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APPLICATION OF RTU MEDIA FOR BIOSAFETY OF *MIE LETHEK*, INDONESIAN BENDO-CASSAVA NOODLES, BASED ON CHROMOGENIC AGAR

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Abstract. Detection of microbial contamination by plating methods, especially coliform bacteria and other pathogens, many still use conventional methods and take about 7 days to find out the results. RTU (*ready to use*) media is one of the selective media to determine microbes more quickly based on the color of the colonies on the agar media. This study used RTU media for determination of biosafety of *Mi Lethek*. *Mi Lethek*, is cassava noodles originated from Bendo, Srandakan, Bantul, Yogyakarta, made from cassava starch and cassava flour. Production of *Lethek* noodles uses traditional machinery and equipments. Biosafety analysis was performed on dried *mie lethek*. The presence of enteric bacteria is known by the presence of blue colonies for *Eschericia coli* and purple/magenta for *Salmonella*. The results showed that microbial contamination in dried *Lethek* noodles was less than 10^5 cfu/g. Contamination of enteric bacteria was less than 10^2 cfu/10g. Indonesian standard (SNI) of dried noodles was 10^6 cfu/g for total of microbial and 10 cfu/g for enteric bacteria (*E. coli*). Although *mie lethek* quality complies SNI quality standards, sanitation should be improved.

Key words: chromogenic agar, enteric bacteria, *Eschericia coli*, RTU, *Salmonella*

1. INTRODUCTION

IsDB's research related to biosafety strongly supports the availability of good quality food, nutritional value and safety as an effort to improve the quality of superior agricultural commodities. The problem of food quality and agricultural commodities in the free market is primarily the national food quality and safety that affect food trade both domestically and globally. Food products that do not meet the food safety quality requirements include high microbial contamination and pathogenic microbial contamination in various food products.

A new method has been developed using chromogenic-fluorogenic synthetic substrates. In this method the substrate will be hydrolyzed by specific enzymes from the test bacteria, enzymatic activity is measured by the presence of color and/or fluorosity. The use of chromogenic-fluorogenic substrates

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results in simple, fast, specific, sensitive and accurate test procedures. Specific enzymes that are only owned by the test bacteria will hydrolyze the chromogenic-fluorogenic substrate, releasing colored chromogeic compounds or fluorogenous fluorogenous compounds. With this media the bacterial groups of coli (total coli) and *E. coli* (coli stool) can be specifically identified through simultaneous testing within 24 hours[1]. Public Health Association's National Commission has endorsed the use of chromogenic substrates for testing microbial contamination in water in 1992[2].

Chromogenic media can inhibit Gram-positive organisms, proteus and coliform because they contain sodium citrate. To identify *Salmonella* species, this chromogenic has a combination of two basic chromogenic substrates that facilitate identification so that it becomes faster. The two substrates are X-gal chromogenes and Magenta-caprilate. X-gal is a substrate whose role is to visualize the enzyme ß-D-galactosidase produced by the organism and gives the colony a blue color. Magenta colonies are the result of the hydrolysis of magenta-caprylate by the negative lactose Salmonella species. Thus, non-Salmonella organisms appear blue or colorles[3].

This study applied the use of RTU media (*ready to use*) for determination of biosafety of cassava product i.e *mie lethek, mi letheg* or *mi lethek*, is one of the culinary noodles originating from Bendo, Srandakan, Bantul, Yogyakarta, made from cassava starch and cassava flour. Production of *mie lethek* is still using the traditional machinery and equipments. The noodle is a murky brown color (not white or bright like normal noodles), without using chemical dyes or preservatives, but dried *lethek* noodles can be preserved until more than three months.

2. MATERIALS AND METHODS

2.1 Materials

Materials for the formulation of chromogenic media per liter are fushin acid (0.1 g), agar (15.0 g), ammonium ferric citrate (1.5 g), bile salt (9 g), bromothymol blue (0.065 g), propylene glycol (10 ml), sodium citrate (8.5 g), meat extract (6.0 g), peptone casein (5.0 g), chromogenic ingredient (5.0 g) and bacteriological agar (12.0 g)[1]. The tools used are autoclaves, laminar flow, and a set of microbiology test kits.

2.2 Preparation of chromogenic media

Material formulated for chromogenic media is dissolved in water. Then it is heated and stirred until it boils and the media is dissolved. After that it is cooled to a temperature of 40°C, it is used for plating of *mie lethek* samples for safety evaluation.

