Journal of Science and Technology, Vol. 10 No. 2 (2018) p. 71-76

Physicochemical and Microbiological Quality of Selected Commercial and Traditional Honey in Klang Valley Market, Malaysia

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Received 30 September 2017; accepted 3 April 2018; available online 1 August 2018

DOI: https://10.30880/jst.2018.10.02.012

Abstract: This study was intended to determine the physicochemical and microbiological quality of commercial and traditional honey in Klang Valley. Eleven honey samples from different origins were obtained and examined. Commercial honey samples were labelled as A, B, C, D, E and F and traditional honey samples consisted of Yemeni Sidr honey, Red Tualang honey, Black Tualang honey, Acacia honey and Fraser Hills Tualang honey. Physicochemical quality such as sugar content moisture content, water activity, pH and colour were measured. The pH value for commercial honey reached from pH 3.48 to 3.97 while the pH value for traditional honey reached from 3.07 to 4.72. The moisture content of commercial honey ranged from 17.53% to 18.93% compared to moisture content for traditional honey ranged from 18.03% to 20.67%. The water activity for commercial and traditional honey was in the range 0.56 to 0.62 a_w and 0.52 to 0.62a_w, respectively. Total sugar content obtained for commercial honey varied from 79.27 to 81.73 g/mL while total sugar content obtained for traditional honey were slightly higher, from 80 to 83.77 g/mL. Colour revealed that commercial honey, D, has the darkest colour compared to other honey (*L=2.11±0.08, *a=-0.02±0.21, *b=1.63±0.15). Standard plate count and yeast and mould were carried out to determined microbiological quality of honey. Generally, honey samples A, B, C, D and Acacia honey were considered safe, as no growth was detected on standard plate count. Less than 10 CFU/g was detected in Honey E, Black Tualang honey and Fraser Hills Tualang honey. There was no growth of yeast and mould count except for Red Tualang honey with not more than 10 CFU/g. Results in this study are within the limits of standards and are comparable with previous reports on honey from various countries.

Keywords: Honey; physicochemical properties; microbiological quality.

1. Introduction

Honey is originated by bees (*Apis mellifera*) from the nectar of flowers. It has a very sweet taste and viscous syrup texture. Honey is teeming with excellent nutritional values and health benefit consequences [1]. This could be due to the presence of about 200 substances considered the essential part of traditional medicine. The chemical composition of honey is complex, consists of sugars, vitamins, minerals, proteins, flavonoids, enzymes phenolic acids, 5-hydroxymethylfurfural (HMF) and volatile compounds [2]. These compositions can be influenced by different factors such as floral types, geographical areaand entomological source [3]. Honey provides numerous phenolic compounds, which are excellent sources of antioxidant. Therefore, it has the capability of posing antiseptic and antibacterial properties. These properties could inhibit the growth and infections of certain bacteria. As a traditional medicine, honey has been used for several purposes such as upset stomach, coughs, and sore throats. Rao, Krishnan, Salleh, and Gan (2016) have reported honey could treat gastrointestinal disorders [4]. In addition, honey is hygroscopic where it can drain out the moisture and dehydrate the bacteria. The low-level of pH and high sugar content also can impede the microbe's growth [5]. Furthermore, low moisture content could inhibits the formation of HMF from sucrose [3].

The quality and safety of honey is influence by the presence of microorganisms. Bacteria, molds, and yeast, e.g., *Pseudomonas,*

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e-ISSN: 2600-7924/penerbit.uthm.edu.my/ojs/index.php/jst

Psychrobacter, *Acinetobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Micrococcus* spp, *Brochothrix*, and *Citrobacter* are found in honey and honeycombs. The microorganisms in honey can be from bees, nectars and external sources, such as environmental conditions, handling, and storage [6]. Honey quality can be analyzed by different characteristics; the physical, chemical, microbiological and sensorial. The quality criteria of honey are referred in the regulatory standards (Codex Alimentarius Standard). Even though the quality of honey is already specified, the report of honey quality around central of Malaysia is still limited. Therefore, this study aims to analyze the physicochemical and microbiological quality of commercial and traditional honey available in Klang Valley market.

