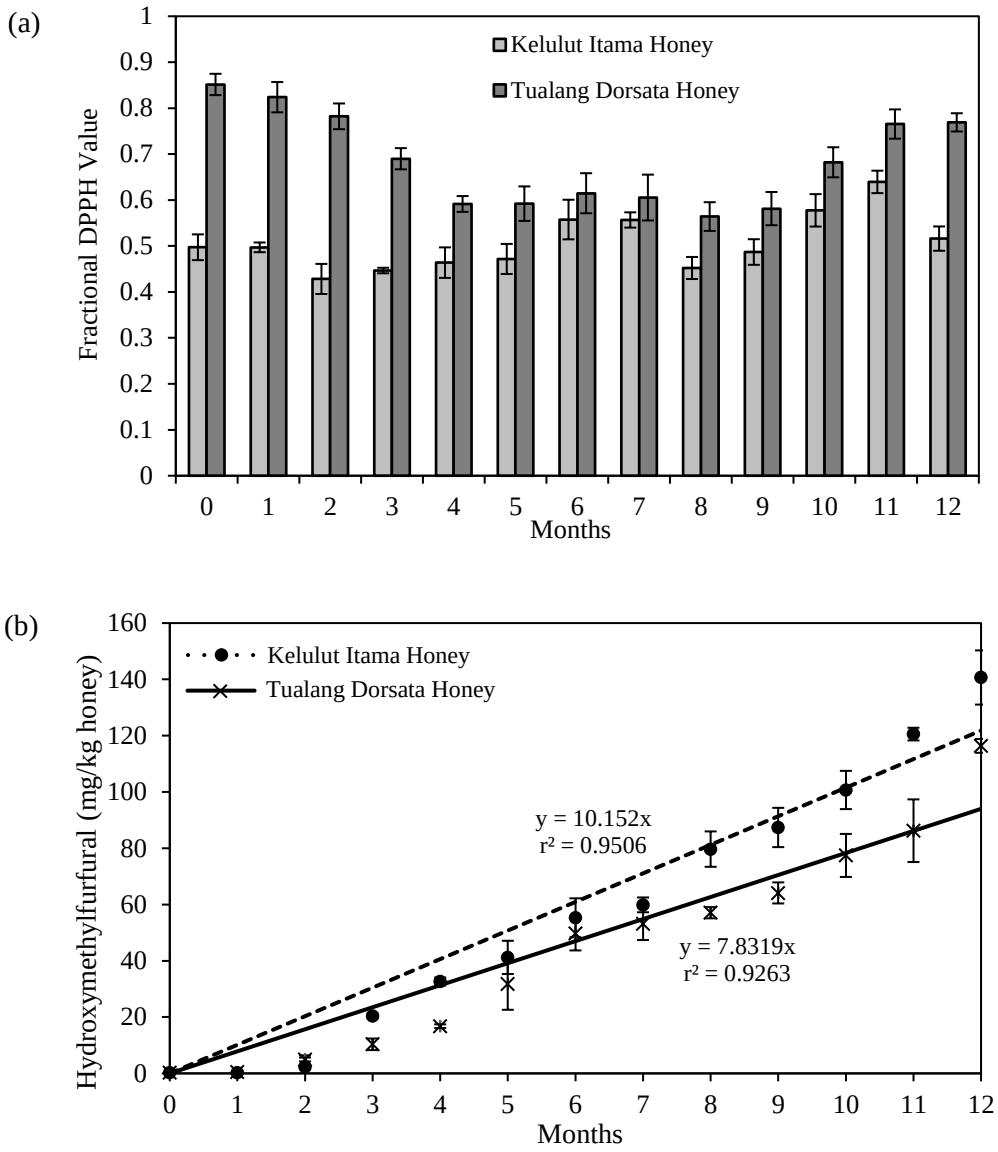

Fig.2 Changes of (a) fractional DPPH and (b) hydroxymethylfurfural (HMF) concentration in honey during storage

While DPPH results indicated inconsistent trends, the accumulation of HMF and its increase in honeys is almost certain. As supported by the *Kelulut* and *Tualang* honey samples, they followed a significant zero-order kinetic increasing trend (Fig. 2(b)). The HMF of honey increased steadily during storage and reached a high concentration of 140.67 and 116.37 mg/kg honey respectively after 1 year of storage. Study by Mouhoubi-Tafinine et al. [50] have also reported high HMF concentration of 100.84 to 353.09 mg/kg honey in 5 honey samples after storing for 9 months. Based on Fig. 2(b), the HMF concentration of *Kelulut* honey increased at a higher rate than *Tualang* honey based on its higher rate constant of 10.152 mg kg^{-1} $month^{-1}$. HMF is formed in honey during storage through the acid-catalysed hexose dehydration process [51]. The more acidic nature and higher moisture characteristic of *Kelulut Itama* honey [33] favoured the production of HMF [51], thereby increasing the rate of HMF formation in *Kelulut* honey.

