



ANALYSIS MINERAL ESSENTIAL (CRHOMIUM, ZINC AND MANGAN) AND BIO-PHYSICAL CHEMISTRY TEST AT HOME HONEY DISTRICTS KINDANG BULUKUMBA

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ABSTRACT

Honey is a sweet liquid that comes from the nectar of plants that are processed by bee. The composition of minerals in honey is an important factor in determining the quality of honey because it can affect the properties of bio-chemical physics honey. This research investigated the essential minerals concentration (Cr, Zn, and Mn), as well as the properties of bio-chemical physics. Three samples were collected from different location in the district Kindang is, Kindang, Pattallasang and Oro Gading. Honey samples were analyzed for essential micro mineral Chromium, Zinc and Manganese by using instrument ICP-OES and also analyzed for common bio-physicochemical parameters like moisture content, acidity, ash, pH, conductivity, protein, fat, carbohydrate and calorie. Ranged concentration manganese content of honey from 0.05 to 0.07 mg/mL, 0.05-0.07 mg/mL and concentration of chromium $\leq 0,1$ ng/mL-0.22 mg/mL, whereas the concentration of zinc is below the limit detection tool that is $\leq 0,1$ ng/mL. The average value of the quality of honey Kindang districts is 19.69% water content, ash content of 0.26%; DHL 0.36 mS/cm; 22.06 acidity meq/kg; pH 4.43; protein of 7.19% (w/w); 0.1301% fat. Total carbohydrate is 72.55%; calories 320.3101 cal; and energy 1340.2289 KJ. The results of this study indicate that manganese has the highest concentration in all samples of honey and showed that honey from Kindang district have the a good quality according to national and international standar of honey.

Key Word : Micro mineral essential, Bio-physic chemical, Honey, District Kindang, ICP-OES

1. INTRODUCTION

Honey is the nectar liquid enzymatic amended by adding the enzyme from hypopharyngeal glands, especially diastase, sucrase and glucose oxidase. Honeybees need some important compounds such as carbohydrates, proteins, fats, sterols, minerals, vitamins and water in the hold of life.^[19] Increasing public awareness of the importance of honey affect the concerns caused by the presence of low-quality honey which is sold freely in the market.^[12]

According to some studies, the content of honey varies greatly depending on the type and origin of honey. According to research conducted by Conti et al., (2014) there are 19 minerals were

analyzed as As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se, Tl, U, V and Zn. According to research conducted by Libertato et al., (2013) on the physicochemical properties of mineral and protein samples of honey from Ceará, Northeastern Brazil showed that the mineral content Cr, Zn and Mn sequentially with a concentration of 22.50×10^{-3} -170.333 ug/kg, 0.07 to 1.85 mg/kg and 0.06 to 1.96 mg/kg and declared free from pollution and environmental pollution.

Minerals in honey such as Al, Ba, Sr, Bi, Cd, Hg, Pb, Sn, W, Sb, Cr, Ni, V, Co, Mo, P, S, Ca, Mg, K, Na, Zn, Fe, Cu and Mn affect the nature of the biological, chemical physics^[6] and the quality such as



voltage surface, the refractive index ^[18] the electrical conduction, ^[10] pH, viscosity, ash content, water content, insoluble solids, color and taste of the honey. ^[14] ^[16] Chromium is an essential mineral for human health, as they can provide proper growth kontirbusi, the process of food digestion, helping the body's metabolic processes. Zinc has many important functions in the body are like the berbagai activity of the enzyme, the synthesis of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), immunity, antivirus, alcoholism, benign prostatic hyperplasia, to prevent warts, regulate appetite, and able accelerate wound healing. While manganese is an essential mineral that controls the enzyme system, which is included in the break down carbohydrates and nitrogen metabolism. Additionally manganese is essential for the formation of chlorophyll (Schulman, 2006). Mineral honey can also affect the antibacterial activity and antioxidants. ^[24] ^[11] The properties are one of the factors that determine the price of honey in the world market ^[11] in ^[13].

The connection with that, the research content of metallic minerals essential deemed necessary were performed using an instrument ICP-OES as well as to test the quality of honey based on the parameters of bio-physics, chemistry, which consist of moisture, ash, protein, carbohydrates, fat, pH, acidity and conductivity of the sample honey originating from sub Kindang Bulukumba district that is only limited to the essential metals Cr, Zn and Mn. Because of them, study metal content deemed essential minerals needed by using ICP-OES instrument and to test the quality of honey based on the parameters of bio-chemical physics which consist of moisture, ash,

protein, carbohydrates, fat, pH, acidity and conductivity of honey samples originating from sub Kindang Bulukumba district that is only limited to the essential metals Cr, Zn and Mn.

