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THE EXISTENCE OF INDIGINEOUS MICROFLORA OF FOREST HONEY FROM SEVERAL REGIONS IN INDONESIA

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ABSTRACT

The existence of indigineous microfloras have been explored in forest honey from several regions in Indonesia. The honey samples collected rondomized from 8 region in Indonesia. The microflora isolated and the proositional counted in Glucose Peptone Agar medium. The research has been done by survey method and the data were analyzed descriptively. The results of research showed that the composition of microflora in the honey from each regions were vary. The Honey sample of Lima Kaum of West Sumatra consisted the highest proportion of bacteria (55.60 $x10^9$ cfu/ml) while the forest honey from West Pasaman of west Sumatra sowed the highest total presence of yeast (49.00 $x10^8$ cfu/ml), the highest total of mold was found in the sample of fores Honey of Situbondo of East Java (60.00 $x10^7$ cfu/ml).

KEYWORDS: Forests Honey, Indigineous Microflora, Yeast, Mold.

1. INTRODUCTION

Honey is a natural substance in the form of viscous and sweet liquids produced by bees and other insects from the flower nectar. If the Hornet honey is already in the nectar nectar removed from the abdominal honeybag and chewed together with another wasp, if the nectar is finely placed on the cell, if the cell is full it will be closed and fermented (Nelson and Couto, 2009). The sweetness of honey is caused by the elements of the monosaccharide fructose and glucose contained within the honey itself, and has a sweetness similar to sugar (Keeling and Gonyou, 2001).

Honey is a mixture of sugars and other compounds. In relation to carbohydrates, especially fructose honey (about 38.5%) and glucose (about 31.0%), so it is similar to synthetic sugar syrups produced inversely, which is about 48% fructose, 47% glucose, and 5% sucrose. The remaining carbohydrates of honey include maltose, sucrose, and other complex carbohydrates. Like all other nutritious sweeteners, honey contains mostly sugar and contains only a small amount of vitamins or minerals. Honey also contains small amounts of some compounds considered to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin. The specific composition of a number of honey depends on the interest available for bees that produce honey (Martos, Ferreres and Tomas-Barbera, 2000).

Honey can be divided based on the plants that are the source of nectar, one of which is forest honey, the honey taken nectar more than one plant or also called poliflora honey. All honey has a sweet taste resulting from flower nectar, where this sweet taste will cause microbial activity that can live in it like yeast. At the time of storage for a long time, the honey will produce parts such as gas or steamy, it shows that in honey also occurs activity caused by enzymes and microbes in addition to yeast in it (Nadhilla, 2014). Honey with high moisture content is easily fermented by yeasts of the genus Zygosaccharomyces that are resistant to high sugar concentrations. The yeast cells will degrade the sugars in honey (especially glucose and fructose) into alcohol (ethanol). When the alcohol reacts with oxygen, the alcohol will form acetic acid that affects the acidity, taste and aroma of honey (Harjo, Radiati, and Rosyidi, 2015).

Research on honey in Indonesia has been widely reported as Wahyuni, Budiarti and Dewi, (2016) reported on the antibacterial activity test on honey. Rio, Djamal and Asterina (2012) have also reported comparative antibacterial effects on some honey as well as oligosaccharide isolation and prebiotic activity on honey by Karimah, Anggowo, Falah and Suryani, (2011). Similarly, according to Sihombing 1997 which states, that honey is a source of sugar that can be used as a source of nutrients for lactic acid bacteria.

Based on the above statement can be seen, that very few reports that states clearly the existence of microflora contained in pure honey, especially forest honey. Therefore it is necessary to conduct research on the existence and diversity of indigineous microflora in some pure forest honey in Indonesia. The purpose of this research is to determine the existence of microflora present in some Indonesian forest honey and compare proportional indigineous microflora to some forest honey in Indonesia.