2. Material and Method

2.1 Honey

Eleven honey used in this research were purchased from Klang Valley Market, Malaysia. They were six commercial honey, labeled as A, B, C, D, E, and F, and five traditional honey, namely as Yemeni Sidr honey, Red Tualang honey, Black Tualang honey, Acacia honey, and Fraser Hills Tualang honey.

2.2 Physicochemical analysis

2.2.1 Determination of pH

Ten percent of the aqueous honey solution is used to test the pH by using a digital pH meter at 28±2°C. The digital pH meter was calibrated first at 4.0 and 7.0 with standard buffer solutions [7].

2.2.2 Determination of moisture content

The refractometric method was used to analyze the moisture content by using Atago handheld refractometer at ambient temperature [8].

2.2.3 Determination of water activity

Water activity assay was conducted by Aqua lab water activity meter. Each sample was analyzed in three-panel determinations [9].

2.2.4 Determination of honey colour

The colour assessment of honey was carried out using a Hunter Lab, model D25 L optical sensor (Hunter Associates Reston, VA, USA). A 10 g of diluted honey was placed into a cylindrical optical cell. 45mm viewing aperture was using to measure the reflectance values [10].

2.2.5 Determination of total sugar content

Twenty-five percent (w/v) of honey solution was suspended in distilled water. A refractor metric method was used to determine the total sugar content for each honey sample. Ambient temperature required in measuring the refractive indices of honey samples by using an Atago handheld refractometer. Meanwhile, percentage of sucrose content was calculated per g/mL honey [11].

2.3 Microbiological analysis

2.3.1 Standard plate count

Ten grams of honey was suspended in 90 ml of 0.1% phosphate buffer solution. A series of dilutions were then carried out and 0.1 ml was spreaded on Plate Count Agar (PCA) (OXOID). The culture were incubated for 72h at 37°C.

2.3.2 Yeast and mold count

Ten grams of honey was suspended in 90 ml of 0.1% phosphate buffer solution. A series of dilutions were then carried out and 0.1 ml was spreaded on Potato Dextrose Agar (PDA) (OXOID). The culture were incubated for 5 days at 25°C.

3. Result and Discussion

3.1 Determination of pH

Acacia honey resulted as the most acidic honey with pH 3.07 ± 0 ($p<0.05$) among all the honey types, (Table 1). Normally, high-level acid of honey can induce the fermentation process of sugars into organic acids. However, Yemeni Sidr honey was slightly acidic (pH 4.72 ±0.01). This study discovered that all the investigated honey samples were not exceeding the allowed limit of pH, which then considered as an index of freshness. pH is an applicable indicator in determining any feasible microbial contamination [12]. It could be a significant factor in the preservation of honey because it can control the microbial spoilage and the shelf life of product [13]. Instinctively, most bacteria and moulds can grow in mildly alkaline and neutral conditions, respectively [14]. Meanwhile, yeasts required an acidic condition (pH range of 4.0 to 4.5). The pH

values of the honey samples were acidic (pH 3.07 to 4.72), which obey the recommended limits (pH 3.4 -6.1) for fresh honey [15]. The pH values of Turkish, Spanish and Algerian honey were discovered to be in the range of 3.67 to 4.57, 3.63 to 5.01 and 3.49 to 4.53, respectively [16]. Even though honey is considered to be acidic, the high sugar content masks the acidity in the honey taste. Naturally, honeyis dominated by formic acid and citric acid. However, it was recently discovered that gluconic acid is the predominant acid compound produced from bee secretions under the action of oxidase enzyme on glucose [17]. The high-level acid of honey is required due to its benefit in promoting the wound healing through the release of oxygen from hemoglobin [18].