2. METHODS

Materials Research

The materials used in this study is the honey origin District of Kindang, Bulukumba, HNO₃, H₂C₂O₄, NaOH, chloroform, distilled water, akuabides, indicators pp, buffer pH 4, the mother liquor BSA 1 mg / mL, Lowry B (Na₂CO₃ 2% in 0.1 N NaOH, a solution of Na-K-Tartrate solution CuSO₄.5H₂O 2% and 1%), A Lowry (Folin Ciocalteu solution and distilled water) and filter paper.

Tools

The tools used consisted of glassware are common in the lab, ICP-OES, furnaces isotherm muffle furnace 182, porcelain dish, desiccator, analytical balance Mettler AE 100, clamp, waterbath, Lutron pH-meter 201, hotplate Maspion S-300, spektronik 20D+, refractometer Atigo, konduktometer Bench Mi 180, 260 Binder WTC oven, freezer and stone boiling.

Procedure

Intake and Sample Preparation Honey

Sampling was done subdistrict Kindang honey, Bulukumba. Sampling sites consisting of 3 points, 1 point from Ivory Oro, Kindang and Sofa. Samples were taken subsequently placed in a container that is free of contamination and stored in a freezer at a temperature of 4-5 ° C prior to analysis.

Mineral Analysis Cr, Zn and Mn in Honey (AOAC International, 1990)



A total of 1 g of honey put in a 50 mL beaker and then added 2 mL of 0.1 M HNO₃, stirred over the bath. Then added another 10 mL of 0.1 M HNO₃ with stirring. After that, the sample solution is put in a 100 mL volumetric flask and diluted with aquabides to mark boundaries, then analyzed by ICP-OES.

Analysis of Bio-Physical Chemistry Honey¹³¹ Water Content

Several samples of honey dripped on the surface of the refractometer prism and then closed. Refractometer temperature set to 20 °C and noticed a clear zone clear and dark. Furthermore, refractive index measurement is recorded.

The water content in honey samples determined by comparing the value of the refractive index and water on the table relationships refractive index (Appendix 2). If the refractive index value are not on the table, then determined based on the standard regression.

Ash Content

Porcelain dish dried first for 1 hour in an oven at a temperature of 150 °C, then cooled in a desiccator, and then weighed. The sample is weighed as much as ± 5 g and placed into the cup. The sample burned on a hot plate until no smoke. And then inserted into an electric furnace at a temperature of 600 °C to obtain a white ash. The samples were cooled in a desiccator and then be measuriing. The cup porcelain dried first for 1 hour in an oven at a temperature of 150 °C, then cooled in a desiccator, and then weighed. The sample is weighed as much as ± 5 g and placed into the cup. The sample burned on a hot plate until no smoke. And then inserted into an electric furnace at a temperature of

600 °C to obtain a white ash. The samples were cooled in a desiccator and weighed.

pH and Adicity

Prior to the titration acidity, standardization of NaOH first performed using a solution H₂C₂O₄. In addition, the pH meter calibration is done by using buffer pH 4, pH 9 and pH 14. Each before and after use pH-meter rinsed with akuabides.

Honey samples weighed 5 g, and the pH was measured using a pH-meter readings to remain for 10 seconds and pH noted. Afterwards, diluted with 35 mL akuabides CO₂ free and then inserted into a 250-mL Erlenmeyer. After that, a sample of honey added 4-5 drops indicator PP and dititar with 0.1 N NaOH solution until titration end point is marked by changes in color and showed pH 8.3 for 10 seconds. Volume 0.1 N NaOH used for titration is recorded to calculate the acidity of honey.

Electrical conductivity

A total of 10 gram sample with akuademineralisasi honey dissolved in 50 mL volumetric flask then matched. Samples of 20 mL pipette and put into a test tube. Then cell electrode is dipped into the test sample and set the temperature to 20 °C and test results are recorded. Each before and after use cell electrode rinsed with aquabides

Protein Content (Lowry method)

A total of 10 g of honey samples were weighed and himpitkan in 50 mL volumetric flask. Then pipette as much as 0.1 mL and dihimpitkan in a 100 mL volumetric flask with distilled water. Then also made a solution of Bovine Serum Albumin as a standard solution with a concentration of 0.02; 0.04; 0.06; 0.08



0.10 and 0.12 mg/mL, and distilled water as a reference solution. Each solution was put into a test tube that is different by 5 mL and added with 6.87 mL of reagent Lowry B, then shaken and allowed to stand for 15 minutes. Then added with 0.63 mL of Lowry A, shaken and allowed to stand at room temperature for 30 minutes. Spektronik its absorbance was measured with the 20D+.

