PharmacologyOnLine

Archives • 2021 • vol.2 • 223-233

ANTIOXIDANT ACTIVITY AND PHYSICOCHEMICAL CHARACTERIZATION OF HONEY OF REGIONS IN INDONESIA

Akhmad Endang Zainal Hasan[1*], Heny Herawaty[2], Purnomo[3], Rizki Rinda Sari[1]

[1]*Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor, Indonesia.*

[2]*Post-Harvest Research Institute, Agricultural Research, Bogor, 16000, Indonesia*

[3]*Forest Plant Fiber Technology Research Institute, Kuok Km.09, Bangkinang, Kampar, 28463 Indonesia*

**pakzainalhasan@gmail.com*

Abstract

Honey has been consumption since hundreds of years ago and is believed to have high nutritional value and has healing properties. This research was conducted in order to characterize the honey samples from several regions in Indonesia base on SNI 2013, name through the water content, ash content, reducing sugar, and HMF, followed by the measurement of antioxidant activity of selected samples. The method used to measure the antioxidant activity of honey samples in DPPH method. There are five samples honey that pass the test of physicochemical characterization, namely honey samples S1, TK1, RMBTN, KLKNG, and MRV. IC50 value of the five samples of the honey was 18,18 µg/mL, 20,47 µg/mL, 15,08 µg/mL, 14,78 µg/mL, and 15,94 µg/mL. The results of measuring the antioxidant activity in all five elected honey proved that honey contains antioxidant.

Keywords: *honey, nutritional, healing, antioxidant, reducing sugar*

Introduction

Honey is a natural food product that has been consumed by the general public. Honey has a distinctive sweet taste produced by honey bees [1]. The basic ingredients of honey production are plant flower extracts in the form of floral nectar or other parts of the plant, namely extra floral nectar[2]. Floral nectar works as a carbohydrate source for honey, while pollen or extra floral nectar works as a protein source [3] [4]. Honey components vary widely depending on the nectar source and the external conditions of honey fermentation [5][6]. According to [7] Indonesia has several types of honey nectar sources, ranging from monoflora to multiflora. So that the components of honey in one area may be different from other areas, but the basic components must remain the same. According to the 2013 Indonesian National Standard concerning honey, it is stated that some basic characteristics of honey are water content not more than 22% w/w, maximum ash content is 0.5% w/w, minimum reducing sugar is 65%, and maximum hydroxymethylfurfural content. is 50 mg/kg.

Physiologically, honey is a thick liquid like syrup with a distinctive sweet aroma, when heated, the honey aroma will be stronger. Consumption of honey has been carried out for hundreds of years and is believed to have high nutritional and healing properties. Research on honey has been done since ancient times and still continues today. This is done to exploit the benefits of honey. Along with the development of technology, several benefits of honey have been revealed, including as a food sweetener, functional food, antioxidant, antibacterial, and anticancer [8]. Consumption of honey can significantly increase the antioxidant capacity in the body [9]. Antioxidants can prevent or reduce damage caused by free radicals that can cause oxidative stress, one of the effects of oxidative stress itself is that it can trigger cancer [10].

Every day the human body always interacts with free radicals that can come from exposure to UV rays, cigarette smoke, air pollution, radiation, and by-products of the body's metabolism. Free radicals can cause oxidation of carbohydrates, lipids, proteins, and DNA. If the number of free radicals in the body exceeds the body's capacity to neutralize them or it can also be called oxidative stress, there will be oxidation out of control in cells that can cause damage and mutations to the cells themselves [11]. This mutation can cause the proliferation of cells to become uncontrolled, this is the forerunner of the formation of cancer cells. Naturally, but the body produces antioxidants, if the number of free radicals that enter the body is much greater than the amount of natural antioxidants, it will cause oxidative stress. To avoid this, it is necessary to take additional antioxidants, one of the natural products that may be a source of natural antioxidants is honey.

This study aims to measure the antioxidant activity of selected honey from several regions in Indonesia. honey is done by selecting based on its physicochemical components in the form of measuring water content, ash content, reducing sugar concentration, and the amount of hydroxy methyl furfural contained in twelve honey samples. The best honey that has passed the standard physicochemical test will be tested for its antioxidant activity.

Methods

Materials

The materials used in this study were in the form of twelve honey samples from various regions in Indonesia as the main raw material (S1, SLP, TK1, TK2, TK3, SNTRM, RMBTN, KPK, KLKNG, PLBNG, and MRV) honey samples. While the materials needed for testing the water content and ash content include spiritus, tissue and dH2O. Then for the determination of reducing sugar content, materials in the form of NaOH, 3,5-disodium nitro salicylate, KNa-tartrate and dH2O are needed. In addition, in the process of determining the value of HMF, additional materials are used in the form of potassium ferrocyanide for the manufacture of Carrez I reagent, zinc acetate for Carrez II reagent, sodium bisulfate as a standard, and the last ingredient is alcohol to remove foam in the sample. Furthermore, the materials used for testing antioxidant activity include DPPH, absolute methanol, and dH2O.

