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Analysis of Total Reducing Sugar Content, Acidity Value, and Hydroxymethylfurfural (HMF) Content of Various Honey Types

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ABSTRACT

The aims of this research are to determine the total reducing sugar content, acidity value, and hydroxymethylfurfural (HMF) content of various honey types in Special Region of Yogyakarta and Central Java. Those types sample of honey are sengon, randu, kelengkeng, rambutan, and kopi honey from several places in Yogyakarta and Central Java. This research using analytical method, there are iodometry titration, acid-base titration, and UV-Vis spectrophotometry. The result of total reducing sugar content from sengon honey 74.40%, randu honey 61.42%, kelengkeng honey 62.07%, rambutan honey 88.14%, and kopi honey 87.04%. Acidity value of sengon honey 71.33 mL/Kg, randu honey 116.67 mL/Kg, kelengkeng honey 41.33 mL/Kg, rambutan honey 32.00 mL/Kg, and kopi honey 70.00 mL/Kg. HMF content of sengon honey 14.7 mg/Kg, randu honey 39.7 mg/Kg, kelengkeng honey 45.2 mg/Kg, rambutan honey 47.5 mg/Kg, and kopi honey 38.8 mg/Kg. In spite of the result of this analysis is more than the number of Indonesian National Standard of honey, those honeys are still good and doesn't mean there are forbidden to consume it.

Keyword: Honey, Analytical method, Indonesian National Standard

1. INTRODUCTION

Honey comes from nectar that bee processed to serve as food stored in the nest. Nectar is a complex compound produced by the plant's "necterifier" glands in the form of a variety of sugar solutions. The main components of nectar are sucrose, fructose, and glucose and other sugar substances such as maltose, melibiose, raffinose, and other carbohydrate derivatives. In addition to its delicious taste, honey is also believed to have various kinds of health benefits. Beekeeping products, especially honey, have long been known by the community as a nutritious food that restores stamina, maintains health and is able to cure various diseases, such as rheumatism, high blood pressure and low blood, lumbago, and burns (Sayyid, 2006).

Honey has a variety of types. Based on the source of nectar honey differentiated into honey monoflora and multiflora honey. Monoflora honey comes from one kind of flower nectar, while multiflora honey comes from various kinds of nectar (Molan, 1999). Pure honey is a honey that has the composition of pure honey without any additional other ingredients. Natural honey also contains many enzymes, which are very complex protein molecules produced by living cells and serve as a catalyst that is: a reaction speed conversion agent in a chemical process that occurs in the body of every living creature.

The quality of honey is determined by several things such as the time of harvesting honey, water content, honey color, taste and aroma of honey. Honey harvesting time should be done at the right time, when the honey has matured and the honey cells begin to close by the bees. In addition, the water content contained in honey also greatly affect the quality of honey. A good honey is a honey that contains moisture content of about 17-21 percent (Sihombing, 1997).

So far, people still have minimal knowledge about how to know the quality of a honey. Pure honey has the criteria listed in Indonesian National Standard (SNI) number 3545:2013. In the SNI mentioned criteria that need to be fulfilled so that pure honey has a good quality such as total reducing sugar content, acidity value, and Hydroxymethylfurfural (HMF) content. Based on SNI, honey has total reducing sugar content below 65%, acidity value below 50 ml NaOH/Kg and has maximum content of HMF 50 mg/Kg.

Therefore it is necessary to conduct research to find out the quality of pure honey in the market, in this case SNI honey will be used as a parameter. The sample of honey studied was pure monoflora honey covering honey of Kelengkeng *(Dimocarpus longan)*, Rambutan *(Nephelium lappaceum)*, Sengon *(Albizia chinensis)*, Kopi *(Kopia sp.)* and Randu *(Ceiba pentandra)*. There are many parameters of pure honey which is good based on SNI, but in this research will only be analyzed total reducing sugar content, acidity value and hydroxymethylfurfural (HMF) content. The analysis of total reducing sugar content was done using Luff Schrool method, acidity value analysis was done by titration of alkalimetry and also the analysis of hydroxymethylfurfural (HMF) content was done by UV-Vis spectrophotometry. The results of these three analyzes will be compared to the existing criteria in SNI to determine the quality of honey simply because there are many other criteria that are not analyzed.

2. RESEARCH METHOD

Honey Samples

There are 5 kind samples of monofloral honey which used at this research, was Sengon Honey, Kelengkeng Honey, Randu Honey, Rambutan Honey and Kopi Honey. All of the samples bought from collector of honey at Kaliurang, Yogyakarta. That collector collected the samples honey from beekeeper at area of honey farm in season which each samples of honey production.