The synergistic effects of DPPH changes and HMF accumulation have resulted reduction of health impact of both honey samples with storage as measured by the HHIM (Fig. 3) indicating deterioration during storage. Both honeys deteriorated from their initial excellent grade to a non-compliant grade after one year of storage. The HHIM trend in Fig. 3 was well-fitted to the zero-order kinetics model which gave rate constants of 0.0941 for the *Kelulut Itama* and slightlier lower value of 0.0811 for the *Tualang Dorsata* at high r^2 of 0.9747 and 0.9739 respectively. This indicated that the rate of quality deterioration of *Kelulut* honey was slightly higher than *Tualang* honey. In general, honey acidity [52], moisture content [53], botanical sources and fructose/glucose ratio [54] can affect the rates of honey quality deterioration, thereby causing faster deterioration of its quality.

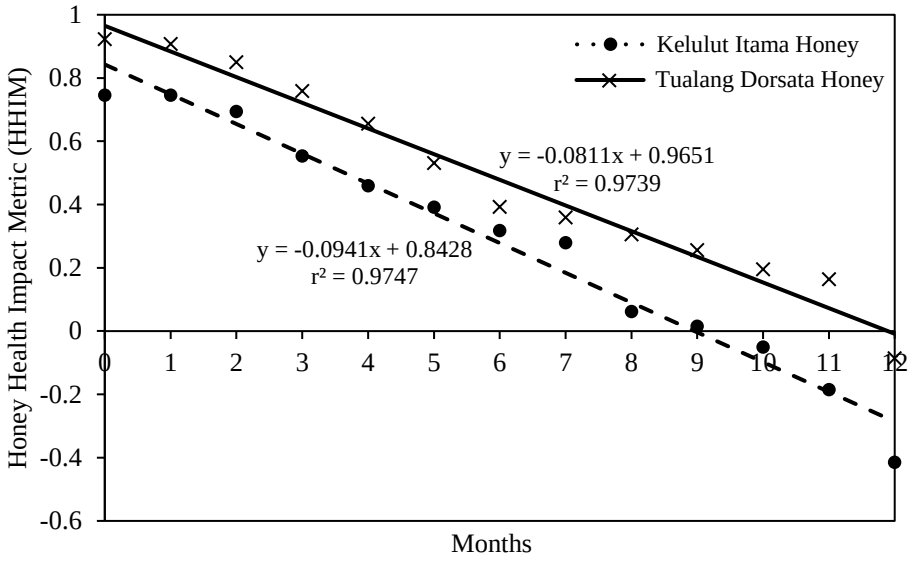

Fig.3 Changes of Honey Health Impact Metric (HHIM) of *Kelulut Itama* and *Tualang Dorsata* honey

The HHIM values of *Kelulut Itama* honey reached a negative value after ten months of storage, which was two months earlier than *Tualang Dorsata* honey. This provided information that storage period is good for about 10 months for the *Kelulut Itama* and 12 months for the *Tualang Dorsata* honey following conditions of storage of dark room between temperatures of 23 to 26ºC. Khalil et al. [41] did recommend honey shelf life of 1 year for all types of honey studied based on the changes of HMF content during storage. Study by Fallico et al. [23] suggested a longer shelf life of 15-20.3 months for 4 different honey samples based on their diastase activity and HMF concentration. The suggested shelf life is far shorter than 36 months which is normally declared on honey labels [23]. However, according to Fallico et al. [23], the 36 months shelf life of honey was set solely based on common practice and customer requirements and it does not consider the product characteristics, especially its health impact characteristics. The HHIM is giving consistent indication that

honey stored for 36 months will give potentially adverse health effects as supported by other studies reporting the same [21, 55].