Procedure

Determination of Water Content

Water content testing is done by using a moisture balance tool. A total of 2 grams of honey samples were dropped onto the measuring plate. The tool is closed, then the water content measurement is started by pressing the "start" button. The measured moisture content of the sample will be displayed on the moisture balance screen.

Determination of Ash Content [2]

The porcelain cup to be used is cleaned first, then baked in the oven for 1 hour. Porcelain dishes that have been baked are weighed and the weight is recorded as the weight of the empty dish. Then as much as 3 grams of the sample was put into a porcelain dish and ashes by means of roasting at a temperature of 550℃ for 4 hours or until all samples formed ash. After all the samples formed ash, the cup containing the sample ash was cooled and then weighed and recorded as the weight of the cup and ash.

Reducing Sugar Test [2]

DNS reagent generation. A total of 1 gram of DNS was added with 50 mL of ddH2O, after being homogenized, it was added with 30 grams of KNa tartrate and 20 mL of 2 N NaOH. Then the homogeneous solution was put into a 100 mL volumetric flask and calibrated using ddH2O.

Preparation of glucose standards. Glucose standard was made from glucose 1 gram, 0.8 gram, 0.6 gram, 0.4 gram, and 0.2 gram dissolved in 20 mL ddH2O. 0.1 mL of the solution was sampled and then 1.5 mL of DNS reagent was added. Followed by homogenization with the help of a vortex, then the solution was heated at 100C for 5 minutes. After the solution cooled, 0.5 mL of the solution was sampled and added with 15 mL of ddH2O. The last step is to homogenize the solution and then measure the absorbance using a UV-Vis spectrophotometer at a wavelength of 540 nm.

Determination of sample reducing sugar. 1 gram of honey sample was homogenized with 20 mL of ddH2O, then 0.1 mL was taken to be mixed into 1.5 mL of DNS reagent. The solution was homogenized using a vortex, then heated at 100℃ for 5 minutes. After removal from the water bath, the solution is cooled by washing with running water. This step must be done carefully so that water does not enter the test tube. Then 0.5 mL of the solution was taken and added with 15 mL of ddH2O. Followed by homogenization by vortex, and ended with measuring the absorbance of the solution using a UV-Vis spectrophotometer at a wavelength of 540 nm.

Hydroxy methyl furfural Test [2]

A total of 5 gram of honey from each sample was weighed carefully (to an accuracy of 1 mg), then dissolved in water and put into a 50 mL volumetric flask, added with ddH2O until the volume of the solution was 25 mL. Added with 0.5 mL of Carrez I solution, shaken and added with 0.5 mL of Carres II solution, shaken again and then added with ddH2O to the limit of tera. A drop of alcohol is added to remove the foam that forms on the surface. Then filtered with filter paper, the first 10 mL of the filter was discarded. A total of 5 mL of the filter was put into 2 different test tubes, in each tube 5 mL of ddH2O and 5 mL of 0.2% sodium bisulfide were added to the other tube. The two tubes were then shaken until well mixed, and the absorbance was measured at a wavelength of 284 nm and 336 nm. If the absorbance is higher than 0.6 then it is diluted using dH2O for the sample and 0.20% NaHSO3 for the comparison solution.

Antioxidant Test

Preparation of DPPH reagent. A total of 1 mg of DPPH was weighed with an accuracy of 0.2 mg then dissolved into 15 mL of absolute methanol by stirring with a stirrer for 30 minutes. Then the reagent solution was diluted using ddH2O as much as 15 mL.

Sample testing. A total of 0.1562 grams of sample was dissolved in 25 mL of absolute methanol, then the solution was shaken until homogeneous. The solution formed has a concentration of 6.25 L/mL which acts as the sample with the highest concentration as well as the stock. Then, the dilution was carried out in stages to form solutions with concentrations of 5 µL/mL, 3.75 µL/mL, 2.5 µL/mL, 1.25 µL/mL, and 0 µL/mL (as blanks). A total of 0.6 mL of each concentration was pipetted into a test tube that had been filled with 0.9 mL of 0.4 mM

DPPH. Followed by shaking until homogeneous, then incubated in a dark room for 20 minutes. Absorption was measured using UV-Vis spectrophotometry at a wavelength of 517 nm.

Results

The moisture content of honey was measured using a moisture balance device with a weight range of 2 grams for each sample. The percentage of water content measured from twelve honey samples is between 14% to 25% w/w. The honey sample with the highest moisture content was RHU2 of 24.88%, while the lowest water content was found in the MRV honey sample with a moisture content of 14.45%. The moisture content of honey as shown in Figure 1.

