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Bacteriological Quality Test of Southeast Sulawesi Honey to Support Functional Food

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ABSTRACT

Honey is widely utilized as a food and beverage additive in the industrial field, as a cosmetic ingredient, and as a medicinal ingredient from ulcer disease, coronary heart disease. However, honey does not always have guaranteed quality, because the processing process is less hygienic, so that microorganisms can grow and multiply, and become less safe if consumed. The purpose of the study was to test the bacteriological quality of processed honey in the people of southeast Sulawesi.

This type of research is an experiment using the MPN (*Most Probably Number*) method, identification test, gram staining, biochemical tests. The results showed that out of 20 honey samples, 6 honey samples were found to be positive for Coliform bacteria, of the 6 positive samples, biochemical testing was continued and 1 sample was found to be positive for *E.coli* bacteria. The conclusion of the study is that the Colifrom and *E. coli* bacteria found in honey samples in dunga come from the containers used that are less hygienic.

Keywords: Bacteriology Test, Honey, Functional Food, Southeast Sulawesi

INTRODUCTION

Honey is widely used as a food and beverage additive in the industrial field, as a cosmetic ingredient, food ingredient and medicinal ingredient (Fona *et al.*, 2022). Furthermore, Rohmi (2014) explained that honey that is packaged and consumed does not always have guaranteed quality, which can be caused by processing that pays less attention to cleanliness in the production process and cleanliness of tools so that microorganisms can grow and multiply. The danger of microorganisms is related to packaging materials in honey because some materials may be contaminated with bacteriology. Storage conditions must be standardized and hygienic so as to reduce the possibility of contamination (Muna, *et al*., 2020). In some cases the packaging or container needs to be sterilized before use or sterilized after the container is filled. Another risk is the introduction of toxic components from the packaging material into the food or the transfer of odors from the packaging material to the honey product (Rohmi *et al*., 2014).

According to BPOM RI (2009), to maintain the safety of a product so that it can still be consumed safely by the public, one of the efforts that can be made is through bacteriological quality testing. Testing the quality of a food ingredient requires various tests which include physical tests, chemical tests, bacteriological tests, and organoleptic tests. Chairunnisa (2019) explained that bacteriological testing is one of the most important tests, because in addition to being able to predict the shelf life of a food, it can also be used as an indicator of food sanitation. Bacteriological testing includes qualitative tests and

quantitative tests of pathogenic bacteria to determine the level of safety, and indicator bacteria tests to determine the level of food sanitation (Trisnaini, *et al*., 2018).

In the process, honey obtained directly from the forest is stored in plastic bottles that are not sterilized. The packaging is taken from used beverages which are then cleaned with unsterilized water. Contamination caused by coliform bacteria can disrupt the digestive system. Generally, coliform bacteria are nonpathogenic but if excessive in the digestive tract can cause acute and chronic diarrhea (Hutasoit, *et al.*, 2020). Standardized and hygienic processing and production of food ingredients will produce food products that are safe and free from contamination of disease-causing bacteria (Nurlila, *et al*., 2019).

RESEARCH METHOD

This research is an experimental study by conducting bacteriological quality tests on honey. The work procedure for examining honey samples using the MPN method consists of 3 stages: dilution, estimation and confirmation.

Sample dilution

Honey samples were weighed as much as 25 grams and then dissolved with distilled water in 250 ml of erlenmeyar until homogeneous then pipetted honey samples of 10 ml1 ml and 01 ml each in the estimation test. (BPOM RI.2013)

Estimation test

Pipetted 10 ml '1'ml'0'1ml sample (10-1, 10-2, 10-3) into each: 3 tubes of Lactose Broth Double Strength, each 10 mL, 3 tubes of Lactose Broth Single Strenght 1 mL, 3 tubes of Lactose Broth single Strength 0.1 mL. Put the tubes into an incubator at 37 ^{O}C for 24 hours. Each Lactose Broth (LB) tube that shows positive gas is recorded (Nisha, 2017).

