MICROBIAL CONTAMINATION IN *LARU* (LOCAL BEVERAGE OF EAST NUSA TENGGARA)

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ABSTRACT

This study aims to describe the microbiological contamination (*E.Coli*) in the local beverage of Timorese community in Naimata village, Kupang city, East Nusa Tenggara. Three white *laru* samples were taken from different sellers and analyzed at the Technical Implementation Unit of East Nusa Tenggara Province Health Laboratory. The results of the analysis showed that the white *laru* samples are free of microbial contamination of *E.Coli* whereas other microbial contamination in the packaging has exceeded the maximum limit based on Indonesian National Standard. *Laru* contains a number of organic acids, amino acids, and alcohols.

Keywords: laru, e.coli

INTRODUCTION

The palm trees have many benefits for the people of Timor. Palm leaves can be formed into a variety of containers, such as bowl or basin, plate, bucket, even as hats called *Ti'i Langga*, as well as materials for making musical instruments of *sasando*. Palm fruit, commonly called *saboak* also be consumed. The flesh is slightly juicy if it is still young, and it will be slightly chewy and will be hard if it is old. Palm flowers have nectar that is usually tapped and serve a variety of drinks, from palm wine (nectar of freshly tapped palm) (Borassus flabellifer L.), *gula aer*, palm sugar, powdered sugar, even alcoholic beverages such as *laru* and *sopi*, and vinegar. Palm sap can be used to heal scars.

Laru is very popular among the people of East Nusa Tenggara, especially those from the Timor, Rote and Sabu tribes. Generally, *laru* is always available in almost every event, both family and formal even though it is not officially served. If it is drunk a little, it will provide warmth to the body because of fermented alcohol-containing products. If consumed in excessive amounts, it will cause interference with gesture coordination, mental ability, ability to make decisions and talk. If consumption is increasing, alcohol can cause fainting or death.

In Kupang City, white *laru* sales can be found in Naimata sub-district. The observation of the preliminary finding was the lack of concern about food hygiene and sanitation and personal hygiene when processing or selling the *laru*. The problem of hygiene for both containers and laru is still undoubtedly a safe level, especially from microbial contamination such as *Escherichia coli*. This study aims to describe the microbiological contamination (E. coli) in the local beverage of Timorese community in the Naimata sub-district, Kupang city.

MATERIALS AND METHODS

This type of research is descriptive type to clarify the picture content of the bacteria contained in white *laru*. Researcher analyzed the content of E. coli and Total Plate Count of bacteria in samples of white *laru* and packaging, and then compared with the Indonesian National Standard.

Sampling of white *laru* and tool swab specimens on white *laru* packaging was carried out at 3 random sellers located on the edge of the highway in Naimata Sub-district, Kupang City in September 2018. The sample of this study was a white laru and a container/packing swab used to sell the white laru. Analysis of E. coli and TPC content was carried out at the Technical Implementation Unit of NTT Province Health Laboratory.

Tools and materials for sampling in the field were white *laru*, bottles for filling new samples, sterile cotton sticks (lidi water), namely sticks which end is folded with cotton, sterile/clean gloves, lowercase markers, small scissors, label paper, spirit lights, ice flasks, and alcohol.

Equipment and materials for examination of E. coli were test tubes, bunsen, Petri dish, scales, 1 ml measuring pipette, label paper, knives, mortars, bottle, white *laru*, Medium EA, Medium SS-Agar, and Medium S110 Agar.

Tools and materials for checking Total Plate Count of bacteria are Medium Nutien Agar, dilution/0.9% NaCl, 4 pieces of Sterile Petri dish for each examination, 2 pieces of sterile pipettes, 4 pieces of spirit lights/bunsen, matches, and test tubes, and a wooden shelf.

The sampling procedure is as follows: Prepare sterile gloves to start taking samples. Prepare a sterile stick, then open the bottle cap and put a sterile cotton stick in it. Sterile cotton sticks in the bottle are pressed into the bottle wall to remove the liquid, newly removed and rubbed on the container used to fill and sell the white *laru*. The rubbed surface of the container/package is: the outer and inner surfaces of the bottle lips from jerry cans or a bottle of aqua packaging as high as 6 mm. The way to do swabs is by sweeping around the bottle lips. Each field of surface that is rubbed is carried out 3 times in a row, and one cotton stick is used for one container/package of white *laru* examined. After the container has been wiped, the cotton stick is inserted into the bottle, the *lidi* stick is broken or cut, and the lip of the bottle is heated with spirits fire, and then covered with cotton. Stick the label paper that has been provided. Write the label with a marker stating the name of the tool and the place where the sample is taken (code). Samples are stored in ice flasks and sent immediately to the laboratory with cold temperatures to be examined.