Measurement of honey ash content was carried out using the gravimetric method. Ashing was carried out using a furnace at a temperature of 550°C for approximately 4 hours to obtain a constant weight. The ash level of honey as shown in Figure 2.

The reducing sugar content in honey was measured using the DNS method. The results obtained showed the amount of reducing sugar content in honey ranged from 67% to 96% of the total weight of honey. The measurement of reducing sugar in honey was carried out using the DNS method. The basic principle of this method is the calculation of the reduction and oxidation reactions in samples and reagents. The reducing sugar contained in honey when placed under alkaline conditions can reduce 3,4-dinitrosalicylate (DNS) which is yellow to orange in color. This color change is proportional to the amount of reducing sugar in the sample. The higher the amount of reducing sugar in honey, the more concentrated the orange color will be. This color change can be measured using UV-Vis spectrophotometry at a wavelength of 540 nm. The reducing sugar content of honey as shown in Figure 3.

Hydroxy methyl furfural or commonly abbreviated as HMF was measured using the Carrez method based on colorimetry. Colour readings were carried out using a UV-Vis spectrophotometer at a wavelength of 284 nm and 336 nm. The hydroxy methyl furfural of honey as shown in Figure 4.

Measurement of antioxidant activity was carried out using the DPPH method. The samples whose antioxidant activity was measured were only samples that had passed the physicochemical quality test using the 2013 SNI standard on honey. The number of honey samples that managed to pass was only five honey samples out of twelve total samples. The antioxidant axtivity of honey as shown in Table 1,

Discussion

The 2013 Indonesian National Standard (SNI) has set the maximum value for the water content of honey, which should not be more than 22% w/w. If the water content exceeds this value, it can be said that the quality of the honey tested is not good. The water content value will represent the amount of water in the honey. The higher the water content, the more water content in honey. Referring to 1 show that there are five honey samples that have a moisture content of more than 22%, while the rest are below 22%. Honey that has a value above 22% is a sample of SLP honey, TK2, TK3, SNTRM, and PLBNG with water content values of 25.00%, 27.50%, 24.21%, 30.31%, and 24.40%. According to ANOVA statistics with Tukey's further test, moisture content positively affects the quality of a honey, so that the five samples of honey that have high water content will certainly not proceed to the final test of antioxidant activity. High water content will cause honey to be easily damaged. The trigger for this damage can be caused by the occurrence of fermentation by microorganisms. Honey that has a moisture content of more than 20% is prone to fermentation because the high water content can trigger the proliferation of yeast cells [12].

The high-water content in the five honey samples could be caused by several factors. water contained in honey comes from nectar that has been ripened by bees and its concentration on weather conditions, nectar moisture content, and post-harvest handling of honey [13][14]. According to [15] and [14] honey is hygroscopic or absorbs air. Harvesting ripe honey is done by removing the honey from the cells, when the honey is in direct contact with the air, the honey will absorb air from the surrounding environment until it reaches a balance. This is because honey is a highly saturated solution of sugar, the sugar in honey ranges from 65% to 80% [12]and [13].

The moisture content of honey is also influenced by the relative humidity of the environment. If an environment has a relative humidity of about 66%, the moisture content of honey harvested in that environment will reach 21.5% [16]. According to [7], Indonesia is a tropical area with relative humidity ranging from 60% to 90%, so it is natural that the water content level in Indonesia is higher than 21.5%. And [7] also explained that to meet the SNI standard for honey, post-harvest handling must be carried out in the form of reducing air content. One example of a post-harvest method to reduce water content is to use adsorption drying [16].

The post-harvest processing of honey in Indonesia is still not evenly distributed. There are several areas that have used tools for honey processing, such as using extractors and reducing water content. However, there are still many areas in Indonesia that use manual processing for honey. Farmers outside Java generally squeeze honey manually, then it is packaged immediately without post-harvest processing. Meanwhile, honey originating from Java Island has undergone more modern processing, so that it has a water content that is in accordance with SNI quality standards.

The ash content of honey was measured using the gravimetric method which was carried out using a furnace at a temperature of 550°C for approximately 4 hours or until a constant weight of the ash was obtained. The basic principle of measuring ash content is to compare the weight of the initial honey and the final honey. The final honey weight is obtained by heating the honey until all the water and organic components have evaporated, from which the ash content of the honey can be measured. According to the 2013 SNI standard, the ash content of honey should not be more than 0.5% w/w. Referring to Figure 2 which describes the data from the analysis of the ash content of 12 honey samples, the ash content value is in the range of 0.010% to 1.205%. SNTRM honey has the highest ash content of 1.205% and the lowest ash content is RHU5 honey sample with an ash content value of 0.010%.

