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Research Article

Analysis of Microbial Content in Local Timorese Drinks (Sopi) in Several Community Businesses in Sikumana Village, Kupang City in 2024

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Abstract

This research aims to describe the microbial content in the local drink of the Timor Community (Sopi) in several community enterprises in Sikumana Subdistrict, Kupang City, in 2024. Three samples of sopi were taken from different sellers and analyzed by the Health Laboratory Unit of NTT Province. The analysis results showed that the sopi samples were free from *E. coli* contamination, while contamination from other microbes on the equipment and the hands of the sopi distillers exceeded the maximum limit based on the Indonesian National Standard (SNI).

Keywords: *Escherichia coli*; ALT

Introduction

The lontar tree has many benefits for the people of East Nusa Tenggara, which is why it is commonly called the tree of life. Lontar leaves can be shaped into various containers, such as haik or containers for holding sap, woven into food containers, footwear, bed mats (called tikar), and even hats called Ti'i Langga, as well as being used in making the musical instrument Sasando. The fruit of the lontar, known as siwalan, commonly called saboak, is also consumed. Its flesh is slightly watery when young, and it becomes slightly chewy and hardens as it matures. The lontar flower produces sap that is typically tapped and served in various beverages, including fresh sap from the lontar tree (*Borassus flabellifer L.*), palm sugar, gula lempeng, gula semut, and even alcohol-containing drinks known as sopi.

Sopi is a popular traditional Timorese drink that has been produced by the community for a long time through the distillation of sap that has undergone a fermentation process. The word sopi comes from the Dutch word zoopje, which means liquid alcohol, colorless, and with a distinctive odor. Generally, sopi in Timor is used in rituals, traditional ceremonies, religious events, and so on. The alcohol content of sopi produced by farmers or sold in the market varies between 40%-70%, depending on the fermentation method and the length of the distillation process. The price of sopi in the market is highly fluctuating, depending on its quality and alcohol content. The higher the alcohol content, the higher the selling price of the sopi product. Ethyl alcohol (CH_3CH_2OH), also known as alcohol, has the following physical properties: It is a liquid, colorless, volatile, miscible with water and ether in all proportions, boils at 79°C, freezes at -117°C, has a specific gravity of 15°C, and a molecular weight of 46 g/mol. Alcohol is mostly produced through fermentation using yeast, specifically *Saccharomyces cerevisiae*. During the fermentation process, only a dilute ethanol solution is formed, as yeast cells die when the alcohol concentration exceeds 12%-15%. Sodium benzoate is a type of substance at 0.1% or 1000 ppm.

Dominggus Elcid et al. (2013), in their research, revealed that in On the Ethnology of Timor-Laut, published in the Journal of the Anthropological Institute of Great Britain and Ireland in 1882, exactly 136 years ago, an ethnologist named H.O. Forbes found common practices of sopi production and consumption. Sopi became an economically valuable luxury item with significant social, political, and cultural value. The article beautifully describes how a midwife assisting in the birth of each child would receive a plate placed in a turtle shell along with 10 betel leaves, 20 areca nuts, and one sarong. Meanwhile, the person who bathed a newborn child would receive a bottle of sopi, sourced from the palm tree. It is further explained that William Blight, in A Voyage to the South Sea, indicated that the Timorese were already drinking arak when they arrived in Kupang on June 12, 1789 [1-5].

Methods

This type of research is descriptive to clarify the bacterial

content found in laru putih (a distilled liquid). The researcher analyzed the content of *E. coli* and the Total Plate Count (TPC) of bacteria in the sopi samples, the distillation equipment, and the distiller's hands, then compared the results to the Indonesian National Standard (SNI). Sampling of sopi and specimen swabs from equipment and hands was conducted at 3 distillation sites and the hands of 3 distillers in June 2024. The research samples included the sopi, the distillers' hands, and the equipment used in the distillation process. The analysis of *E. coli* and TPC content was performed by the Technical Implementation Unit of the Health Laboratory of East Nusa Tenggara Province.