Fat Content (Batch Solvent Extraction Method)

A total of 1 g of honey was extracted with chloroform as many as 10 mL. Meanwhile, porcelain dish dried in an oven at a temperature of 110 °C for 1 hour, then cooled in a desiccator and weighed weights and equipment. After that, the sample as much as 5 mL pipette and put into a porcelain dish and heated in an oven at a temperature of 105 °C for 4 hours. After that, cooled in a desiccator and weighing is done to obtain permanent weight.

Determination of Carbohydrate Content.

The formula used to determine the levels of carbohydrates are:
% Carbohydrate = [100 - levels (protein + fat + ash + water)]%.

Determination of Value Calories

Value calories per 100 g sample:
Value Calories (cal) = (9 x% fat) + (4 x% protein) + (4 x% carbohydrate) cal.

Determination of Value Energy

Formula :

Energy = 4.186 x [(9 x% fat) + (4 x% protein) + (4 x% carbohydrate)]

3. RESULTTS AND DISCUSION

Based on the analysis of data essential minerals (Cr, Zn and Mn) in the districts of

origin honey Kindang Bulukumba district showed that of the three samples, mineral manganese has a concentration ranging from 0.05 to 0.07 mg/mL. Concentration chromium was 0.22 mg/mL in the B sample (Oro), while samples A (PTS) and C (KND) below the limit of detection equipment with a range of 0.1-100 ng/mL. In this study, three samples of the honey are not mineral zinc. or were below the detection limit ICP-OES range 0.1-100 ng/mL. Picture No. 2 shows that the largest concentration of the mineral chromium contained in the sample B (Oro) with a concentration of 0.022 mg/mL, while samples A (Pattalassang) and sample C (Kindang) below the limit of detection equipment. Then for the mineral manganese, the biggest concentration present in the sample A (Pattalassang) with a concentration of 0.075 mg/mL, while the lowest concentration found in the sample B (ORO) with concentrations 0.055 mg/mL.

The concentration of manganese minerals in samples of honey origin Kindang districts was higher than the concentration of minerals chromium and zinc. It caused by soil mineral content of manganese is very abundant. In a study conducted by Eremia (2010), contained manganese minerals in the soil at 8.78%. It is much larger than the minerals chromium and zinc, which only reached 0.10 to 0.32%. Manganese minerals play an active role in various functions in the body, for example in the process of metabolism, enzyme reactions, respiration and conversion of solar light in the chloroplasts in plants.^[21]

The mineral content of chromium gained quite low because of the three samples studied only in one sample, that sample B (ORO) at a concentration of



0.022 mg/mL, while the zinc from the three samples studied everything there is no mineral zinc. Climatic conditions, vegetation and plant species bees are important factors that tmempengaruhi various properties of honey. Different characteristics of the area and species of land plants visited by bees and other factors such as factor botanical, nectar honey, pollen and water is very influential on the mineral content of honey, so that the composition, quality and quantity of micro elements honey is very varied.^[23]

The development of research on the importance of essential micro minerals have progressed, marked by numerous researchers from inside and outside the country. Mineral nutrition of honey by Table 4, the mineral content of honey

origin Kindang districts classified as high-quality honey, because it meets the minimum stretch nutritional intake of honey.^[2]

Essential mineral that is needed in the human body intake is quite varied, such as the need for intake of children aged 1-4 years to adults. Needs mineral intake of manganese, zinc, and chromium respectively for children aged 1-4 years of a maximum of 1.5 mg, 3 mg and 0.06 mg. As for age adults a maximum of 5 mg of the mineral manganese, chromium mineral 1.5 and 10 mg zinc. Samples of honey origin Kindang districts that have met the needs of intake of mineral for the human body.^[2]

Table 1. Mineral Content Analysis (Cr, Zn and Mn) in the District of Kindang

Sampel	Mineral (mg/L)		
	Kromium (Cr)	Seng (Zn)	Mangan (Mn)
PTS	0	0	0,075
ORO	0,22	0	0,055
KND	0	0	0,061
Trayek Mineral madu ^[2]	0,01-0,3	0.05-2	0,02-2
Commite Eropa Product Bee (Matei, dkk., 2004)	0,001-0,80	-	0,1-12
Min	0	-	0,075
Max	0,22	-	0,550
Mean	-	-	0,0637

Description: PTS (Pattallassang); ORO (Oro d'Ivoire); KND (Kindang).