2. RESEARCH METHODS

The research was conducted using survey method with several stages of bacterial isolation in some forest honey and the data obtained were analyzed descriptively.

Sampling

Forest honey samples are taken from the providers of pure forest honey in Batang Hari (Jambi), Bengkulu (Bengkulu), Pagai Utara (Mentawai), Lamandau (Kalimantan), Pasaman Barat, Lima Kaum (Batusangkar), Pangkep (Sulawesi Selatan) and Situbondo (Surabaya)

Sterilization Tools

All the tools to be used such as Erlenmeyer, petridish, and testube are first washed and dried, petridisk then wrapped in peanut paper, and testube filled with aquades as much as 9 ml and wrapped in peanut paper. Then the tools used in this study were sterilized by using autoclave at 1210C, 15 lbs pressure for 15-20 min. This is done to prevent contamination of the tools to be used.

Isolation of Microflora from Honey

Prepare tools and materials to be used aseptically, taken 1 ml of honey using a pipette, and diluted on five honey to 10-9, and grown 10-5, 10-6, 10-7, 10-8, 10-9 on a petridisk containing each of the Glucose Peptone Agar (GPA) mediums prepared by the pour plate method, then incubated at 37 ° C for 24 to 48 hours. Observe and calculate the amount of bacterial colonies in the medium.

3. RESULT AND DISCUSSION

Based on research on indigenous microflora biodiversity from 8 samples of forest honey in Indonesia, it is known that there are microflora in honey sample. The state of pH and sugar content of the forest honey itself can be seen in Table 1.

Table 1: Sugar Level and pH Value of some Indonesian Forest Honey.

No	Sample	Sugar Content (%Brix)	pH Value
1	MJB	47,5	2,92
2	MBK	51,0	2,17
3	MMI	36,5	3,07
4	MSR	50,0	3,55
5	MKB	25,0	2,74
6	MPB	20,0	3,20
7	MBS	43,5	2,92
8	MSL	49,0	3,88

Description: (MJ: Jambi), (MBK: Bengkulu), (MM: Mentawai), (MK: Kalimantan Barat), (MS: Surabaya), (MPB: West Pasaman), (MBS: Batusangkar), (MSL: Sulawesi South).

In Table 1. above, it can be seen how the pH and sugar content of each forest honey. The pH value of forest honey ranged from 2.17 to 3.88, where the highest pH was found in Sulawesi honey samples and the lowest in Bengkulu honey samples. This indicates that the pH of the forest honey sample is classified as acid, in accordance with the SNI (2013) which states the acidity of honey itself is maximally only on a scale of 5. While Belitz and Grosch (1987) state the pH value of native honey ranges from 3.4 -6.1.

The sugar content of each forest honey can also be seen in Table 2, where the highest sugar content is found in Bengkulu honey sample of 51% brix and the lowest sugar content in the sample of West Pasaman honey by 20% brix. This is quite different from the SNI (2013) which states that the sugar content in honey is at least 65% brix. While at the maximum sugar content obtained only 51% brix, this can be caused by the content of honey, which can be caused by the process during storage in addition to the possibility of not pure honey itself.

The presence of microflora in each medium is also influenced by the content of honey itself both sugar and acidity. When associated with the pH value of each honey can be said that in honey there are bacteria that can grow at a low pH called by the term asidophilic bacteria. Because the pH value of forest honey ranged from 2.17-3.88 that is acidic. This is in accordance with the opinion of Hidayat, Padaga and Suhartini (2006) which states that the acidophilic is a microbial that grows on the acid or low ph is the range of pH 2-5.

Sugar levels are also one of the factors of growth of bacteria from forest honey. Where some groups of bacteria can live on high levels of honey sugar forests. It can be said that the bacteria that can grow on honey belong to osmofilik bacteria. Garbutt (1997) in Suryadi, Nurwantoro and Mulyani (2012) explains that bacteria that can grow at high sugar levels are bacteria that enter into the osmofilik class.