Confirmation Test

Gas positive results on LB media were transferred into 2% BGLB media as much as 1 - 2 ose eyes then incubated at 37 ^{o}C for 24 hours. The observation results on BGLB media showed positive gas read in the MPN table, to get the Coliform index. After that, it is planted on EMBA and SSA media, then incubated at 37 ^{o}C for 24 hours (Nisha, 2017).

Clarification:

Preparation of Eosin Methylen Blue Agar (EMBA) media :

Preparation of tools and materials: Weighing 3.75 grams of EMBA media, put in a suitable erlenmeyer then dissolve with distilled water up to 200 mL, then shake until homogeneous. Checked pH 7.1 ± 0.2 then cover with cotton and parchment paper or aluminum foil. Homogenized using a waterbath until the powder is completely dissolved. Then sterilize in an autoclave at 121 ^{o}C for 15 minutes. Cool to a temperature of 40-50 ^{o}C, then stored in the refrigerator (Fardiaz, 2010).

Preparation of Salmonella Shigella Agar (SSA) media :

Preparation of tools and materials: Weighing 12.6 grams of SSA media then put it in an Erlenmeyer. Dissolve SSA powder in Erlenmeyer with 200 mL of distilled water, stirring until homogeneous. The solution is heated while stirring until completely dissolved. Measure the pH of the SSA pH solution is 7

± 0.2. Media was poured into the plate as much as 15-20mL with the fixation process. The media is labeled with the name and date of manufacture. The media is ready for use (Fardiaz, 2010).

Gram Stain Test

Take a colony with a round ose on EMBA and SSA media and then place it on a glass object that has been given 0.95% NaCl, flatten it with an ose. After drying, it was heated on a Bunsen flame. The preparation was stained with gentian violet carbolic dye for 1 minute, then rinse with running water. Drip with lugol solution for 1 minute, rinse running water and flood with 96% alcohol for 30 seconds. Pour safranin solution for 1-3 minutes. Rinse with running water, dry and then examined using a 100x magnification microscope using oil emersion solution.

Biochemical Test

A. Take TSIA media, open the tube with the little finger and then fix the surface of the tube, take the colony using an ose needle and then stab the ose that contains the bacteria to the bottom, do not damage the media and then scratch with a zigzag shape and cover the tube again with cotton.
B. Take SIM media, open the cotton cover, fix the mouth of the tube, puncture the ose needle to the bottom, but do not hit the bottom of the tube and close the tube again with cotton.
C. Take the citrate medium, open the cotton swab, fix the tube mouth, scratch the ose needle from bottom to top with the zig zag method, but do not damage the agar and re-cap the tube with cotton.
D. Fix the ose needle until red and then cool.
E. Take MR media, open the cotton cover, fix the tube mouth, stick the ose needle, shake the tube and cover the tube with cotton again.
F. Take VP media, open the cotton swab, fix the tube mouth, insert the needle ose, shake the
G. tube and cover the tube with cotton swab.
H. Incubate the media for 24 hours at 37 ^{o}C
I. Observe the bacterial colonies formed on each media. (Amiruddin, 2017).

Interpretation of Results

Positive ; Coliform bacteria identified in honey samples

Negative ;No Coliform bacteria identified in honey samples.

MPN Index Formula:

MPN Index ;MPN Table x 1 / Center dilution facto. (Nisha, 2017)

The following are the results of the MPN (*Most Probably Number*) test of southeast Sulawesi honey samples as functional food.