For information, Sengon Honey was get from Pati region; Kelengkeng Honey from Salaman, Magelang region; Randu Honey from Jepara region; Rambutan Honey from Srumbung and Purwodadi region: and Kopi Honey was get from Magelang region. Almost all of the samples was produce in months May till Augusts except Rambutan Honey which produced in months September till Desember.

All of the samples packed with transparent pack and sealed. Before and during research the samples was keep in the package with normal temperature.

Research procedure

Quality of honey which sell in Indonesia have standard of SNI. Indonesian National Standard (SNI) was national standard of many things like food, materials or etc. which selling or used in Indonesian. Indonesian government was made the standard.

Quality of honey which sell in Indonesia have standard of SNI 3545:2013. The standard gave information of parameter quality for good honey. How to analysis quality and preparation of samples honey also wrote in SNI 3545:2013.

So the research used the standard of SNI for manual analysis. The research used 3 kind parameters quality of honey there are: total reducing sugar content, acidity value and hydroxymethylfurfural (HMF) content.

The research was analytical chemistry research with produce quantitative data. All of the analysis parameters which did in this research was did 3 repeated.

Materials and equipment

All the chemical materials except the samples were got from Chemical Laboratory of Mathematics and Science Faculty Yogyakarta State University. All of the equipment included spectrophotometer UV-Vis also borrowed from Chemical Laboratory of Mathematics and Science Faculty Yogyakarta State University.

Even in Indonesian had national standard for quality of honey, other countries also had standard for quality of honey which was international standard. According to the standards of the Codex Alimentarius Committee on Sugars (2001), the minimum amount of reducing sugars was 60 g 100 g or 60% for floral honey (Codex Standard for Honey.2001). That standard was like SNI, the minimum of reducing sugar (glucose) in SNI was 65% or 65 g 100 g. In general, the sugar composition of honey is affected by the types of flowers used by the bees, as well as regions and climate conditions (Tornuk et al., 2013).

In this research of total reducing sugar content was present of totally reducing sugar because in this research was not do specific analysis of one kind of reducing sugar like glucose.

Luff Schrool method used for determination of total reducing sugar content. The principle of this method was reducing sugar in honey can reduction metal Cu in Luff Schrool reagent. This method used analytical method like alkalimetri titration, so data of this analysis was volume. Therefore the data must had analysis with formula in SNI to make data meaningfulness, gave meaning about present of reducing sugar.

Acidity was one of characteristic of organic compound which can use to qualified quality of honey, it also one of parameters standard in SNI. Good quality of honey had not too low acidity level or too high, even the characteristic of honey had acidity level which kind of acid.

The acidity value analysis was used analytical method of alkalimetri titration. Like analysis of reducing sugar, the data was volume and it must analysis with formula in SNI for valuating acidity of samples honey.

Hydroxymethylfurfural (HMF) compound can made from decomposition reaction of reducing sugar. It may happen because long time storage or over heating process. Hydroxymethylfurfural (HMF) is widely used as an indicator of honey quality deterioration produced by excessive heating or inadequate storage conditions. The HMF fresh honey is low. However a high concentration of this compound is present in old honeys, honey that has been heated, stored in non-adequate conditions, or adulterated with invert sugar or syrup (Nozal, et al. 2001). Other condition like low acidity level of honey also can made increase present of Hydroxymethylfurfural (HMF) compound (Capuano & Fogliano.2011).

Determination of Hydroxymethylfurfural (HMF) content in honey used analytical method spectrophotometry. Spectrophotometry that used in this research was spectrophotometer UV-Vis with range of absorbance 284 and 336. Data was get from this analysis was absorbance value of samples honey, and that convers to present of Hydroxymethylfurfural (HMF) compound in honey with formula which wrote also in SNI.

3. RESULTS AND ANALYSIS

The aims of this research are to determine the total reducing sugar content, acidity value, and hydroxymethylfurfural (HMF) content of some honey types in Special Region of Yogyakarta and Central Java based on Indonesian National Standard. Those types sample of honey are sengon, randu, kelengkeng, rambutan, and kopi honey from several places in Yogyakarta and Central Java. This research using analytical method, there are iodometry titration, acid-base titration, and UV-Vis spectrophotometry.