The equipment and materials for field sample collection included: Bottles for new samples, sterile swabs, sticks with cotton tips, sterile/clean gloves, small markers, small scissors, label paper, a spirit lamp, ice thermos, and alcohol. The equipment and materials used for *E. coli* testing included test tubes, a Bunsen burner, Petri dishes, a scale, a 1 ml pipette, label paper, a knife, a mortar, a bottle, laru putih, EA medium, SS-Agar medium, and S110 Agar medium. The equipment and materials for checking the Total Plate Count of bacteria included Nutrient Agar medium, 0.9% NaCl dilution, 4 sterile Petri dishes for each test, two pieces of sterile pipettes, spirit lamps/Bunsen burners, matches, test tubes, and a wooden rack.

The sample collection procedure was as follows: Prepare sterile gloves before taking the samples. Prepare a sterile stick, open the bottle, and insert the sterile cotton swab inside. The sterile cotton stick in the bottle is pressed against the side of the bottle to extract the liquid, then removed and rubbed onto the container used to store and sell laru putih. The surfaces swabbed included the outer and inner lips of the bottle or packaging, such as a jerry can or an aqua bottle, at a height of 6 mm. The swabbing process was carried out around the bottle's lip 3 times in succession, with one cotton stick used for each container/package. After the swabbing was done, the cotton swab was placed back into the bottle, and the stick was broken or cut off. The bottle's lip was heated with a soft flame, then sealed with cotton. Label the sample with the provided label paper. Write the label with a marker specifying the name of the equipment and the location where the sample was taken (code). The samples were stored in an ice bottle and immediately sent to the laboratory at a cold temperature for examination.

The procedure for testing *E. coli* content was as follows: Take 11 grams of the material and place it in a bottle containing 99 ml of sterile water, then shake it (dilution 10-1). 1 ml is taken from the bottle and placed in test tube 1, containing 9 ml of sterile water, then shaken (dilution 10-2). 1 ml is taken from dilution 10-2 and placed in test tube 2, containing 9 ml of sterile water, then shaken (dilution 10-3). Dilution is carried out up to 10-4. Duplicate plating was done on Petri dishes by taking 0.1 ml from each dilution for each *E. coli* test, 10-3, 10-4, and 10-5. The plates were incubated for 2 × 24 hours, and then the *E. coli* colonies were observed and counted.

The procedure for testing the TPC of bacteria was as follows: Prepare 0.9% NaCl dilution solution for 4 test tubes and 4 Petri dishes labeled with 10-1, 10-2, 10-3, and control. Take 1 ml of the sterile NaCl solution from the control code and place it into the appropriately labeled Petri dish. Take 1 ml of the sample with a sterile pipette and place it into test tube 10-1. Pipet 1 ml from tube 10-1 and place it into the Petri dish labeled 10-1 and the remaining 1 ml into test tube 10-2. Continue this process up to test tube 10-3. Pour 15 ml of nutrient agar (at 55°C-56°C) into the Petri dishes containing the samples, shake until even, and allow it to solidify. Place the Petri dishes upside down in an incubator at 37°C for 1 x 24 hours and observe the gas formation in Durham tubes. The *E. coli* and TPC results from the laboratory were analyzed descriptively to explain the bacterial content found in the distilled sopi in the Sikumana subdistrict area.

Results

Profile of Sikumana village

Sikumana Village is located within the Maulafa Sub-district of Kupang City. The distance from Sikumana Village to Maulafa Sub-district is approximately 7 km, and it is less than 8 km from the center of Kupang City. The location of this sub-district within Kupang City facilitates easy and quick access for the community to healthcare facilities. Currently, Sikumana Village has very smooth transportation access both to the city center and vice versa, making it easier for community mobility.

As of 2024, the population of Maulafa Sub-district is 109,993 people, with the majority being young individuals. The education level of the population varies, but there are more elementary school graduates and high school graduates. Daily occupations include civil servants (1.3%), farmers (12.5%), laborers (16.74%), and those who are unemployed or housewives (69.46%). The population categorized as unemployed includes housewives and children who are still in school. Residents of Maulafa Sub-district come from various ethnic groups, both from East Nusa Tenggara (NTT) and outside NTT. The majority of the population originates from Timor and Rote, with others coming from Sabu, Sumba, Flores, Alor, Java, Batak, and Sulawesi.