The procedure for examining E. coli content is as follows. Take 11 grams of material and put it in a bottle containing 99 ml of sterile water then shaken (dilution 10-1). 1 ml was taken from the bottle and put in the test tube 1 containing 9 ml of sterile water then shaken (dilution 10-2). 1 ml was taken from 10-2 dilution and put in a test tube 2 containing 9 ml of sterile water then shaken (10-3 dilution). Dilution was carried out up to 10-4. Making plating in duplicate in Petri dish by taking as much as 0.1 ml from each dilution for each test of E. coli 10-3, 10-4, 10-5. Incubate for 2x24 hours, then observe in Petri dish about the appearance of E. coli and then count the amount.

The procedure for examining TPC of bacteria is as follows. Prepare a solution of 0.9% NaCl diluent as much as 4 tubes each and 4 Petri dishes which were coded 10-1, 10-2, 10-3, and controls. Take 1 ml of sterile NaCl solution with the control code and put it in the Petri dish that has been tagged with control code. Take 1 ml of the sample with a sterile pipette and put it in a test tube with a code of 10-1 dropper pipette and should not be closed. 1 ml pipette from tube 10-1 and put it into Petri dish which is coded 10-1 and the remaining 1 ml into tube 10-2 dropper pipette 25 times or many times. 1 ml pipette from tube 10-2 put 1 ml into the Petri dish which has been coded 10-2 and insert it into the 10-3 tube dropper 25 times or many times. 1 ml pipette from tube 10-3 put into Petri dish given code 10-3. Pour the Petri dish containing the sample with Nutrient agar 55° C - 56° c as much as ± 15 ml. Shaken until blended and left frozen. Add the Petri dish in the incubator to be covered and the Petri dish is reversed at 37° C for 1 x 24 hours and examined for gas formation in the Durham tube.

The results of bacterial E. coli and TPC content from the laboratory were then analyzed descriptively to explain the description of the bacterial content contained in the white *laru* sold in the Naimata sub-district area.

RESULTS

Profile of Naimata village

Naimata village is included in the administrative area of Maulafa subdistrict and Kupang City. The distance from Naimata village to the center of Maulafa subdistrict is around 3 km and to the center of Kupang City is less than 10 km. The location of this village within the city of Kupang makes it easy for people to access health facilities and public places quickly. At present, the Naimata village area has also been serviced by public vehicles in the form of Damri buses so as to facilitate community mobility.

The population of Naimata village in 2013 was 3,059 people, consisting of 1,592 men and 1,467 women, and the majority of the populations were young. Education from the population also varies, but more are elementary and high school educated. The daily occupations are farmers (14.74%) and unemployed (64.79%). The populations included in the non-working category are housewives and children who are still in the education bench.[1]

Residents domiciled in Naimata village come from various ethnic groups, both from East Nusa Tenggara (NTT) and outside NTT. Most of the population is from Timor and Rote, the rest are from Sabu, Sumba, Flores, Alor, Java, Batak, and Sulawesi.

White *laru* seller

People who live as sellers of white *laru* generally come from Rote and Timor. This is because the culture of drinking *tuak* (palm wine) or *laru* is true in the traditions of both ethnic groups. White *laru* sold are generally produced by themselves. The production of white *laru* starts from slicing palm wine (palm tree flowers) in the early hours of the morning and then the sap is collected so that at around 08.00 WITA (Indonesia Central Standard Time), it is ready for sale. In the Naimata village, there are still palm trees which grow close to community settlements, making it easier to do the process of slicing palm wine. Palm wine (*tuak*) or white *laru* collected are then poured in packs in the form of bottles of 1 liter mineral water or in 5-liter jerry cans. Each bottle containing 1 liter of white *laru* is sold for Rp. 5,000 - Rp. 7,000. The seller claims to be able to get up to IDR 70,000 per day, but the amount of income varies every day.

The sellers of the white *laru* who peddle daily along the highway that crosses the Naimata village ranges from around 15 people. The selling time starts from around 8:00 a.m. until the afternoon at 5:00 p.m. and takes place from Monday to Friday every week. The activity of selling white *laru* is not carried out on Saturdays and Sundays because it is a time

of work holiday so the streets that are traversed are usually deserted so that the number of buyers is reduced.

The observation of the completeness of the dressings of the white *laru* sellers at Naimata village showed that all of them wore everyday clothes and did not use the fittings when selling white *laru*, such as headgear, gloves, aprons and masks.

White l*aru* sales package

The equipment used to sell white *laru* is quite simple. White *laru* that is ready for sale are accommodated in a large 20-liter jerry can or in a large bucket and measured into bottles of 600 ml and 1 liter mineral water using the bailer. The package for this white *laru* is obtained from used bottles that are washed and dried, then used.