3.2 Determination of moisture content

The analysis of moisture content is used to measure the water presence in honey, as it is a dominant parameter to determine honey quality. The moisture content must be within the limit (≤20%) based on the international regulations. Various factors contributed to the water content in honey such as the degree of maturity reached in the hive, harvesting season and climatic factors [19].The moisture content of honey F and Yemeni Sidr honey samples shows a significant different, ($p< 0.05$). Honey F shows the lowest moisture content by 17.53 %±0.31 and Yemeni Sidr honey shows the highest moisture content by 20.67 %±1.15 (Table 1). The moisture content of honey from different origins shows varied differences, ranged from 13% to 29% [16]. The low moisture content in honey samples was indicating longer shelf life during storage, which facilitates a good storage ability and quality. It can be an important factor to resist fermentation and granulation during storage [20].

3.3 Determination of water activity (a_w)

The water activity (a_w) in honey can be a significant factor in determining the survival or growth of microorganisms. Normally, water activity levels of honey fall below 0.60.This study resulted, a_w levels of the honey samples fall between 0.52 and 0.62 (Table 1). However, commercial honey labeled as C, F, and Fraser Hills Tualang honey had a_w levels exceeding 0.60, which may allocate a suitable medium for yeast growth. The water activity is a vital factor that controls the stability of food by impeding and restraining microbial growth. The shelf life of honey and growth of undesirable microflora, especially osmotolerant yeast can be influenced by the increasing of water activity. This is because the osmotolerant yeasts can grow at a low-level water activity of 0.60 [16]. Although osmolality plays a significant role in the antimicrobial activity of honey, yet, there are other factors in honey that also have a tremendous role in the antimicrobial effect of honey. Water condition in honey could be depended on factors such as source of nectar, the location of the flowers, the storage time, and preservation method [21]. The quality of honey is usually influenced by the water activity owing to its stability, viscosity, and crystallization [16]. For instance, in the crystallization process, water is set free by the formation of glucose monohydrate. Thus, it can lower the liquid concentration and increases the water activity.

3.4 Determination of total sugar content

The total sugar content is the sum of all monosaccharides, disaccharides and oligosaccharides. The total sugar content of the honey samples was ranging between 79.27 and 83.77 g/ml, which are slightly above the maximum limit (≥60%) based on the European honey directive (Table 1). Yemeni Sidr honey was determined as the highest total sugar content (83.77±0.05). Meanwhile, the reported sugar content of Algerian honey was in a range of 62.80 to 70.00 g/ml [22]. High sugar content in honey samples may aid to low moisture content and high acidic nature, which hinder the emergence of HMF, mainly in glucose and fructose. Additionally, honey is hygroscopic, which can drain out the moisture and dehydrate the bacteria. The high sugar content can prevent and inhibit the growth of microbes, thus impede fermentation [2].

3.5 Determination of colour

In food product, colour is an essential attribute since it is perceived immediately by the consumers. The colour of the untreated honey depends on its botanical origins. Therefore, colouris crucial in the categorization of monofloral honey for commercial activities [20]. The maximum lightness among all honey samples isexhibited by Yemeni Sidr honey (L= 13.02±0.57) while

the minimum value is Honey D (L=2.11±0.08) (Table 2). Redness was found to be highest for Honey F (+a=7.59±0.15) and Honey B represented by a lowest value (-a = -1.59±0.11). Meanwhile, both Honey E and Yemeni Sidr were having the range of yellowness (+b=12.16±0.38) and (12.11±0.79) and the minimum value was in Honey D (+b= 1.63±0.15). Colour data revealed that Honey D has the darkest colour compared to other honey (*L=2.11±0.08, *a=-0.02±0.21, *b=1.63±0.15). Fahim et al.(2014) reported that the darker colour of honey resembles the higher levels of polyphenols content [23]. It has been known that honey darkens with age. Furthermore, the various conservation methods and beekeeper's interference such as exposure to high temperatures or light and contact with metals might cause colour changes.