3.1. Total Reducing Sugar Content

Table 1. Data from the Result of Total Reducing Sugar Test

No	Sample of Honey	V1 / Volume titrant (mL)			V (mL)			W1 (mg)			W (mg)	fp
		V1	V2	V3	1	2	3	1	2	3		
1	Sengon Honey	3.1	4.1	3.9	12.4	11.4	11.6	31.38	28.68	29.22	2000	50
2	Randu Honey	5.5	6	5.5	10	9.5	10	25	23.7	25	2000	50
3	Kelengkeng Honey	5.7	5.8	5.2	9.8	9,7	10.3	24.48	24.22	25.78	2000	50
4	Rambutan Honey	1.8	1.9	1.3	13.7	13.6	14.2	34.89	34.62	36.26	2000	50
5	Kopi Honey	1	1.5	3	14.5	14	12.5	37.1	35.7	31.65	2000	50

Blank (aquades and other reagents except samples) = 15.5 mL (V2)

General formula :

$$\% = \frac{(W1 \times fp)}{W} \times 100\ \%$$

information :

W1 = conversion mL Sodium thiosulfate to mg glucose

(V (ml) = mg glucose)

V = titrant volume (V1) – blank volume (V2)

W = 2 gram = 2000 mg

fp = dilution factor (50 times)

Based on the research data, and through the above calculation can be obtained data of total reducing sugar content of several samples of honey as follows:

Table 2. Data of Total Reducing Sugar Content of some Honey

No.	Sample of honey	Total Reducing Sugar Content (mean)
1.	Sengon Honey	74,4 %
2.	Randu Honey	61,42 %
3.	Kelengkeng Honey	62,07 %
4.	Rambutan Honey	88,13 %
5.	Kopi Honey	78,99 %

Based on **Table 2**., it can be seen that the average total reducing sugar content of honey samples studied. The largest content of total reducing sugar is owned by Rambutan honey with the content of 88.14% and the smallest is Randu honey with a content of 61.42%. Based on SNI, reducing sugar content possessed by a honey at least 65%. Honey that meets SNI is Rambutan honey, Kopi honey, and Sengon honey. For Randu honey and Kelengkeng honey doesn't meet the SNI, but only close to reducing sugar content according to SNI.

Sugar levels in each honey may be different may affect the efficacy of honey, especially in the treatment process. The sugar content in honey is also influenced by the long storage process of honey. Sucrose found in honey will decompose to form glucose and fructose. In addition, factors that affect sugar levels are moisture content, moisture, harvest time, and also areas where the honey is harvested (climate).

Sugar levels in honey are also influenced by the ratio of glucose, fructose, and sucrose contained by honey. Honey is easier to crystallize means to have a higher glucose ratio, because glucose in honey is not easily soluble in water. However, because honey also contains other sugars that can inhibit the crystallization of honey.

3.2. Acidity Value

Blank (aquades and other reagents except samples) = 0.1 mL

Table 3. Data of from the Result of Acidity Value Test

No.	Sampel of Honey	Test 1	Test 2	Test 3
1.	Sengon Honey	3.3	4,2	3.2
2.	Randu Honey	6.3	5.9	5.3
3.	Kelengkeng Honey	2.1	2.2	1.9
4.	Rambutan Honey	1.7	1.5	1.6
5.	Kopi Honey	3.5	3.5	3.5

$$\text{General formula} = \frac{\text{A volume of titrant NaOH x B(normality of titrant NaOH (0,1 N))}}{\text{C (mass of sample (gram))}} \text{ x } 1000$$

Based on the research data, and through the above calculation, acid values obtained in some honey samples as follows :

Table 4. Data of Acidity Value of some Honey

No.	Sample of honey	Acidity value (mL NaoH/Kg)
1.	Sengon Honey	71,33
2.	Randu Honey	113,33
3.	Kelengkeng Honey	41,33
4.	Rambutan Honey	32
5.	Kopi Honey	70

Based on applicable SNI, good pure honey has a level of acidity of not more than 50 mL NaOH/Kg of honey. From the table above, the results of research can be seen, the honey that meets the standard is rambutan honey with an average acidity of 32 mL NaOH / kg and kelengkeng honey with an average acidity of 41.33 mL NaOH/Kg.

Differences in acidity value between honey samples with each other are so far apart difference, this is because the five samples of honey have a different source of interest. Characteristics of flower nectar inhaled by these bees that affect the differences in the acidity of each type of pure honey. The variations in acidity among the studied honey samples were also due to differences in their harvest season. The low acidity value indicates the freshness of the honey sample while the high acidity indicates the fermentation of sugar to organic acids.

In addition, the texture of honey also affects the acidity of pure honey, honey that has a thick texture tend to have a low acidity because the fermentation of free acid formation is more difficult to occur and vice versa with liquid texture will have a high acidity as well. The length of storage also affects the value of acidity of honey, old stored honey tends to be more acidic because of the fermentation that occurs also possible more so as to cause the formation of free acids.