Conclusions

The normalised HMF and fractional DPPH radical scavenging activity have been combined to define a Honey Health Impact Metric (HHIM) which can be experimentally determined for any given honey sample to yield an antioxidant- and hydroxymethylfurfural-based index for indication of health impact in honey. The HHIM of any sample with acceptable quality varies between 0 and 1. Its values tend to decrease with storage time which follow a zero-order kinetic and the rates of decrease depend on storage conditions and the chemical composition of the sample. A shelf life of about a year is recommended for both the studied honeys, *Kelulut Itama* and *Tualang Dorsata* based on the deterioration rates of HHIM.

Declarations

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

Funding

This work was supported by Universiti Putra Malaysia (UPM) [Grant Number UPM.RMC.800-3/3/1/GPB/2021/9695600].

References

1. Codex Alimentarius Commission, Codex Stand. **12**, 1 (2001).
2. European Union, Off. J. Eur. Communities **10**, 47 (2002).
3. Department of Standards Malaysia, *Kelulut (Stingless Bee) Honey- Specification* (2017).
4. A. Thrasyvoulou, C. Tananaki, G. Goras, E. Karazafiris, M. Dimou, V. Liolios, D. Kanelis, and S. Gounari, J. Apic. Res. **57**, 88 (2018).
5. United States Department of Agriculture, Fed. Regist. 1 (1985).
6. M. Glasnović, K. Marković, S. Drakula, and N. Vahčić, Hrana u Zdr. i Boles. Znan. Časopis Za Nutr. i Dijetetiku **10**, 1 (2021).
7. O. G. E. Arowosoge, Int. J. Dev. Sustain. **7**, 1376 (2018).
8. M. Shahezwan, A. Wahab, N. Othman, N. Hazwani, I. Othman, A. A. Jamari, and A. A. Ali, J. Appl. Pharm. Sci. **7**, 144 (2017).
9. R. Zanchini, S. Blanc, L. Pippinato, G. Di Vita, and F. Brun, Br. Food J. **124**, 4372 (2022).
10. A. Girma, W. Seo, and R. C. SheI, PLoS One **14**, e0224495 (2019).
11. Unique Manuka Factor Honey Association, (n.d.).
12. S. Ahmed and N. H. Othman, Malaysian J. Med. Sci. **20**, 6 (2013).
13. T. K. Zae, A. Azlan, A. Abu Bakar Sajak, E. K. Hock, L. C. Nyuk, M. F. Md Noh, and N. Mustafa Khalid, Food Res. **4**, 205 (2020).
14. M. Moniruzzaman, S. A. Sulaiman, M. I. Khalil, and S. H. Gan, Chem. Cent. J. **7**, (2013).
15. O. O. Erejuwa, S. A. Sulaiman, and M. S. Ab Wahab, Molecules **17**, 4400 (2012).
16. J. Alvarez-Suarez, S. Tulipani, S. Romandini, A. Vidal, and M. Battino, Curr. Anal. Chem. **5**, 293 (2009).
17. A. R. Pérez, M. T. Iglesias, E. Pueyo, M. González, and C. De Lorenzo, J. Agric. Food Chem. **55**, 360 (2007).
18. N. Gheldof, X.-H. Wang, and N. J. Engeseth, J. Agric. Food Chem. **50**, 5870 (2002).