Sopi distillers

The community members who work as sopi distillers in Sikumana Village generally come from Rote and Timor. This is due to the drinking tradition of sopi in those communities, which is typically observed during significant events such as funerals and other celebrations. The sopi produced through distillation is usually made for personal consumption. The production of sopi comes from the sap of the lontar tree, known as tuak, which is then cooked with various spices according to local distillation customs. The distillation process results in several categories: The first distillation is called "sopi kepala," and the subsequent distillation is referred to as "sopi kelas dua." After distillation, the sopi is poured into used 750 ml mineral water bottles, ready for sale. Each bottle containing 750

ml of sopi is sold for IDR 10,000-IDR 30,000, depending on the quality of the sopi. Distillers claim they can produce up to 30 bottles per day, although the number varies among distillers daily.

The sopi distillers start their work after preparing all the necessary raw materials, with about 20 households involved. Distillation begins at around 08:00 AM and continues until 05:00 PM, from Monday to Saturday each week. Distillation activities are not conducted on Sundays, as it is a day for religious observance. Observations of the distillers' clothing show that there is no difference from their everyday attire, and they do not use personal protective equipment such as masks, boots, gloves, etc.

Materials and tools for sopi distilation

The equipment used for sopi distillation is quite simple. It includes at least ½ balek of sugar (15 bottles) for one distillation, which is stored in a plastic drum, lontar tree bark, forest betel leaves, wood for burning, a jerrycan for collecting the distillate, bamboo and copper pipes, water in a basin for cooling, and a filter net.

Result product of distilation

The research results from 3 sopi distillation sites indicate that only 5 liters of sopi kepala (first distillation) are produced per day, with an alcohol content of over 40%. In contrast, 30 liters of regular sopi are produced daily with an alcohol content of around 20%, as presented in Tables 1 and 2.

Table 1: Sopi production amount per day

Distiller	Total production per day	>30 liters per day	< 30 liters per day
1	3 Times	Yes	-
2	3 times	Yes	-
3	2 times	-	No cooked per 2 times

Table 2: Alcohol content per distillation

Distillers	Total production per day	Distillation	Alcohol contains
1	Distiller 1	Firstt	40%
		Second	24%
		Third	21%
2	Distiller 1	First	41%
		Second	30%
		Third	25%
3	Distiller 3	First	40%
		Second	24%
			23%

The amount of *E. coli* in swabs of tools and materials as well as distillation results

The results of the analysis of microbial contamination from swabs of tools, materials, and the hands of sopi distillers based on the *E. coli* content in Sikumana Village are shown in Table 3.

Table 3 indicates that the swab samples of tools and materials, as well as the swab from the hands, are free from *E. coli* microbial contamination, showing a value of 0 and remaining below the maximum contamination limit of <3/ml. This indicates that the sopi samples are not contaminated by *E. coli* bacteria.

Total plate count of bacteria on swabs from the hands of sopi distillers

The results of the analysis of microbial contamination in sopi based on the total plate count of bacteria from several distillers' hands are shown in Table 4.

Table 3: Microbial *E. coli* contamination in sopi based on *E. coli* content

No	Sopi distiller	Microbial contamination levels of based on *E. coli* content					Average	Limit contamination
		Drum for soaking sopi ingredients	Cooking drum for sopi	Sopi distribution pipe	Sopi storage place	Distiller's hand		
1	Distiller 1	0	0	0	0	0	0	*E. coli*: 0/cm
2	Distiller 2	0	0	0	0	0	0	
3	Distiller 3	0	0	0	0	0	0	

Table 4: Microbial contamination usap tangan penyuling total plate count

No	Sopi distiller	Microbial contamination levels of based on *E. coli* content			Average	Limit contamination
		Worker 1	Worker 2	Worker 3		
1	Distiller 1	19	0	0	0	TPC: 2×10^2 colonies/ml
2	Distiller 2	0	54	12	33	
3	Distiller 3	105	0	0	105	