3.6 Microbiological quality of honey

The results of microbiological examination of the honey samples were shown in Table 3. Honey A, B, C and D demonstrated the absence of bacteria and mold growth due to the antimicrobial properties it possesses. The high osmotic pressure of honey due to its concentrated sugar solution contributes to the unsuitable condition for microbial growth [20]. Meanwhile, Honey E, Black Tualang honey, and Fraser Hills Tualang honey displayed microbial growth less than 10 CFU /g. A study by Iurlina and Fritz (2005) reported the higher levels of microbial content in commercial honey samples for aerobic mesophiles counts were (average 244 CFU/g) while mould and yeasts counts(average 34 CFU/g) [24]. The presences of moulds in this study might due to unhygienic practices during harvesting, packaging, andstore of the honey samples. Molds are known as xerophiles since they thrive in samples with low water contents between 16.2 to 17.0%.

4.0 Conclusion

The physicochemical and microbiological quality of selected commercial honey (A – F) and traditional honey available in the random area of Klang Valley market was evaluated. In overall, the traditional honey have a higher range of pH value, moisture content, and total sugar content. The water activity of both commercial and traditional honey was in the same range. The microbiological quality of commercial and traditional honey present in this study had shown no growth of bacteria by standard plate count of honey A, B, C, D, and Acacia. Meanwhile, less than 10 CFU/g of bacterial growth were observed in E, Black Tualang, and Fraser Hills Tualang honey.

Table 1 pH value, moisture content, water activity and total sugar content of selected honey

Honey	pH	Moisture content	Water activity	Total sugar content
Honey A	3.72±0.01	18.93±0.12	0.56±0	81.73±0.06
Honey B	3.48±0.01	18.80±0.00	0.59±0	80.47±0.25
Honey C	3.75±0.01	18.40±0.20	0.61±0	80.17±0.06
Honey D	3.48±0.01	18.47±0.20	0.60±0	80.07±0.25
Honey E	3.97±0.02	18.60±0.26	0.57±0.01	80.64±0.12
Honey F	3.61±0.02	17.53±0.31	0.62±0.04	79.27±0.06
Yemeni Sidr Honey	4.72±0.01	20.67±1.15	0.54±0.03	83.77±0.06
Red Tualang Honey	3.52±0.01	19.47±0.31	0.55±0	82.20±0.1
Black Tualang Honey	3.56±0.01	19.50±0.00	0.52±0.01	83.73±0.06
Acacia Honey	3.07±0	18.80±0.00	0.57±0.01	80.47±0.31
Fraser Hills Tualang Honey	3.43±0.02	18.03±0.06	0.62±0.01	80±0.1

Table 2 Colour value of selected honey

Honey	**L***	**a***	**b***
Honey A	9.67±1.20 c	3.46±0.35b	10.26±0.43b
Honey B	9.46±0.35c	-1.59±0.11f	7.98±0.28c
Honey C	3.41±0.15ef	0.97±0.07d	2.26±0.24ef
Honey D	2.11±0.08f	-0.02±0.21e	1.63±0.15f
Honey E	11.22±0.33b	0.30±0.14e	12.16±0.38a
Honey F	10.61±0.52bc	7.59±0.15a	9.68±0.36b
Yemeni Sidr Honey	13.02±0.57a	3.58±0.15b	12.11±0.79a
Red Tualang Honey	4.52±0.21e	1.69±0.19c	2.86±0.18ef
Black Tualang Honey	3.4±0.04ef	1.75±0.14c	3.38±0.1e
Acacia Honey	2.26±0f	1.37±0.24cd	2.33±0.18ef
Fraser Hills Tualang Honey	6.97±0.51d	0.30±0.09e	6.17±0.45d

Table 3 Standard plate count and yeast and molds count of selected honey

Honey	Standard Plate Count(CFU/g)	Yeast and Mould Count(CFU/g)
Honey A	ND	ND
Honey B	ND	ND
Honey C	ND	ND
Honey D	ND	ND
Honey E	$<1.0 \times 10^1$	ND
Honey F	2.1×10^3	ND
Yemeni Sidr Honey	7.5×10^2	ND
Red Tualang Honey	7.0×10^2	$<1.0 \times 10^1$
Black Tualang Honey	$<1.0 \times 10^1$	ND
Acacia Honey	ND	ND
Fraser Hills Tualang Honey	$<1.0 \times 10^1$	ND

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