Based on the research that has been carried out the observations show that the microflora that grows in the same medium in different samples, got the total number of different microflora. The results of the existence of natural microflora of forest honey in general medium and specific medium can be seen in the following Figures and Tables:

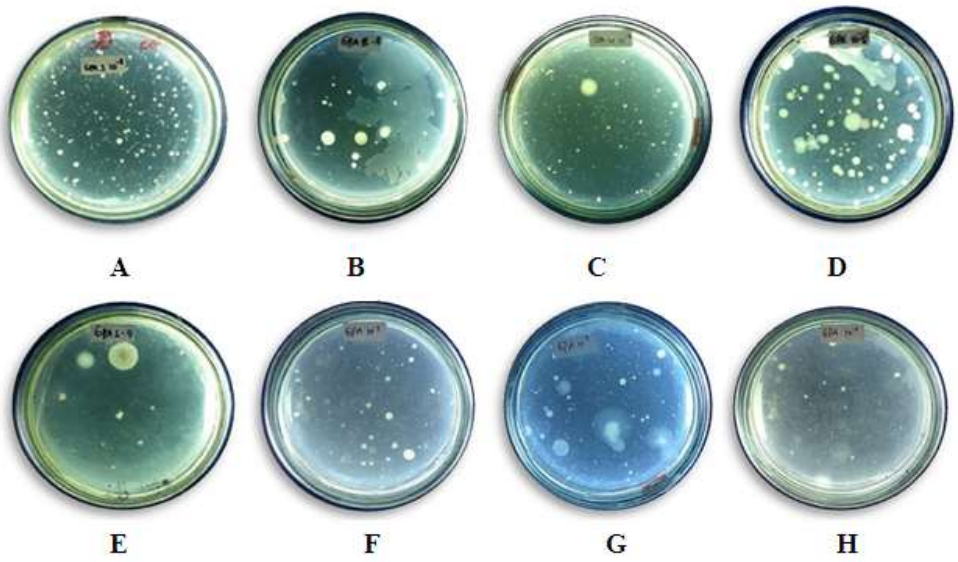

Figure 1: The existence of natural microflora of 8 samples of forest honey on GPA medium. (A) MJI. (B) MBK (C) MMI (D) MKB (E) MSR (F) MPB (G) MBS (H) MSL.

Figure 1. above shows the existence of all the microflora contained in forest honey. In each sample of honey there are bacteria, yeasts and molds in different proportions. The average of each microflora that is in honey can be seen in Table 2 as follows:

Table 2: Average Presence of Natural Hydroflora of Forest Honey.

No	Sample	Total Bacteria (..x10^9.cfu/ml)	Total Yeast (..x10^8.cfu/ml)	Total Mold (..x10^7.cfu/ml)
1	MJB	0,05	0,23	0
2	MBK	0,05	0,03	0
3	MMI	61,70	0,01	0
4	MSR	0,26	1,50	60,00
5	MKB	0,85	0,18	0
6	MPB	0,93	49,00	0,03
7	MBS	55,60	18,00	6,00
8	MSL	0,28	0,50	0

Descrption : (MJ: Jambi), (MBK: Bengkulu), (MM: Mentawai), (MK: Kalimantan Barat), (MS: Surabaya), (MPB: Pasaman Barat), (MBS: Batusangkar), (MSL: Sulawesi Selatan).

Based on Table 2. we can see the average existence of microflora in some samples of forest honey on GPA medium. The types of microflora contained in forest honey are bacteria, yeasts and molds. The highest total bacteria were Batusangkar honey samples of 55.60 x109 cfu / ml, and the lowest in Bengkulu and Jambi honey were 0.05 x 109 cfu / ml, which had exceeded the APM limits of bacteria (SNI, 2013) ie <5 x103 colony / g. The highest total yeasts were found in West Pasaman honey, 49.00 x108 cfu / ml and the lowest in Mentawai honey 0.01 x108 cfu / ml. While the highest number of shrimp found in Surabaya honey is 60 x107 cfu / ml. This can be caused by various factors that exist around the honey as well as contained in the honey itself, such as natural factors that occur when the process bees produce honey and honey storage factor after being matured and removed from the cell.

