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The Sucrose Contents of Four Honey Types from *Apis mellifera* Beekeepers in Java

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Abstract

Honey is a sweet thick liquid made by honeybees as their main products. Due to the increasing demand for honey, the risk of counterfeiting is on the rise, mainly when the production is limited. In term of quality standards, the sucrose content is one of the benchmarks in determining the authenticity of honey. This study aims at determining the sucrose contents of the honey from six regencies in Java. Ten honey samples that consisted of two samples of kapok honey, three samples of mango honey, three samples of rubber honey, and two samples of coffee honey were obtained from Apis mellifera beekeepers. The results showed that the averages of sucrose contents in mango honey, rubber honey, coffee honey, and kapok honey were respectively 4.94%, 3.47%, 10.49%, and 1.02%. The sucrose contents of the ten honey samples averagely met the benchmark. Still, two honey samples exceeded the criterion outlined in SNI-2013 (maximum of 5%), i.e., mango honey from Sragen Regency and coffee honey from the Pasuruan Regency. The high sucrose content of coffee honey was allegedly induced by honey counterfeiting through the addition of sugar solutions.

1. INTRODUCTION

Honey is the natural sweet substance produced by honey bees from the nectar or other living parts of plants or excretions of plant sucking insects through the physicochemical process (Gebremariam and Brhane, 2014). Honey contains different types of sugar, i.e. glucose, fructose, and maltose (Ratnayani et al., 2012). The other sugar contents in honey are disaccharides,

namely sucrose and maltose. The dominant types of sugar found in honey are glucose and fructose which are approximately 70–80%, 10-20% water, and other components such as organic acids, minerals, vitamins, proteins, enzymes, volatiles and flavonoids (Nayik and Nanda 2015) .

Due to the varied landscapes in Indonesia, each region produces honey in accordance with the characteristics of dominant plants. The physico-chemical compositions of honey in each region differ depending on the soil types, the climatic conditions, and plant species (Buba et al., 2013).

Indonesia is a honey-producing country with an average annual production of approximately 4,000 tons and nearly 25% of total honey production comes from *Apis mellifera* (Asmanah and Kuntadi, 2012). Honey consumption tends to increase so that boost the demand (Saefudin et al., 2014). The demand for honey in Indonesia is still not fulfilled by local products, as evidenced by the circulation of counterfeit honey from Thailand and China in the market. Currently Indonesia still imports 1,500-2,500 tons of honey per year.

Along with the increase of honey consumption, the counterfeiting has been developed by certain parties to generate profits (Susanto, 2007). The relatively expensive price of honey leads to the counterfeiting of honey by adding granulated sugar solutions at a cheaper price. The counterfeit honey will harm consumers and result in different benefits compared with pure honey (Sumantri et al., 2013).

Since the circulation of counterfeit honey that increasingly widespread in the market, honey quality testing is required. The criteria for honey quality in Indonesia is determined based on the Indonesian National Standard (SNI) Number 01-3545-2013. The honey quality is used as a reference so that the honey circulated in the market can be quality and safety guaranteed public consumption. Savitri et al., (2017) suggested that the quality of honey can be identified based on water content, total sugar content, and acidity.

The physicochemical characteristics of honey in many studies can be references in identifying honey counterfeiting. Saefudin et al. (2014) suggested that the characteristics of pure honey can be identified by the contents of glucose, fructose, sucrose, water, pH, color, and aroma. However, it is difficult to distinguish pure honey and counterfeit honey based on the tastes and aromas. Generally the counterfeit honey has an almost similar color as pure honey, making it difficult to distinguish (Suranto, 2004). The sucrose content in honey is essential in determining the authenticity of honey (Sumantri et al., 2013). The Indonesian National Standard 01-3545-2013 limits sucrose content in honey to a maximum of 5% b/b. The high sucrose content in honey can be induced by the addition of very large amounts of sugar solution or the addition of sucrose directly into honey. A study conducted by Endah (2009) regarding the quality of honey circulated in the market based on their sucrose contents by using iodometry method proved that 90% of honey did not meet the SNI. Studies on the quality of various types of monofloral honey from *Apis mellifera* circulated in the market based on the sucrose contents are still limited. According to this situation, sucrose content testing of honey circulated in several centers of honey production is necessary to ensure the authenticity and quality of honey. This study aims to determine the sucrose contents of four types of monoflora honey of *Apis mellifera* from the beekeepers in various honey production centers in Central and East Java.