19. M. Dzugan, M. Tomczyk, P. Sowa, and D. Grabek-Lejko, Molecules **23**, (2018).
20. M. Lewoyehu, M. Amare, and F. Yildiz, Cogent Food Agric. **5**, 1685059 (2019).
21. U. M. Shapla, M. Solayman, N. Alam, M. I. Khalil, and S. H. Gan, Chem. Cent. J. **12**, 35 (2018).
22. I. N. Pasias, I. K. Kiriakou, and C. Proestos, Food Chem. **229**, 425 (2017).
23. B. Fallico, E. Arena, and M. Zappala, J. Food Qual. **32**, 352 (2009).
24. A. T. Thrasyvoulou, J. Apic. Res. **25**, 186 (1986).
25. R. C. Weisell, Asia Pac. J. Clin. Nutr. **11**, S681 (2002).
26. M. L. Fernandez and D. Webb, J. Am. Coll. Nutr. **27**, 1 (2008).
27. K. Y. Chong, N. L. Chin, and Y. A. Yusof, Food Sci. Technol. Int. **23**, 608 (2017).
28. J. W. White, J. Assoc. Off. Anal. Chem. **62**, 509 (1979).
29. P. J. Molnár, Food Qual. Prefer. **6**, 185 (1995).
30. M. I. Khalil, M. Mahaneem, S. M. S. Jamalullail, N. Alam, and S. A. Sulaiman, J. ApiProduct ApiMedical Sci. **3**, 4 (2011).
31. M. Hunter, R. Ghildyal, N. M. D'Cunha, C. Gouws, E. N. Georgousopoulou, and N. Naumovski, Curr. Res. Food Sci. **4**, 532 (2021).
32. R. K. Kishore, A. S. Halim, M. S. N. Syazana, and K. N. S. Sirajudeen, Nutr. Res. **31**, 322 (2011).
33. S. K. Yap, N. L. Chin, N. N. A. K. Shah, L. T. L. Than, and K. Niranjan, J. Sci. Food Agric. (2022).
34. Y. Ranneh, F. Ali, M. Zarei, A. M. Akim, H. A. Hamid, and H. Khazaai, LWT - Food Sci. Technol. **89**, 1 (2018).
35. Z. Jubri, N. B. Abdul Rahim, and G. J. Aan, Clinics **68**, 1446 (2013).
36. S. Ahmed, S. A. Sulaiman, and N. H. Othman, Evidence-Based Complement. Altern. Med. **2017**, Article 5904361 (2017).
37. S. B. Almasaudi, N. A. El-Shitany, A. T. Abbas, U. A. Abdel-Dayem, S. S. Ali, S. K. Al Jaouni, and S. Harakeh, Oxid. Med. Cell. Longev. **2016**, (2016).
38. O. O. Erejuwa, S. A. Sulaiman, M. S. Wahab, K. N. S. Sirajudeen, M. S. M. D. Salleh, and S. Gurtu, Ann. Endocrinol. (Paris). **71**, 291 (2010).
39. J. White, Bee World **75**, 104 (1994).
40. S. Ajlouni and P. Sujirapinyokul, Food Chem. **119**, 1000 (2010).
41. M. I. Khalil, S. A. Sulaiman, and S. H. Gan, Food Chem. Toxicol. **48**, 2388 (2010).
42. A. Choudhary, V. Kumar, S. Kumar, I. Majid, P. Aggarwal, and S. Suri, Toxin Rev. 1 (2020).
43. S. N. Sabireen, S. A. Sulaiman, R. M. Salleh, and W. E. M. Fuad, Asian J. Med. Biomed. **4**, 10 (2020).
44. X. H. Wang, N. Gheldof, and N. J. Engeseth, J. Food Sci. **69**, fct96 (2004).
45. G. Šarić, K. Marković, N. Major, M. Krpan, N. Uršulin-Trstenjak, M. Hruškar, and N. Vahčić, Food Technol. Biotechnol. **50**, 434 (2012).
46. P. Missio da Silva, L. V. Gonzaga, F. C. Biluca, M. Schulz, L. Vitali, G. A. Micke, A. C. Oliveira Costa, and R. Fett, LWT **129**, 109521 (2020).
47. L. C. A. Ázquez, M. C. D. Í. A. Z. Aroto, M. A. G. O. Iñas, E. D. E. L. A. F. Uente, M. S. P. É. Oello, L. Castro-Vázquez, M. C. Díaz-Maroto, M. A. González-Viñas, E. De La Fuente, and M. S. Pérez-Coello, J. Agric. Food Chem. **56**, 1999 (2008).
48. F. Braghini, F. C. Biluca, L. V. Gonzaga, A. S. Kracik, C. R. W. Vieira, L. Vitali, G. A. Micke, A. C. O. Costa, and R. Fett, J. Food Process. Preserv. **43**, e13954 (2019).
49. M. Zarei, A. Fazlara, and N. Alijani, Funct. Foods Heal. Dis. **9**, 593 (2019).
50. Z. Mouhoubi-Tafinine, S. Ouchemoukh, M. Bachir Bey, H. Louaileche, and A. Tamendjari, Int. Food Res. J. **25**, 1044 (2018).
51. W. Yang, C. Zhang, C. Li, Z. Y. Huang, and X. Miao, J. Food Sci. Technol. **56**, 2417 (2019).
52. B. Fallico, M. Zappalà, E. Arena, and A. Verzera, Food Chem. **85**, 305 (2004).
53. L. Bulut and M. Kilic, J. Food Process. Preserv. **33**, 22 (2009).
54. A. Kesić, A. Crnkić, Z. Hodžić, N. Ibrišimović, and A. Šestan, J. Sci. Res. Reports **3**, 1057 (2014).
55. M. N. Islam, M. I. Khalil, M. A. Islam, and S. H. Gan, J. Appl. Toxicol. **34**, 733 (2014).