The function of mineral essential is generally quite variation, such as the activation of the enzyme, coenzyme activity and synthesis of chlorophyll in plants. One important discovery is the process of RNA synthesis involving zinc so that the process of RNA synthesis can occur. The discovery proves the importance of minerals essential to human

health so as to encourage further research tentang minerals.^[8]

Analysis of Bio-Physical Chemistry

Analysis of bio-chemical physics is divided into two stages, namely the test of chemical physics such as moisture content, pH, ash, conductivity, and acidity. While the biochemical tests include the levels of



fat, calories, carbohydrates and protein. Honey quality standards according to ISO is very important, especially for the labeling and marketing of honey both national and international scale. So that the analysis is intended to give an idea of the quality, quantity and nutritional value of honey origin Kindang districts.

Based on the analysis of chemical physics at the honey that comes from three different sampling points in Kindang district (Table 2), there are significant differences on several parameters: water content, ash content and acidity of honey

Table 2. Analysis of Chemical Physics Honey Origin District of Kindang

Parameter	PTS	ORO	KND	Min	Maks	Mean	Standar IHC (2002)
Kadar Abu (%)	0,19	0,14	0,44	0,14	0,19	0,26	$\leq 1,2$
Kadar Air (%)	24,20	17,75	17,12	17,12	24,20	19,69	≤ 25
Keasaman (meq/kg)	24,75	20,65	20,78	20,65	24,75	22,06	≤ 50
pH	4,19	4,37	4,27	4,19	4,72	4,43	$\leq 3,6-5,6$
Konduktivitas (mS/cm)	0,11	0,24	0,73	0,11	0,73	0,36	$\leq 0,8$

The levels content ash inorganic substances that include metals or minerals contained in honey. The range of ash content in honey origin from Kindang district ranged between 0.1-0.4%. the high variations of ash content contained in honey samples Kindang village with a difference of 0.25 of the sample B (Oro) and 0.3 for sample A (PTS). The lowest content of ash contained in the sample B (Oro), ie 0.14%. According to SNI, maximum ash content of about 0.57% where as honey standard IHC honey ash content ≤ 1.2 ng/100g. Based on standardization body, honey origin Kindang districts have met the national standards of Indonesia and the International Honey Commission. Variations ash content in honey can be influenced by several factors, such as botany, nectar and plant species of bees foraging.

The water content is one determinant of the quality of honey because honey has

a high water content are more prone to fermentation. Indonesian National Standard (SNI)-3545-2004 01 states that the water content of honey is good up to 22%. The water content in honey is able to determine the durability of honey. The water content of honey is low causing spoilage microbes can not live in it, in addition, honey also has a function as an antimicrobial agent. Honey is high water content (moisture content of more than 25%) is fermented by yeast of the genus *Zygosaccharomyces* resistant to high sugar concentrations. So that the water content affects the durability of honey or honey quality. The water content in honey origin Kindang districts an average of 19.69% with a maximum value of PTS contained in the sample which amounted to 24.20% and the minimum levels contained in the samples KND which amounted to 17.12%.

The water content in the districts of origin honey Kindang quite high enough to sample the PTS because it exceeds the maximum limit of 22% according to the



National Standard, but has been meeting international standards with a maximum limit of 25%. This can happen, because at the time of sampling the atmosphere of the rainy season in May according to the statement ^[11] that the weather effects the water content. Honey has matured a low moisture content of 17%, so that the low water levels will keep the honey of damage for a relatively long period. In the rainy season the water content in honey will be higher, it can even exceed 25% because it is influenced by moisture environment.

Acidity indicates the number of free acid contained in the solution. Free acid in honey is sourced from organic acids which are contained in the honey such as acetic

acid and oxalic acid and a fraction of minerals such as Ca, K, Mg, and Na. The average value of the acidity of honey origin Kindang districts of 22.06 meq/kg with a minimum value found in the sample B (Oro) which is equal to 20.65 meq/kg and a maximum acidity in sample A (PTS) with a value of 24.75 meq/kg. According to SNI and IHC, a maximum acidity of honey is 50 meq/kg, which means the overall samples still meet the standards set. Here is a graph of pH and acidity shootout origin District of Kindang, Bulukumba. Honey with high acidity is effective in inhibiting the growth of bacteria, because bacteria are less able to survive and reproduce if it is in a high acidic conditions.