3.3. Hydroxymethylfurfural (HMF) Content

Table 5. Data from the Result of HMF Test

No.	Sample of honey	Concentration (ppm)	Absorbance (wavelength= 284 nm)	Absorbance (wavelength= 336 nm)
1.	Sengon Honey	1	0.204	0.106
2.	Randu Honey	1	0.396	0.131
3.	Kelengkeng Honey	1	0,426	0.124
4.	Rambutan Honey	1	0.419	0.102
5.	Kopi Honey	1	0.379	0.120

$$\text{General formula } \left(\frac{\text{mg}}{100} \text{ gram honey}\right) = \frac{(A_{284} - A_{336}) \text{ x } 14{,}97 \text{ x } 5}{\text{mass of sample (gram)}}$$

$$\text{Factor} = \frac{126}{216830} \text{ x } \frac{1000}{10} \text{ x } \frac{100}{5} = 14{,}97$$

Based on the research data, and through the above calculation, obtained HMF in some honey samples as follows:

Table 6. Data of HMF Value of some Honey

No.	Sampel Madu	*Hidroksimetilfurfural* (HMF) mg/Kg
1.	Sengon Honey	14,7
2.	Randu Honey	39,7
3.	Kelengkeng Honey	45,2
4.	Rambutan Honey	47,5
5.	Kopi Honey	38,8

Hydroxymethylfurfural or HMF is an aldehyde often used as an indicator of honey quality. HMF, as shown in Figure 1, is produced by fructose decomposition under acidic conditions. This happens naturally over time on most honey. Although HMF is not considered a harmful substance, food standards in many countries regulate HMF levels in honey.

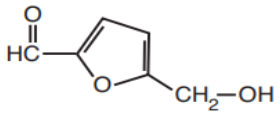

Figure 1. Hydroxymethylfurfural

Usually, fresh honey has a low HMF amount (<15 mg/Kg). The HMF concentration increases when the honey undergoes heat treatment to reduce viscosity and prevent crystallization to facilitate calcification. The EU Codex Alimentarius Standard (ALINORM 01/2000) limits HMF to 40 mg/Kg for honey produced under European Conditions and 80 mg/Kg for honey derived from tropical countries. In fresh honey samples, HMF is determined to be 0.17 μg/mL (which contains 17 mg HMF/Kg of honey). According to the Indonesian National Standard, the recommended HMF levels are contained in honey at 50 mg / kg of honey.

Based on the results of this study obtained HMF levels in some honey in Special Region of Yogyakarta till Centre of Java region according to the table results of the research in Table 6. In the table shows that Rambutan honey has the highest HMF levels among other honey, which amounted to 47.5 mg/Kg and Kopi Honey has the lowest levels of HMF among other honey sample, amounting to 38.8 mg/kg. Honey samples in this study, all already meet the Indonesian National Standard on honey, especially at HMF levels owned by honey. High levels of HMF may be due to inadequate storage, counterfeiting with sugar additives, or storage at too high a temperature, low pH of honey, and so on.

Honey that has a lower pH then the HMF content will be high, and vice versa. The high HMF can affect the color of a honey. The higher the HMF the honey color will darken, and lower HMF will cause the honey to be lighter colored.

4. CONCLUSION

The quality of honey is determined by several things such as the time of harvesting honey, water content, honey color, taste and aroma of honey. Pure honey has the criteria listed in the SNI No. 3545 of 2013. In the SNI listed criteria that need to be fulfilled so that pure honey has a good quality like total reducing sugar content, acidity value and HMF levels.

Based on SNI honey, total reducing sugar content possessed by a honey is at least 65%. Honey that meets the SNI of Rambutan honey is 88.14%, Honey Kopi is 87.04%, and Sengon honey is 74.40%. Total sugar content of Randu honey is 61.42% and honey Kelengkeng 62.07% has not fulfilled SNI, but only close to total reducing sugar content according to SNI.

Pure good honey has a level of acidity of not more than 50 mL NaOH/Kg. From the results of the study, honey that meets the standard is rambutan honey with an average acidity of 32 mL NaOH/Kg and honey longan with an average acidity of 41.33 mL NaOH/Kg. While the value of

acid honey Sengon of 71.33 mL NaOH/Kg, Randu honey of 116.67 mL NaOH/Kg, and kopi honey 70 mL NaOH/Kg not meet the SNI standards.

All analyzed honey has HMF level according to honey SNI that is maximal 50 mg/kg. The order of honey which has the highest HMF content is 47,5 mg/Kg honey, then Kelengkeng honey is 45.2 mg/kg, Randu honey is 39.7 mg/kg, kopi honey is 38,8 mg/kg and Madu Sengon has the most HMF low among other honey samples of 14.7 mg/kg.

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