Table 4 indicates that the highest microbial contamination in the hand swabs was found in the 3rd distiller, with a Total Plate Count (TPC) of 105, exceeding the maximum contamination limit of 2×10^2 colonies/ml. The high level of microbial contamination on the hands may be due to the workers' lack of attention to hygiene while distilling sopi. In contrast, the lowest TPC was recorded for the first distiller, which was 0. This is likely because the first distiller prepared a handwashing station at the distillation site and consistently washed their hands with soap. Additionally, the sampling was conducted in the morning, shortly after the distiller had bathed, and the tools and materials were freshly washed. Likewise, the container for collecting the sopi was also cleaned right before use, and since the sopi was freshly distilled, many microbes may have been killed during the process.

Total plate count of bacteria on swabs of tools and materials as well as distillation results

The results of the laboratory tests to determine the Total Plate Count (TPC) of the equipment and materials used for distilling sopi are presented in Table 5. Table 5 shows that microbial contamination on the equipment and materials for distilling sopi, with the highest contamination found on the equipment and soaking materials, is observed in the 3rd vendor's distillation site, with an average of 121 colonies/ml, and the lowest in the 2nd vendor's distillation site, with an average of 18 colonies/ml. The average contamination level based on TPC from the equipment swabs, materials, and distillation results has exceeded the maximum limit of 2×10^2.

Table 5: Microbial contamination usap alat in sopi package based on TPC content

No	Sopi distiller	Microbial contamination levels of based on *E. coli* Content				Average	Limit contamination
		Drum for soaking sopi ingredients	Cooking drum for sopi	Sopi distribution pipe	Sopi storage place		
1	Distiller 1	22	0	0	0	22	TPC: 2×10^2 colonies/ml
2	Distiller 2	18	0	0	0	18	
3	Distiller 3	121	0	0	0	121	19

Discussion

Chemical contents of lontar tree

The products of the lontar tree include sap and sugar water, which contain sugars consisting of simple glucose and disaccharides in the form of fructose and sucrose. Sugar water has a higher sugar content compared to sap. Therefore, the people of Timor and Rote often consume sugar water, especially in the morning before engaging in activities, as it provides a feeling of fullness and generates energy. Sopi is traditionally a locally fermented alcoholic beverage. The alcohol content of both types of these drinks can warm the body when consumed, but excessive consumption can lead to intoxication.

Alcoholic beverages have become a significant issue in Indonesia. The problem arises from the frequent emergence of illegal producers who create drinks with alcohol content exceeding 55%. According to Presidential Regulation No. 74 of 2013, alcoholic beverages are defined as drinks containing ethyl alcohol or ethanol (C_2H_5OH) processed from agricultural materials containing carbohydrates through fermentation.

Microbial contamination of *E. coli* and total plate count in swabs from tools, hands, and sopi

Generally, *E. coli* bacteria are present in the digestive tracts of humans and animals. Their presence outside the human body serves as an indicator of sanitation, indicating whether food and beverages have been contaminated by human waste. The presence of *E. coli* in water or food is also considered to have a high correlation with the detection of pathogens in food. *E. coli* can grow optimally at a pH of 7-7.5, with a minimum pH of 4 and a maximum pH of 8.5. This bacterium is sensitive to heat and to foods being heated. Freezing in cold storage inhibits bacterial growth but does not kill the bacteria. However, freezing in a deep freezer at temperatures below -10°C can drastically reduce the population and slowly kill *E. coli* bacteria.

Escherichia coli is a gram-negative rod-shaped bacterium, non-capsulated, and is a normal flora in the digestive tract of animals and humans, making it easy to contaminate water. *E. coli* can become opportunistic pathogens when living outside the intestines, such as in urinary tract infections, wound infections, and mastitis. *E. coli* is one of the groups of coliform bacteria and can grow at temperatures between 10°C-40°C, with an optimal temperature of 37°C. The optimal pH for growth is between 7.0 and 7.5, with a minimum of 4.0 and a maximum of 9.0. This bacterium is relatively sensitive to heat and can be inactivated at food pasteurization temperatures or during cooking. Therefore, to prevent bacterial growth, food should be stored at low temperatures. Low temperatures do not kill organisms but inhibit their proliferation (dormancy). Freezing causes minimal damage to microorganisms, and this damage can be reversible or result in cell death. This damage depends on the type of microorganism and the speed of the freezing process. Rapid freezing at very low temperatures causes minimal or no bacterial cell damage, so under favorable conditions, bacteria can resume activity, while slower freezing at relatively high freezing temperatures (up to -10°C) can cause severe damage to bacterial cells and result in bacterial death.