Table 3. Biochemical Analysis of the District of Origin Honey Kindang

Parameter	PTS	ORO	KND	Bogdanov (2008)
KH (%)	77,82	77,44	77,39	70-80,5
Lemak (%)	0,1342	0,0394	0,2169	-
Protein (%)	5,59	8,85	7,14	-
Kalori (kal.)	302,8478	327,2334	330,8493	-
Energi (KJ)	1266,1218	1369,6299	1384,9351	-

Thorough analysis of nutrient content includes not only the moisture content, ash content, and pH, but also the levels of protein, lipid, and carbohydrate content. Carbohydrates are typically analyzed by difference. This analysis is important to know the nutritional composition of a food that can later be used to develop nutrition fact included in food packaging label (Bieber et al., 2013). Table 3 shows the results of an analysis of several biochemical tests (proximate) the origin of honey Kindang districts.

The nutritional content of honey is an important determinant of quality and the quality of honey. In general, the highest nutrient content contained in honey are carbohydrates. Carbohydrates are one of the organic compound biomacromolecules which plays a major role in the living body. Carbohydrates serve as the main source of energy for living things. Carbohydrates in honey can reach 85% mainly monosaccharides (fructose and glucose). Fructose and glucose during digestion, can be quickly transported into the blood so quickly also utilized the body



as an energy source. Carbohydrates are typically analyzed by difference. Carbohydrates contained in the District of origin honey Kindang have a range corresponding to the foreign origin of honey is between 70-80%. Highest total carbohydrates contained in the samples A (PTS) (77.82%) and lowest in the sample C (KND) ie (77.39%). Carbohydrate is a nutrient that functions as an energy producer, which produces 4 calories per gram (Hutagalung, 2004). Carbohydrates contained in honey in the form of reducing sugars (glucose and fructose) with a minimum content of 65% (SNI., 2004).

The results of the analysis of the origin of honey fat content District of Kindang ranged between 0.1-0.2%. Fat content of honey origin Kindang District Subdistrict Bulukukmba lower than the fat content of Nigerian origin honey by 0.1 - 0.5% with an average by Buba et al., (2013). But bigger than the original fat content Sinjai Feels village of .01-.05%^[15]. Some foodstuffs in Indonesia may contain 10% or more of ether extract (fat) storage influence will cause rancidity and reduces the value of these materials. In general, honey has a low fat content.

Protein is the main component in all living cells, whose primary function is as a maker of cell structures like cell membranes, connective tissue, collagen, hair and so on by Patong., (2013). In addition to antioxidants, protein also plays an important role in preventing coronary heart disease. Enough protein requirement of 10-20% of the total energy needs by Cahyani., (2015). The results of the analysis of protein content of honey samples showed that the sample B (ORO) has a protein content of the most 8.85% (w / w) than other samples. Therefore, based on the protein content in the District of

origin honey Kindang indicate that honey is effective in treating the health of the body.

Honey is very large calorific value is 3,280 cal/kg. 1 kg of honey calorific value equivalent to 50 chicken eggs, 5.7 liters of milk, 1.68 kg of meat, 25 bananas, 40 oranges, and 4 kg of potatoes Kusuma., (2009). In the study conducted, the calorific value of honey origin Bulukumba district Kindang districts ranged between 330-350 kcal. The maximum caloric value present in the sample B (ORO) that is equal to 345.5146 kcal, while the minimum calorific value contained in the sample A (PTS) is 334.0478 kcal. The need for calories daily by humans is very dependent on its activities, which can range between 2000-3500 kcal per day. Calorie requirement in humans is influenced by activity and age factors in an individual.^[20]

Energy is one result of the metabolism of carbohydrates, proteins and fats. Energy serves as an energy for metabolism, growth, temperature control and physical activity. Honey origin keccamatan Kindang have a range of energy by 1400 KJ-1450 KJ with a maximum energy contained in the sample B (ORO) is 1446.1546 KJ and minimum energy value contained in the sample A (PTS) is 1446.1546 KJ. The energy contribution from fat daijurkan in children aged 1-3 years of approximately 35%, 30% at the age of 4-18 years and 25% in adults. Therefore, honey is one of the most recommended foods as a potential energy source for humans.

4. CONCLUSION

Based on the research that has been done, it can be inferred mineral manganese



concentrations in honey ranged from 0.05 to 0.07 mg/mL and the concentration of chromium $\leq 0,1$ ng/mL-0.22 mg/mL, whereas the concentration of zinc is below the limit of detection equipment that is $\leq 0,1$ ng/mL. The average value of the quality of honey origin Kindang districts is 19.69% water content, ash content of 0.26%; DHL 0.36 mS/cm; 22.06 acidity meq/kg; pH 4.43; protein of 7.19% (w/w); 0.1301% fat. Total carbohydrate is 72.55%; 320.3101 cal calories; and energy 1340.2289 KJ. The results showed that honey origin Kindang districts have the appropriate quality standard ISO and the International Honey Commission

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