There are two honey samples that have higher ash content than the standard requirements of SNI, namely SNTRM and PLBNG honey samples. The high ash content of the sample indicates that the inorganic components in honey have a large enough amount. According to [17] the value of ash content is significantly influenced by the type of bee species. In addition, the source of nectar and harvesting techniques can also affect the ash content of a honey [18]. Twelve honey samples tested had different places of fermentation, sources of nectar, types of bees, and processing methods. This is what causes the ash content values are very diverse.

One of the important factors that affect the ash content of honey is the method of harvesting. There are two types of honey harvesting, namely by manual extortion and by using an extractor. Based on the value of ash content, it is predicted that the samples of SNTRM and PLBNG honey are harvested using manual squeezing. This can be seen from the high value of the ash content of the two samples. Manual harvesting allows the honey to mix with the pollen in the honeycomb. According to Krell 1996, pollen contains various minerals such as K, Na, Ca, B, Cl, I, Mn, and the ash content value is 3-4%. The pollen mixed with honey causes the ash content of the SNTRM and PLBNG samples to be higher than the honey quality standard in SNI 2013. So the two samples will not proceed to the final antioxidant activity test because they do not pass the phytochemical quality test.

Referring to the experimental results shown in Figure 3, all honey samples contained more than 65% w/w reducing sugar. This value is in accordance with the rules of the Indonesian National Standard on honey in 2013, that honey that has good quality must have a reducing sugar content above 65% w/w. The results of the sample measurement showed that the lowest honey content was owned by the PLBNG honey sample with a reducing sugar content value of 67.97%, while the highest reducing sugar contained in the KPK honey sample was 92.03%. The other honey samples contained reducing sugars between 67% to 92%.

The main components of honey are glucose and fructose which are formed from the fermentation of nectar by honeybees. So that the sweet taste of natural honey actually exceeds the sweetness of white sugar. The dominant type of sugar in honey is fructose, which is pure sugar derived from fruit juices, while white sugar is made from sucrose, which is sugar that is processed by humans from

sugar cane trunks [19]. The level of sweetness of honey compared to ordinary sugar can reach one and a half times. However, the sweet taste of honey does not have the same bad effect as white sugar, because the carbohydrates in honey are simple carbohydrates in the form of monosaccharides, so they are more easily absorbed by the body.

Measurement of reducing sugar content in honey can be used as a reference to see whether there is dilution in honey or not. If the honey has been previously diluted, the reducing sugar content contained will be below the SNI threshold. However, testing for reducing sugars cannot be used as a reference for the total amount of glucose and fructose in honey samples, because the reducing sugars contained in honey are not only glucose and fructose. The disaccharides present in honey can also be measured as reducing sugars.

Hydroxy methyl furfural (HMF) is the result of the decomposition of glucose, fructose, and other monosaccharides which have six C atoms in an acidic atmosphere and their formation can be accelerated with the help of heat [20]. HMF is usually not found in fresh food but is commonly found in processed foods. An example of food processing that can form HMF is heating. Pasteurization and cooking of foods containing fructose and glucose can also produce hydroxy methyl furfural [21]. The content of HMF in honey is usually used as an indicator to see the level of honey quality.

Measurement of HMF levels in honey samples was carried out using the Carrez method. The HMF molecules contained in the sample will react with the reagents and form a color complex. The more concentrated the color formed, the higher the HMF value contained in the sample. The HMF values contained in the twelve honey samples showed fluctuating values. As shown in Figure 4, the highest HMF value was found in the KPK honey sample, which was 70.43 mg/kg and the lowest was the SNTRM honey sample with an HMF content of only 1.38 mg/kg. According to the standards set by SNI 2013 regarding good honey quality, the maximum value of HMF of a honey is 50 mg/kg. If the HMF content is above this value, it can be said that the honey sample has been processed by heating, so that the sugar contained in the honey is dehydrated to form HMF.

The presence of HMF in a food does not have a bad effect on the body. High levels of HMF are common in processed foods. However, for honey itself, HMF is used as a quality reference. The higher the HMF content in honey, the lower the quality of honey. There are two honey samples that have HMF values higher than the standard (50 mg/kg), namely RHU5 and KPK honey samples with values of 61.29 mg/kg and 70.43 mg/kg respectively. The high value of the HMF content of the sample indicates that the sample has below standard honey quality and is suspected to have undergone processing involving heat. Through the statistical calculation of ANOVA and continued with Tukey's further test, the P value of 0.000 rejected H0 which means that the HMF value positively affects the quality of a honey. So that the RHU5 and KPK honey certainly will not proceed to the antioxidant test.