Table 1. MPN (*Most Probably Number*) Test Results

N o	**Sample type/code**	**Media used LB 37^{0}C**			**BGLB 37^{0}C**			**able value MPN/100ml Colifrom**	**Formula value 1/FPT**	**Bacteria E.coli**
		10 ml	**1 ml**	**0,1 ml**	**10 ml**	**1 ml**	**0,1 ml**			
1	MD.01	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)

2	MD.02	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
3	**MD.03**	**3/1**	**3/0**	**3/1**	**3/1**	**3/0**	**3/1**	**7**	**700**	**Negative (-)**
4	MD.04	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
5	MD.05	**3/1**	**3/0**	**3/0**	**3/1**	**3/0**	**3/0**	**4**	**400**	**Negative (-)**
6	MD.06	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
7	**MD.07**	**3/2**	**3/0**	**3/0**	**3/2**	**3/0**	**3/0**	**9**	**900**	**Negatifve(-)**
8	MD.08	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
9	MD.09	3/0 **3/2**	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
10	**MD.10**		**3/1**	**3/1**	**3/2**	**3/1**	**3/1**	**20**	**2.000**	**Negative (-)**
11	MD.11	3/0 3/0		3/0	3/0	3/0	3/0	0	0	Negative (-)
12	**MD.12**	**3/3**	**3/0**	**3/1**	**3/3**	**3/0**	**3/1**	**39**	**3.900**	**Negative (-)**
13	MD.13	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
14	MD.14	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
15	MD.15	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
16	**MD.16**	**3/3**	**3/3**	**3/1**	**3/3**	**3/3**	**3/1**	**460**	**46.000**	**Positive (+)**
17	MD.17	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
18	MD.18	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
19	MD.19	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)
20	MD.20	3/0	3/0	3/0	3/0	3/0	3/0	0	0	Negative (-)

Based on the table above, of the 20 honey samples analyzed, there were 6 positive samples of Colifrom that did not meet the maximum limit of Coliform bacteria set in the regulation of the Minister of Health of the Republic of Indonesia No. 492/MENKES/Per/IV/2010, which is the maximum level of

Coliform bacteria 0/100 ml. The following are the results of bacterial identification and biochemical tests on southeast Sulawesi honey samples.

Table 2. Results of Bacterial Identification and Biochemical Test

No	Sample Code	Gram staining	Colony Media		Biochemical Test							
			EMBA	SSA	D	L	H^2S	Gas	Sitrat	SIM	MR	VP
1	MD.03	Gram-negative rods	(+)	(-)	A	A	(-)	(-)	(+)	(-)	(-)	(+)
2	MD.05	Gram-negative rods	(+)	(-)	A	A	(-)	(-)	(+)	(-)	(-)	(+)
3	MD.07	Gram-negative rods	(+)	(-)	A	A	(-)	(-)	(+)	(-)	(-)	(+)
4	MD.10	Gram-negative rods	(+)	(-)	A	A	(-)	(-)	(+)	(-)	(-)	(+)
5	MD.12	Gram-negative rods	(+)	(-)	A	A	(-)	(-)	(+)	(-)	(-)	(+)
6	**MD 16**	**Gram-negative rods**	**(+)**	**(-)**	**K**	**K**	**(-)**	**(+)**	**(-)**	**(+)**	**(+)**	**(-)**

Description: A; acid (red), K; Alkali (yellow), Positive (+) the presence of bacterial growth, Negative (-) no bacterial growth.

Based on the table above, 6 samples grew colonies on positive EMBA media, while SSA media did not grow colonies. While the biochemical test on TSIA media obtained results at the base of alkaline media, the slope is alkaline, H2s there is no black precipitate, the gas is positive. In citrate media there is no color change, in positive SIM media a red ring is formed, Methyl red media is positive and in Voges proskauer media negative results are obtained.

Figure 1. EMBA media Metallic Green Colonies

DISCUSSION

The results of the study by analyzing bacteriological tests on 20 honey samples in southeast Sulawesi obtained the results that bacterial growth as many as 6 positive samples contained Coliform bacteria, namely in the sample code MD. no 16 with the highest number of Coliform bacteria, namely in MD. no 16 with the number of Coliform bacteria, namely 460 MPN/100 ml in the calculation of the number of 46,000 cells / ml, MD.no 12 is 39 MPN/100 ml, the calculation value of 1 / FPT 39,000 cells / ml. MD no 10 is 20 MPN/100 ml, calculation value 1/FPT 2000 cells/ml, MD no 07 is 9 MPN/100 ml, calculation value 1/FPT 900 cells/ml. MD no.3 is 7 MPN / 100ml, the calculation value of 1 / FPT 700 se / ml while the lowest value is 4 MPN / 100 ml, the value of 1 / FPT 400 cells / ml from the MPN table value, then the calculation is carried out by entering the MPN table value into the formula 1 / factor of the middle dilution shows results that do not meet the health requirements based on SNI-01-3546-2004 regarding the limits of food and beverage contamination.