2. Materials and Methods

The research materials were four types of honey collected from various locations i.e. mango honey, rubber honey, coffee honey, and kapok honey, standard sucrose solution, Pb acetate, ethanol, and sodium oxalate. The equipment used in this study included analytical balance, micropipettes, laboratory glassware, and Shimadzu Rid-10 HPLC - UFLC.

The study was conducted in July until October 2015 by collecting honey samples produced by *Apis mellifera* from several honey production centers in Central and East Java. Honey samples were tested based on their types and origins as shown in Table 1. Honey is directly obtained from *Apis mellifera* beekeepers at each harvesting season to avoid counterfeit honey. Ten honey samples were analyzed including 3 samples of mango honey, 2 samples of coffee honey, 3 samples of rubber honey, and 2 samples of kapok honey.

The methods were survey and observation. The study locations were selected through purposive sampling based on the honey production centers in Central and East Java. The characteristic of honey used as a benchmark for testing the occurrence of honey counterfeiting is sucrose content. The tests took place at Bogor Chemistry Analysis Laboratory in October 2015 to January 2016. Honey sucrose content was qualitatively tested by using the HPLC method (High performance liquid chromatography) through matching the retention time of each peak on the sample chromatogram with the retention time of comparative raw material. Data were analyzed and presented descriptively and tabulated.

Sucrose content analysis

Samples Preparation:

A 5 grams of sample honey was weighed and a 50 ml was transferred into the polyethylene centrifuge tube. The sample was added with 20 ml of absolute ethanol and water mixture with ratio (80:20), put into the centrifuge tube, then heated in a water bath at a temperature of 80□C for 30 minutes. Centrifuged with a minimum speed of 2000 rpm for 10 minutes. Two ml of the supernatant obtained was added with 10 ml of Pb-acetate solution. Centrifuged again and separated from the supernatant. The deposit resulted was added with 20 ml of absolute ethanol and water mixture with ratio (80:20), shaken and centrifuged, its supernatant was combined with the previously obtained supernatant. Then was evaporated with the rotary evaporator to a volume up to 10 ml. The excess of Pb-acetate was removed by adding 5% Na-oxalate until no deposit formed. Twenty five ml of sample was put into the volumetric flask and then added with the mixture of absolute ethanol : water (80:20) to the line marked. Shaked until homogeneous and filtered and sample was ready to be injected into the HPLC. The standard curve of sucrose with the injection volume of 20 µl was prepared. The sucrose content calculation was based on the interpolation of standard curve.

Sucrose content = (sample area /standard area) x standard concentration x dilution factor

Standard curve preparation

A 20 µL of 0.10% standard sucrose solution was injected by using an auto syringe injector. Let the component out and separated from the column. The retention time for the sucrose component was recorded. The correlation between the concentration of the standard solution with the peak area of the sucrose component was plotted. Sucrose content in honey was calculated based on this formula:

Table 1. Honey sampling location in several honey production centers

No	Bee species	Honey type	Nectar source	Sample code	Sampling location		
					Village	District	Regency
1	*A. mellifera*	Monoflora	Mango	M2	Panti	Panti	Sragen
2	*A. mellifera*	Monoflora	Mango	M3	Wonokerto	Sukorejo	Pasuruan
3	*A. mellifera*	Monoflora	Mango	M4	Pancur	Lumbang	Pasuruan
4	*A. mellifera*	Monoflora	Coffee	KP 1	Bangsalsari	Bangsalsari	Jember
5	*A. mellifera*	Monoflora	Coffee	KP 2	Tambaksari	Purwodadi	Pasuruan
6	*A. mellifera*	Monoflora	Rubber	K4	Curahlele	Balung	Jember
7	*A. mellifera*	Monoflora	Rubber	K5	Tanggul	Tanggul	Jember
8	*A. mellifera*	Monoflora	Rubber	K6	Kertosari	Plelen	Kutosari
9	*A. mellifera*	Monoflora	Kapok	R14	Tamatan	Tongas	Probolinggo
10	*A. mellifera*	Monoflora	Kapok	R21	Wongsokerjo	Wongsokerjo	Banyuwangi

3. RESULTS

Eight of the ten honey samples analyzed in the market from Apis mellifera beekeepers according to the SNI (Indonesian National Standart) 2013. There were two samples of honey that exceeded the sucrose contains set forth in SNI, i.e. mango honey from Panti District, Sragen Regency and coffee honey from Tambaksari Village farmers, Purwodadi District, Pasuruan Regency. The averages of sucrose content, i.e. 4.21% in mango honey, 3.71% in coffee honey, 3.46% in rubber honey, and 1.01% in kapok honey.