For freshwater at the 3 vendors, the results showed no

E. coli or other types of bacteria were found (TPC Test). As a gram-negative bacterium, *E. coli* has the ability to withstand environmental conditions that do not benefit their survival. In this case, the laru stored for a relatively long period will become sour. A very low pH level does not generally permit bacterial survival; however, for some bacteria, including *E. coli*, they can survive.

The sample testing results indicate that *E. coli* colonies and other groups of bacteria were not found in the sopi samples. The absence of *E. coli* in the laru and other species at distilleries 1, 2, and 3 is due to the samples of tools, materials, and hand swabs being taken early in the morning just after the distillation process began, resulting in all samples being clean. The presence of *E. coli* in the laru may be due to the use of inadequately clean new containers and the cleanliness of the distillation area itself (personal hygiene) as well as exposure to dirty air from the surroundings (sanitation). This finding is consistent with research conducted by the Research Agency of the Ministry of Health of the Republic of Indonesia in collaboration with the Health Office of DKI Jakarta on beverage vendors, which showed that 55% of vendors did not wash their hands before handling beverages, 28.2% did not wash their hands with soap after defecation, 23.3% used dirty cloths as cleaning rags, 23.3% of beverage handlers had unclean hands, 17.1% of food vendors had long nails, and 61.54% had positive *E. coli* contamination in street food beverages.

Microbial contamination based on total plate count of sopi in hand swabs and workers' tools

Laboratory results indicate that the highest microbial contamination in the equipment used for cooking sopi, based on Total Plate Count (TPC), was found in the 3rd distiller, with a bacterial count of 105 colonies/ml on the workers' hands at that location. In contrast, the lowest count was observed in the first distiller, which recorded 0 colonies/ml. The highest contamination from swabs of the tools used in the 3rd distiller amounted to 121 colonies/ml, while the lowest count was in the tools used by the 2nd distiller, with only 18 colonies/ml.

The elevated levels of microbial contamination in the drum used for soaking sopi ingredients in the 3rd distillation are attributed to insufficient washing practices, resulting from daily cooking activities, which neglects the cleanliness of the drum. Additionally, the soaking area is outdoors, allowing for direct contact with dust and dirt carried by the wind. The distillers believe that washing the soaking drum frequently might reduce the functionality of the sopi ingredients. Failing to wash thoroughly leaves behind significant nutrients (glucose), which, if exposed to non-*E. coli* bacterial spores, can thrive and develop into flourishing colonies (logarithmic phase).

Moreover, the open nature of the distillation area facilitates contamination from consumers who may touch the cooking equipment. According to Slamet (1994) in Meilisnawaty, food can become contaminated with microbes for several reasons: Handling food with dirty hands, using unclean equipment, maintaining a dirty environment, exposure of food to animals such as insects and rodents, and storing raw and cooked materials together or in dirty water [6].

Further research is necessary to identify the types of microbes present in the TPC content of new reservoirs to determine whether these species are parasitic to humans or harmless. Food and beverage hygiene and sanitation are crucial efforts to control factors related to food, people, places, and equipment that may cause illness or health issues. Sanitation and hygiene requirements are technical provisions established for cooking products and restaurants, personnel, and equipment, encompassing bacteriological, chemical, and physical standards [7-13].

Conclusion

Sopi contains a variety of organic acids, amino acids, and alcohol. The sopi samples were free from *E. coli* microbial contamination, while contamination from other microbes on the cooking equipment and the hands of the distillers exceeded the maximum limits based on the Indonesian National Standard (SNI).

Conflict of Interest

The authors declare that they have no conflict of interest.

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None.

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