Table 1. White *Laru* Sales Package in Naimata village 2018

No.	Package of white laru	Package	
		Mineral water bottles	Jerry can
1.	Seller 1	Yes	Yes
2.	Seller 2	Yes	Yes
3.	Seller 3	Yes	Not

About 5-6 bottles of white laru were put on the wooden table placed on the roadside. Another thing that was encountered when observing in the field was that there were sellers who did not use bottle caps that ended up in allowing the dust or other debris to lace the white laru being sold.

Chemical Ingredients Palm Tree Product

Some of the products produced from palm trees are palm wine, *gula aer*, laru and gin (*sopi*). Chemical content of each product are presented in Table 2 and Table 3.

Table 2. Chemical ingredients of palm product

Contents	Palm wine	*Gula aer*	*Laru*	Gin (*sopi*)
Fructose (%)	4.0	4.5	-	-
Glucose (%)	3.5	4.6	-	-

Sucrose (%)	3.6	8.9	-	-
Water (%)	85.2	80.1	-	-
Alcohol (%)	-	-	6.4	7.0
Malic acid (ppm)	-	-	11.5	-
Citrate (ppm)	-	-	4.6	-
Glutamate (ppm)	-	-	7.9	-
Tannin acid (ppm)	-	-	8.4	-

Source: Naiola (2008)

Table 3. Ethanol (alcohol) and acetic acid in the fermented palm wine product

Contents	**White *Laru***	**Red *Laru***	**Gin**
Ethanol (%)	4.8 to 5.8	5.8 to 7.7	19.5 to 20.6
Acetic acid (%)	0.4-0.5	0.35 to 0.4	0.03 to 0.3

Source: Rahmansyah and Kanti (1999)

The amount of e.coli in white *laru*

The results of the analysis of microbial contamination in white *laru* based on E. coli content in several white *laru* sellers in Naimata sub-district are shown in Table 4.

Table 4. Microbial contamination in white *laru* based on E. coli content

No.	**White *laru***	**Microbial contamination levels of based on E. coli content**		**Average**	**Limit contamination**
		Sample 1	**Sample 2**		
1.	Seller 1	0	0	0	*E. coli*: <3 / ml
2.	Seller 2	0	0	0	
3.	Seller 3	0	0	0	

Table 4 shows that white *laru* samples are free of microbial contamination of E.coli indicated value of 0 and is under the maximum limit contamination of <3 / ml. This shows that white *laru* sample is not contaminated by E. coli bacteria.

Total plate count of bacteria in white *laru*

The results of the analysis of microbial contamination in white *laru* based on Total plate count of bacterial content in several white *laru* sellers of Naimata sub-district are shown in Table 5.

Table 5. Microbial contamination in white *laru* based on total plate count

No.	White *laru*	Microbial contamination levels based on total plate count		Average	Limit contamination
		Sample 1	Sample 2		
1.	Seller 1	0	0	0	TPC: 2x102 colonies/ml
2.	Seller 2	0	0	0	
3.	Seller 3	0	0	0	

Table 5 shows that the sample of white laru free from microbial contamination which indicated a value of 0 for TPC (total plate count), and is under the maximum limit contamination of 2x102 colonies/ml. This is presumably because the sampling time in the morning so it is not exposed to dust or contaminated with bacteria due to the behavior of white *laru* sellers.

Total plate count of bacteria in white laru package

The results of laboratory tests to find out TPC on white *laru* package are presented in Table 6.

Table 6. Microbial contamination in white *laru* package based on TPC content

No.	White *laru* package	Microbial contamination levels based on TPC		Average	Limit contamination
		Sample 1	Sample 2		
1.	Seller 1	575	425	500	TPC: 2x102 colonies/ml
2.	Seller 2	300	200	250	
3.	Seller 3	1413	1370	1391.5	

Table 6 shows that the microbial contamination in bottled white laru measured by TPC mostly in white *laru* package of seller number 3 with an average of 1391.5 colonies/ml and the lowest in the white *laru* package seller number 2 with an average of 250 colonies/ml. The average rate of contamination based on TPC from white *laru* package has exceeded the maximum limit, namely 2x102 colonies/ml.

DISCUSSION

Chemical content in palm products

Palm products in the form of sap and *gula aer* contain sugar, which consists of simple glucose and disaccharides in the form of fructose and sucrose. *Gula aer* has a higher sugar content compared to sap. Therefore, Timorese and Rotenese people often consume *gula aer*, especially in the morning before they move because it gives a satiety effect and produces energy.