Honey has been known to have antioxidant capacity that can neutralize free radicals, naturally this antioxidant can protect components in honey cells from harmful activities [22] [23]. Antioxidants are substrates that can protect the body from damage caused by free radicals. Free radicals are chemical molecules that are very reactive and can react with other molecules around them. This results in molecular changes and causes the molecules to not function properly [24]. If the molecule that reacts with free radicals is a protein molecule, it will cause changes in the body and if it accumulates it can cause premature aging, such as hardening of the skin and arteries and the development of cataracts in the eyes. Meanwhile, when free radicals react with lipids in the cell membrane, it will cause damage to the cell membrane and lead to cell death. Free radicals are also inflammatory activators and compounds that can damage enzymes and body organs. If the molecule that is changed by free radicals is a cell's DNA molecule, it can result in mutations in genes. This condition can lead to cancer. In addition, if the DNA is passed on to the next generation through sperm or egg cells, it can cause abnormalities.

Antioxidants work in two ways, the first is that antioxidants are involved in reactions with free radicals so that they stop the chain reaction.

Second, antioxidants stop the formation of free radicals. While the source of antioxidants itself is divided into two, namely antioxidants produced by the body and antioxidants from outside. For example, the body's antioxidants are estrogen which is also a female sex hormone. Antioxidants from outside the body can be in the form of synthetic antioxidants such as supplements and natural antioxidants such as fruits and vegetables. Each source of antioxidants has a different antioxidant activity, as well as honey. The composition and content of honey is strongly influenced by the nectar source, climate, and environmental conditions [25]. One way to measure antioxidant activity is by using the DPPH method. Antioxidant activity will be expressed in the percentage of its inhibition against DPPH radicals [26].

Antioxidants can prevent or reduce damage caused by free radicals that can cause oxidative stress, one of the effects of oxidative stress itself is that it can trigger cancer [10]. Every day the human body always interacts with free radicals that can come from exposure to UV rays, cigarette smoke, air pollution, radiation, and body metabolism. Free radicals can cause oxidation of carbohydrates, lipids, proteins, and DNA. If the number of free radicals in the body exceeds the body's capacity to neutralize them or it can also be called oxidative stress, oxidation will occur out of control in cells that can cause damage and mutations to the cells themselves [11]. This mutation can cause the proliferation of cells to become uncontrolled, this is the forerunner of the formation of cancer cells.

Antioxidants are naturally produced in the body, but if the number of free radicals that enter the body is much greater than the number of natural antioxidants, oxidative stress will occur. To avoid this, it is necessary to take additional antioxidants, one source of natural antioxidants is honey. Based on the experimental results as shown in Table 1, the IC50 value of all selected samples is quite high, ranging from 14.78 µL/mL to 20.47 µL/mL. After going through a phytochemical quality selection with reference to the 2013 SNI, five honey samples were selected which were tested for antioxidant activity, the five honey samples were S1, TK1, RMBTN, KLKNG, and MRV honey. The LC50 values of each honey sample were 18.18 µL/mL, 20.47 µL/mL, 15.08 µL/mL, 14.78 L/mL, and 15.94 µL/mL. This difference in antioxidant activity is influenced by the location of the honey and the type of bee. This is in accordance with [27] research that location affects the value of antioxidant activity.

IC50 is a value commonly used to express the antioxidant activity of a sample. The meaning of IC50 is the number of samples needed to neutralize 50% of the total free radicals. So, from the measurement of antioxidant activity in the five selected samples, it can be seen that the highest antioxidant activity was found in the KLKNG sample with an IC50 value of 14.78 µL/mL. However, the antioxidant activity values of the other four honey samples were still quite high, when compared to ascorbic acid standards. Ascorbic acid has been commonly used as a comparison of antioxidant activity, the IC50 value of ascorbic acid itself is 5.83 [26]. The high value of the antioxidant activity of this sample is not surprising considering that the five honey samples are the best honey samples.

We performed from four parameters of physicochemical test carried out, namely water content, ash content, reducing sugar, and HMF. Through quality testing, five honey samples were selected, namely S1, TK1, RMBTN, KLKNG, and MRV honey. The five samples then entered the final test, namely antioxidant activity testing. The antioxidant activity values expressed in the IC50 of the five selected samples were quite high, namely 18.18 µL/mL, 20.47 µL/mL, 15.08 µL/mL, 14.78 µL/mL, and 15.94 µL/mL. Through this value, it can be concluded that from twelve honey samples from several regions in Indonesia, there are five honey samples that have good quality and are proven to have antioxidant activity.

Acknowledgments

The authors are grateful for the financial support of the Rector of IPB University and Minister of Research and Technology and Higher Education of the Republic of Indonesia. Thanks also to my colleague and my student at Biochemistry, IPB University, Bogor, West Java, Indonesia.

References

[1] F. Aboud, C. De Pasquale, A. Sinacori, S. Massi, P. Conte, and G. Alonzo, "Palynological, physico-chemical and aroma characterization of Sicilian honeys," *J. ApiProduct ApiMedical Sci.*, vol. 3, no. 4, pp. 164–173, 2011, doi: 10.3896/ibra.4.03.4.03.

[2] SNI, "MADU(honey) SNI-3545:2013," 2013.