In the results obtained a small percentage of the sample of forest honey is present which has microflora mold, and most do not. This may be due to the long period of storage of the sample of the forest honey itself before it is processed. According to Rozanska and Osek (2012) states that the amount of yeast and mold in honey is

usually low but in some conditions the organism is able to multiply in honey during storage. Significant increases in the amount of yeasts and molds occur after storage of honey for long periods of time. These organisms can cause fermentation of honey during storage. In normal fresh honey, bacteria are found in small amounts, a high amount indicates the presence of honey contamination from secondary sources. Theoretically the bacteria should not grow in the honey but some of them may persist in it.

The presence of microflora in the GPA medium can be illustrated in the following graphs:

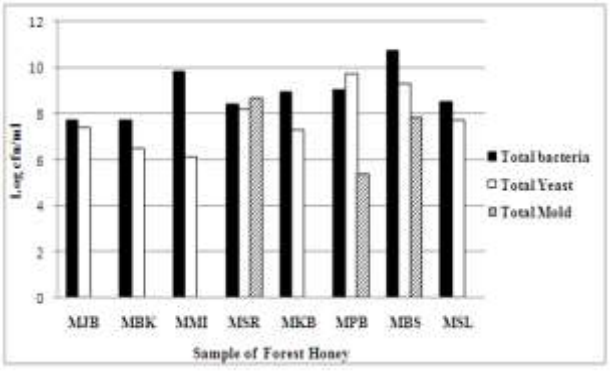

Figure 2: Average number of natural microflora in some Forest Honey on Medium Glucose Pepton Agar.

In the graph above can be seen the comparison of the average presence of bacteria, mold and yeast in forest honey. The growth of one type of microflora will affect the growth of other microflora. One of them is between mold and yeast, which in general can be seen if there is yeast in one honey, it is less likely to grow mold. Because if there is a lot of yeast then there is also a lot of alcohol, while mold is one of the microflora who do not like alcohol.

The GPA medium is used to look at the overall microflora present in a sample of forest honey. As can be seen in Figure 2. The colonies are seen to describe the overall microorganisms that reside in each sample of forest honey. According to (Hadioetomo, 1993) the number of colonies present in petri dishes is the number of organisms that can live and be contained in the sample. To qualify, the petri dish chosen for colony calculations is that it contains 30-300 colonies. Since the number of microorganisms in the previous sample is unknown, to obtain at least one petri dish containing a number of eligible colonies a series of dilutions are performed. Periadnadi and Nurmiati (2010) also stated that basically all bacteria like sugar and a little peptone for its growth, so that in GPA medium is depicted all kinds of bacteria contained in a sample, be it bacteria, yeast, and mold. Variations in the amount of bacteria produced from honey may be due to the type of sample, which can be seen from the freshness of honey, harvest time and analytical techniques used in the process of honey itself (Snowdon and Oliver, 1996).

4. CONCLUSION

Based on the research that has been done can be concluded that:
1. The highest total bacteria was found in Batusangkar Honey sample of 55.60x109cfu / ml, while the lowest was in Bengkulu and Jambi Honey samples of 0.05 x 109 cfu / ml. The highest total yeasts were found in the sample of Western Pasaman Honey, 49.00 x108 cfu / ml and the lowest in Mentawai Madu samples 0.01 x108 cfu/ml. The highest total bulk is found in Surabaya Madu sample of 60,00 x107 cfu / ml and the lowest in sample Madu Jambi, Madu Bengkulu, Madu Mentawai, West Kalimantan Honey and South Sulawesi Honey.

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