The results of the identification test on EMBA media obtained 1 positive sample with sample code MD.no. 16 there is growth of Coliform bacteria with the type of E.coli bacteria, with macroscopic characteristics of metallic green colonies caused by bacteria that can ferment lactose so that there is an increase in acid levels in the media while microscopic rod-shaped, red-colored which is a class of gram-negative bacteria, in Biochemical tests on TSIA media obtained results on the base and slopes of alkaline media, H2S does not form a black precipitate, positing produces gas, in Citrate media no color change occurs, in positive SIM media a red ring is formed after the addition of covac indikataor, and MR media is positive while VP media is negative. Based on these characteristics according to Andriani (2014), it shows the characteristics of E.coli bacteria.

One indicator of the quality of food and beverages is the presence of Colifrom bacteria. (Alifia, *et al*., 2021) The presence of Colifrom bacteria in food and beverages indicates that food and beverages are polluted by human and animal waste. In addition, the processing of food and beverages can also affect quality. Colifrom bacteria are a class of bacteria that live in the human digestive tract, these bacteria are indicators of the presence of other pathogenic bacteria (putra, 2018). Such as E.coli. The presence of Colifrom bacteria in honey samples can be sourced from honey processing processes that still do not pay attention to good standards.

Based on the results of this study, worker hygiene is very important and related to the content of Coliform and E.colli bacteria in honey. This is due to worker hygiene as one of the intermediary media for bacterial contamination of honey in packaging, direct contact between workers and tools and the place used to hold honey is not sterile. The sanitation of equipment used in the packaging of traded honey uses used aqua containers, 20 liter jergen, sieve stirrers to filter honey, or spoons that are not guaranteed cleanliness. In a study conducted by Darna, *et al* (2017) it was found that there was a contamination of Coliform bacteria in the traditional food ingredients analyzed, this illustrates that Coliform contamination can occur in traditional foods that are not properly processed and can even occur up to the unhygienic packaging process. In this study, *E.coli* contamination found in sample MD.No 6 can occur as a result of a less hygienic processing process. Escherichia coli is a gram-negative rod-shaped bacterium in single or paired cells, a member of the Enterobacteriacea family and normal intestinal flora that contributes to normal intestinal function and nutrition but these bacteria will become pathogenic when they reach tissues outside the intestinal tissue. *E.coli* species are motile with their peritrichic flagellum, work because the processing environment is not provided with a place to wash hands and the soap used. Sinarto (2020) states that hand washing has long been recognized as the most effective way to reduce disease transmission

between both lay people and professionals. Hutasoit, (2020) Consumption of food or food ingredients contaminated with bacteria causes health problems and causes infectious diseases in digestion. Furthermore, Whardana (2021) states that Colifrom and *E.coli* contamination in food can be caused by processing and equipment that pay less attention to hygiene. Of the 20 honey samples sold on southeast Sulawesi, 6 positive samples contained Colifrom bacteria which exceeded the maximum limit of 0 MPN / 100 ml and 1 positive sample contained Colifrom bacteria with the type of *E.coli* bacteria, the sample was not suitable for consumption (Ruhi, *et al.*, 2020).

CONCLUSION

Based on the results obtained from this study, it can be concluded that of the 20 honey samples found in southeast Sulawesi, there were 6 positive samples containing Coliform bacteria and 14 samples did not contain Colifrom 0 MPN/100ml bacteria, and of the 20 samples studied, 1 sample contained pathogenic bacteria, namely *E.coli* bacteria which were thought to come from less hygienic honey containers.

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