[3] U. Harahap, Yuandani, Marianne, H. M. Agustya, D. U. Azizah, and S. W. Alfiah, "Subchronic toxicity evaluation of ethanol extract of Picria fel-terrae lour. Leaf in wistar rats," *Scientia Pharmaceutica*, vol. 86, no. 3. 2018, doi: 10.3390/scipharm86030034.

[4] A. Widiarti and Kuntadi, "Budidaya Lebah Madu Apis mellifera L. Oleh Masyarakat Pedesaan Kabupaten Pati, Jawa Tengah," *J. Penelit. Hutan dan Konserv. Alam*, no. November, pp. 351–361, 2012.

[5] C. Liu *et al.*, "Synthesis and anticancer potential of novel xanthone derivatives with 3,6-substituted chains," *Bioorg. Med. Chem.*, vol. 24, no. 18, pp. 4263–4271, 2016, doi: https://doi.org/10.1016/j.bmc.2016.07.020.

[6] A. P. Pereira, A. Mendes-Ferreira, L. M. Estevinho, and A. Mendes-Faia, "Improvement of mead fermentation by honey-must supplementation," *J. Inst. Brew.*, vol. 121, no. 3, pp. 405–410, 2015, doi: 10.1002/jib.239.

[7] A. Suranto, *Terapi madu*. Jakarta: Penebar plus, 2007.

[8] M. Kinoo, F. Mahomoodally, and D. Puchooa, "Anti-Microbial and Physico-Chemical Properties of Processed and Raw Honeys of Mauritius," *Adv. Infect. Dis.*, vol. 02, pp. 25–36, Jan. 2012, doi: 10.4236/aid.2012.22005.

[9] D. D. Schramm, M. Karim, H. R. Schrader, R. R. Holt, M. Cardetti, and C. L. Keen, "Honey with high levels of antioxidants can provide protection to healthy human subjects.," *J. Agric. Food Chem.*, vol. 51, no. 6, pp. 1732–1735, Mar. 2003, doi: 10.1021/jf025928k.

[10] J. M. Alvarez-Suarez *et al.*, "Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage.," *Food Chem. Toxicol. an Int. J. Publ. Br. Ind. Biol. Res. Assoc.*, vol. 50, no. 5, pp. 1508–1516, May 2012, doi: 10.1016/j.fct.2012.01.042.

[11] C. Borek, "Dietary antioxidants and human cancer.," *Integr. Cancer Ther.*, vol. 3, no. 4, pp. 333–341, Dec. 2004, doi: 10.1177/1534735404270578.

[12] Evahelda, P. Filli, M. Nura, and S. Budi, "Uji aktivitas enzim diastase, kadar gula pereduksi dan kadar air pada madu bangka dan madu kemasan yang dipasarkan di kota Palembang," 2015.

[13] A. Gairola, P. Tiwari, and J. Tiwari, "Physico-chemical properties of Apis cerana-indica F. honey from Uttarkashi District of Uttarakhand, India," *J. Glob. Biosci.*, vol. 2, pp. 20–25, Jan. 2013.

[14] J. W. White, "The Role of HMF and Diastase Assays in Honey Quality Evaluation," *Bee World*, vol. 75, no. 3, pp. 104–117, Jan. 1994, doi: 10.1080/0005772X.1994.11099213.

[15] S. Yanniotis, S. Skaltsi, and S. Karaburnioti, "Effect of moisture content on the viscosity of honey at different temperatures," *J. Food Eng. - J FOOD ENG*, vol. 72, pp. 372–377, Feb. 2006, doi: 10.1016/j.jfoodeng.2004.12.017.

[16] S. Maliaentika, S. S. Yuwono, and N. Wijayanti, "Optimasi Penurunan Kadar Air Madu Metode Adsorption Drying dengan Response Surface Methodology (RSM)," *J. Pangan dan Agroindustri*, vol. 4, no. 2, pp. 505–514, 2016, [Online]. Available: https://jpa.ub.ac.id/index.php/jpa/article/view/358.

[17] S. J. Tanuwidjaya, "Karakteristik Kimia dan Organoleptik Madu Dari Lebah Apis mellifera, Apis cerana, Apis dorsata, dan Trigona sp.," pp. 1–27, 2014.

[18] C. Pérez-Arquillué, P. Conchello, A. Ariño, T. Juan, and A. Herrera, "Physicochemical attributes and pollen spectrum of some

unifloral Spanish honeys," *Food Chem.*, vol. 54, no. 2, pp. 167–172, 1995, doi: https://doi.org/10.1016/0308-8146(95)00022-B.

[19] J. R. Purbajaya, *Mengenal dan Memanfaatkan Khasiat Madu Alami*. Bandung: Pionir Jaya, 2007.