Laru and *sopi* are traditionally local alcoholic drinks that are fermented. The alcohol content of both types of drinks can warm the body when consumed, but the excessive amounts of consumption can cause a state of drunkenness. Besides alcohol, *laru* also contains a number of organic acids and amino acids (Table 2). The content of acetic acid in the white *laru* is higher than the red *laru* and *sopi*. The alcohol content in *sopi* is higher compared to the *laru* (Table 3).

Microbial contamination of E. coli and total plate count in white *laru*

E. coli is one species of Escherichia species and is called fecal coliform because it is found in intestinal tracts of animals and humans, so it is often found in feces. These bacteria are often used as indicators of dirt contamination (Fardiaz, 1992). E. coli can grow optimally at pH 7-7.5 with a minimum pH of 4 and a maximum pH of 8.5. These bacteria are sensitive to heat and to foods that are warming up. Freezing in freezer storage (cold storage) inhibits bacterial growth, but does not kill bacteria. But the effect of freezing in deep freezer storage (frozen storage) up to temperatures <-10 0C can reduce the population drastically and kill the Escherichia coli bacteria slowly (Nurwantoro, 1997).

E. coli grows at an air temperature of 10-400C, with an optimum temperature of 370C. The temperature most suitable for bacterial growth is 10-600C. This temperature is called the danger zone. Foods that are still guaranteed to be safe take the longest time in 6

hours, because if more than that the food has been heavily contaminated. Safety zone is < 100C and > 600C. The practice is below 100C, which is in the refrigerator and above 600C, which is in a container that is always on a heating fire.[3]

Low temperatures do not kill organisms but inhibit their proliferation (dormancy). Freezing causes a little damage to microorganisms. This damage can be reversible or cause cell death. This damage depends on the type and speed of the freezing process. Rapid freezing with very low temperatures does not or only slightly causes bacterial cell damage so that in favorable conditions the bacteria can return to activity while slow freezing with relatively high freezing temperatures (up to -10 0C) can cause severe damage to bacterial cells and causing death in bacteria.[4]

For fresh water in the three sellers, the result was zero or no E. coli bacteria and other types of bacteria were found (TPC Test). As a gram-negative bacteria, E. coli bacteria have the ability to defend themselves against environmental conditions that do not benefit their lives. In this case, the *laru* served (stored) in a relatively long period of time will turn sour. PH levels that are too low do not allow bacteria in general, but for some bacteria, they can survive including E. coli bacteria.

The test results from the sample showed that E. coli colonies and other groups of bacteria were not found in the *laru*. The absence of E. coli bacteria in *laru* and other species both in sellers 1, 2 and 3 was due to samples being taken in the morning (taken shortly after being dropped from the tree). This indicated that the source of E. coli was not from the source. The presence of E. coli bacteria in the *laru* was caused by the use of a new container that was less clean and the seller or buyer themselves (personal hygiene) and also the exposure of dirty air entering from the road (sanitation). This is consistent with the results of Kurnia's research, at the Tangerang General Hospital, which shows that 4 out of 10 food samples tested positive for E. coli. The presence of E. coli is due to hygiene and sanitation of

food processing at the Tangerang General Hospital, especially in the protection of food, location and buildings, sanitation facilities, cooking and eating restrictions, food handlers and bacteriological quality of food based on Kepmenkes No. 715/MENKES/SK/V/2003 included in the category of not fulfilling the requirements.[5]

Microbial contamination based on total plate count on white *laru* package

The laboratory results showed that microbial contamination in the package of the white *laru* based on the highest TPC was found in the third white *laru* seller with the amount of germ of 1391.5 colonies/ml, while the lowest was the seller number two with the amount of germ of 250 colonies/ml. The high amount of microbial contamination in the swab packaging of the third seller of white *laru* is due to the lack of washing intensity, which causes the cleanliness of the bottle and jerry cans to remain dirty even getting dirty because there are several bottles and refillers. Clean washing will leave a bulk containing enough nutrients (glucose) which when entered by the total bacterial seeds non-E. coli will live and develop to form fertile colonies (logarithmic phase). In addition, the place of sale is open so that it is easily contaminated by both consumers who hold the packaging when choosing to buy and also the peddled packaging is too close to the roadside with vehicles that every time go through the road making the dust fly and exposed to packaging. Food can be contaminated with microbes because of several things: processing food with dirty hands, using dirty equipment, dirty environment, reachable food for animals such as insects and mice, raw and cooked ingredients are stored together, processing food with dirty water. Further research to identify the types of microbes present in the TPC content of new reservoirs needs to be done to ensure that the species are parasitic for humans or only exist and do not endanger human health.[6]

Hygiene and food and beverage sanitation is an effort to control food, people, places and equipment factors that can or may cause illness or health problems. Sanitary hygiene

requirements are technical provisions stipulated for cook shop and restaurant products, personnel and equipment which include bacteriological, chemical and physical requirements.[7]

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