Table 2. Sucrose contents of honey from several harvesting centers

No	Type of honey	Sample Code	Dilution factor	Honey color (mm Pfund)	Peak area Saccharose	Sucrose standard	Sucrose content (%)
1	Mango	M2	138.35	150	77926	173360	6.39
2	Mango	M3	133.92	141	49212	173360	3.91
3	Mango	M4	135.57	150	56181	173360	4.52
4	Coffee	KP 1	117.18	84	62058	173360	4.31
5	Coffee	KP 2	143.31	43	196141	173360	16.67
6	Rubber	K4	127.03	56	31882	173360	2.40
7	Rubber	K5	145.31	50	56377	173360	4.86
8	Rubber	K6	134.3	41	47985	207274	3.14
9	Kapok	R14	151.1	35	2502	207274	0.18
10	Kapok	R21	134.48	44	23152	173360	1.85

Information: Standard sucrose solution of 0.1028%; HPLC UFLC Shimadzu Rid-10 method

4. DISCUSSION

The analysis results indicated that the sucrose contents of the majority of honey samples met the SNI 2013 (<5%). This shows that most of the honey were additional sugar free. Granulated sugar and artificial sweeteners will increase honey sucrose content and exceed the benchmark of SNI. Sucrose content of honey can be an indicator of honey authenticity (Sihombing 2005).

The high sucrose content in mango honey from Panti District, Sragen Regency is allegedly due to the addition of sugar solution, or the honeycomb still contained sugar solution at the harvest time due to the previous stimulation (the application of sugar solution) during the dry season.

Coffee honey from Tambaksari Village had the highest sucrose content, which was equal to 16.67%. It was presumed that there had been the occurrence of honey counterfeiting through the addition of sugar solution into honey or the intake of a sucrose solution which was not obtained from the nectars to the bees. It is in line with the opinion of Martin and Bogdanov (2002) who stated that honey can be counterfeited by adding sucrose solution which is not obtained from the nectars to the bees.

The averages of sucrose content in honey varied. Al-Nahari et al., (2015) suggested that sucrose content in honey can be influenced by the presence of invertase enzyme that converts sucrose to glucose and fructose with the optimum temperatures of 30-50°C. The results of Wulandari's study (2017) showed that sucrose content in honey stored at room temperature was lower compared to honey stored at cold temperatures. In cold temperatures, the invertase enzyme became inactive, so that the hydrolysis of sucrose to glucose and fructose was inhibited.

Mango honey had a higher average of sucrose content than the three other honey (rubber honey, coffee honey, and kapok honey), while kapok honey had the lowest content of sucrose. The difference in sucrose contents in honey was due to the various nectar sources as the honeybee feed. This is in line with the opinion of Antary et al. (2013) argued that the composition of honey is various and Fitrianingsih et al., (2017) said that various types of honey have different physical and chemical characteristics depend on the nectar sources,

The observation results showed that mango honey was darker than the other three other honey with a color intensity of 141-150 mm Pfund and categorized to dark amber color with the highest sucrose content. The results of this study are in line with the opinion of Elaazu et al., (2013) who stated that darker-colored honey has a high sugar content because it contains high phenolic compared to light-colored honey. The results of the analysis indicated that the regional differences have an effect on the sucrose contents. The same types of honey from different harvesting regions have different sucrose contents. This was proved by three types of honey, i.e. mango honey, kapok honey, and rubber honey.

5. CONCLUSION

All samples of kapok honey, mango honey, coffee honey, and rubber honey collected from Apis mellifera beekeepers in Central and East Java contained sucrose. Two honey samples (20%) of the ten honey samples in the market had sucrose contains that exceeded the benchmark set forth in SNI 2013, i.e. coffee honey and mango honey. The high sucrose contents in mango honey and coffee honey is allegedly due to the addition of sugar solution into honey, or the application of sugar solution to the bees in the form of sucrose sugar solution which was not obtained from the nectars.

The averages of sucrose content in each honey type varied. The differences in sucrose contents in honey were due to the different nectar sources, climate, and processing

methods. The different regions or harvesting locations make the differences in sucrose contents of the same honey types.

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