[20] S. Achmadi, "Analisis kimia produk lebah madu dan pelatihan staf laboratorium pusat perlebahan nasional Parung Panjang," *Fak. Mat. dan Ilmu Pengetah. Alam IPB. Bogor*, 1991.

[21] H. D. Belitz, W. Grosch, and P. Schieberle, *Food chemistry*. Berlin: Springer, 2009.

[22] A. Henriques, S. Jackson, R. Cooper, and N. Burton, "Free radical production and quenching in honeys with wound healing potential.," *J. Antimicrob. Chemother.*, vol. 58, no. 4, pp. 773–777, Oct. 2006, doi: 10.1093/jac/dkl336.

[23] H. A. Alzahrani *et al.*, "Evaluation of the antioxidant activity of three varieties of honey from different botanical and geographical origins.," *Glob. J. Health Sci.*, vol. 4, no. 6, pp. 191–196, Oct. 2012, doi: 10.5539/gjhs.v4n6p191.

[24] A. A. Machado De-Melo, L. B. de Almeida-Muradian, M. T. Sancho, and A. Pascual-Maté, "Composición y propiedades de la miel de Apis mellifera: una revisión," *J. Apic. Res.*, vol. 57, no. 1, pp. 5–37, 2018, doi: 10.1080/00218839.2017.1338444.

[25] M. Küçük, S. Kolaylı, Ş. Karaoğlu, E. Ulusoy, C. Baltacı, and F. Candan, "Biological activities and chemical composition of three honeys of different types from Anatolia," *Food Chem.*, vol. 100, no. 2, pp. 526–534, 2007, doi: https://doi.org/10.1016/j.foodchem.2005.10.010.

[26] L. O. Sumarlin, A. Muawanah, and P. Wardhani, "Aktivitas Antikanker Dan Antioksidan Madu Di Pasaran Lokal Indonesia," *J. Ilmu Pertan. Indones.*, vol. 19, no. 3, pp. 136–144, 2014.

[27] A. E. Z. Hasan, D. Mangunwidjaja, T. C. Sunarti, O. Suparno, and A. Setiyono, "Investigating the antioxidant and anticytotoxic activities of propolis collected from five regions of Indonesia and their abilities to induce apoptosis," *Emirates J. Food Agric.*, vol. 26, no. 5, pp. 390–398, 2014, doi: 10.9755/ejfa.v26i5.16549.

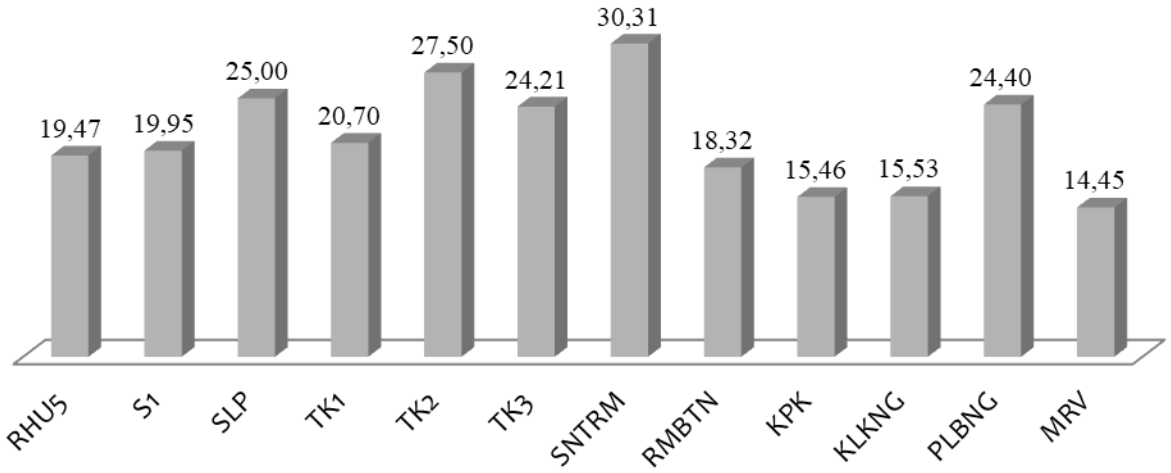

Figure 1 Moisture content of honey sample

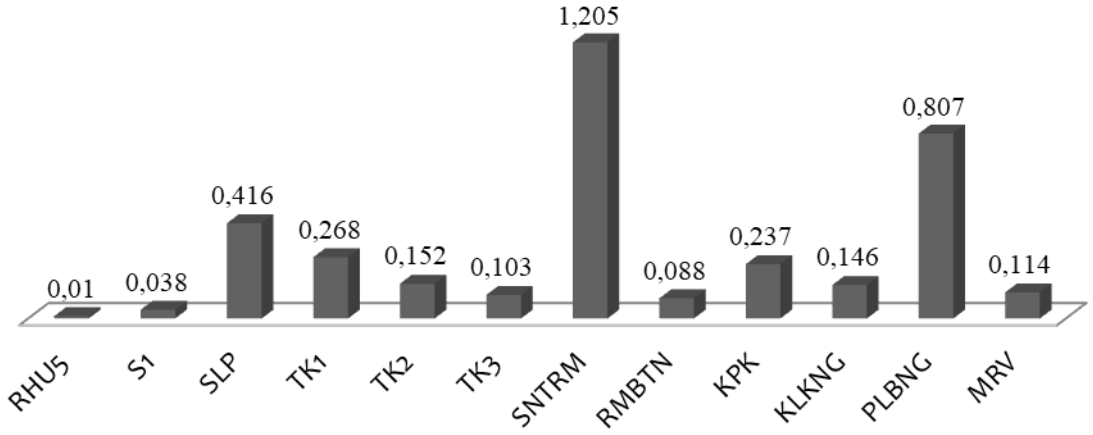

Figure 2 Honey ash content

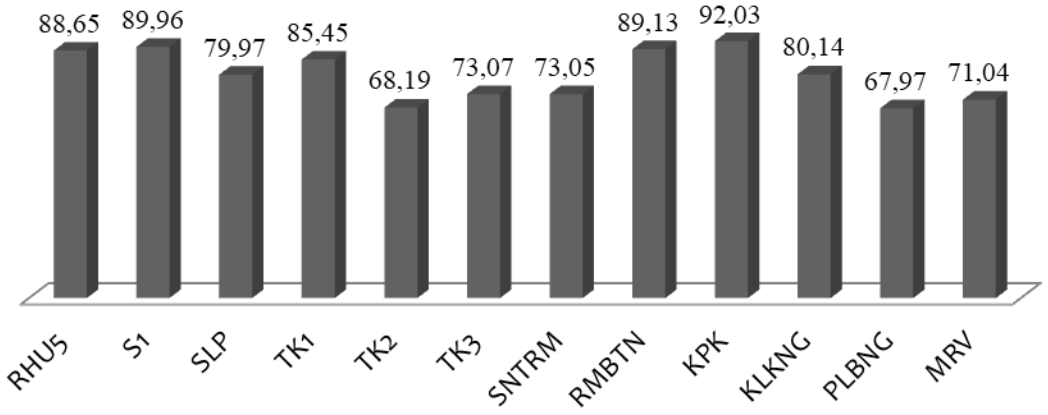

Figure 3 Honey's reducing sugar content

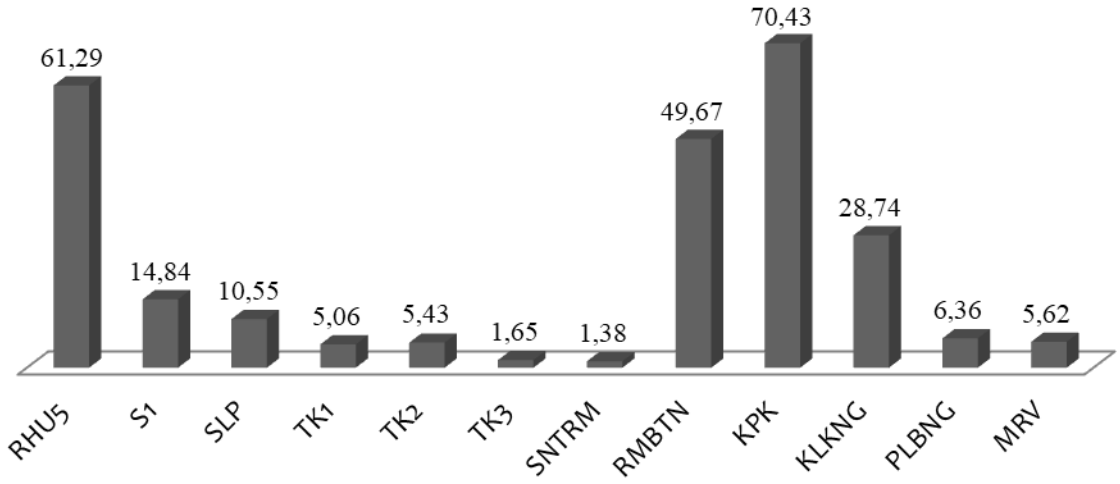

Figure 4 Levels of hydroxy methyl furfural honey

Table 1 Physicochemical Quality Test and Antioxidant Activity of Selected Honey

Sample	Water content (%)	Ash Level (%)	[glucosa] (%b/b)	HMF (mg/kg)	IC50
S1	19,95	0,038	89,96	14,8372	18,18
TK1	20,70	0,268	85,45	5,0561	20,47
RMBTN	18,32	0,088	89,13	49,6680	15,08
KLKNG	15,53	0,146	80,14	28,7375	14,78
MRV	14,45	0,114	71,04	5,6213	15,94