Fig 1. Performance of chromogenic media for *Eschericia coli* (blue color) and *Salmonella* sp (magenta/violet color)

2.3 Biosafety evaluation of mie lethek

Biosafety evaluation of *mie lethek* was conducted at production unit SMEs *Mie Lethek Bendo,* Bantul-Yogyakarta, Indonesia[M1]. Sampling of *mie lethek* was done on the wet noodles and dried noodles of mie lethek. As much as 100 g of sample noodle was dissolved in 1 L of sterile physiological solution containing 0.85% NaCl. Then it was added into serial dilutions of up to 10^{-4}. The last three series were inoculated on chromogenic media then inoculated at 37°C temperature for 24-48h. The growth of

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enteropathogenic bacteria was determined using BAM standards (25 - 250 colonies/plate). Colonies of *Salmonella sp*. were shown as purple-magenta colonies, while colonies of *Eschericia coli sp*. were shown as blue colonies [4].

2.3 Determination of Bacteria Population [5]
Determination of bacterial population was carried out using the BAM (Bacteriological Analytical Manual) namely:
$N = \{\Sigma C / [(1 \times n1) + (0.1 \times n2) \times (d)]\}$
N = number of colonies
ΣC = number of colonies in both dilution series used
n1 = number of plates used in the first dilution series
n2 = number of plates used in the second dilution series
d = series of lowest dilution used.

3. Results and Discussion
3.1 Source of microbial contamination during the mie lethek-processing
There are several stages of process during *mie lethek* making which potentially harbor contamination, namely fermentation, mixing, dough molding, and drying. Opportunities for microbial contamination by tools, assistants, environment, and workers are presented in Figure 2.

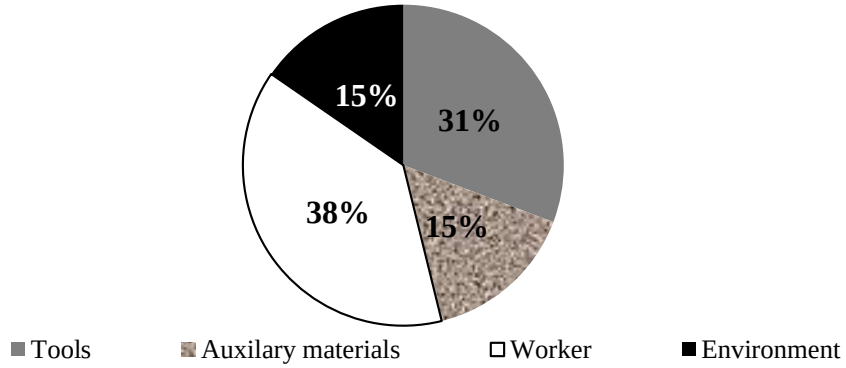

Figure 2. Relative chances of microbial contamination during processing of mie lethekSMEs

The process of making *mie lethek* begins with spontaneous fermentation which is a critical point of contamination in the noodle mixture. Fermentation is carried out by soaking cassava for three days and stirring every 12 hours. Water was replaced everyday. Fermentation changes the textural characteristics of cassava starch to enable it as main ingredient of noodle. Fermentation is carried out is the process of spontaneous aerobic fermentation with the addition of water for 3 days with stirring every 12 hours and the replacement of water in the fermentation tank. Potential contamination occurs from raw materials (flour and cassava starch) as well as water used, tools and workers (Figure 3).

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Figure 3. The conditions of the process of making *mie lethek* that have the potential for cross contamination[6]

The next step is to drain the fermented flour for one day prior to mixing stage. The mixing process used a traditional grinder pulled by walking cow in circle. Potential microbial contamination occurs from livestock that are used for mixing, as well as workers at stirring station. The first stage of molding was done using traditional tools and manuals. First, the dough was cut into cubes using a large knife. This stage has the potential to get bacterial contamination from post-processing workers. The next process is steaming which was carried out in a large oven. Potential contamination can be from the tools used and workers. After that, noodle dough was shaped into small sheets of *mie lethek* using a press machine to form a string of noodles. Potential contamination occurs from workers. The process of separating noodle strips was done manually by workers. This is the biggest opportunity for enteropathogenic bacterial contamination because the product will not pass any thermal process enabling sterilization.

The last stage of processing *mie lethek* before being marketed is sun drying. Potential contamination occurs from the environment in the form of dust and dry leaves, since drying is done in an open space for one day. However, all the dangers of contamination by non-spore pathogenic bacteria will be lost if *mie lethek* are processed for prior to serving. *Mie lethek* was packed in every 5 kg in a plastic bag. Potential contamination occurs due to workers.

3.2 Microbial contamination of mie lethek

The population of total microbial contamination on wet *mie lethek* was less than 10^5 cfu/g, while the enteric bacteria in the range of 10 cfu/g or 1 log cfu/g. The population of microbial contamination is presented in Table 1. The presence of *E. coli* in *mie lethek* in chromogenic media was indicated as blue color colonies (Figure 4).

Table 1. Population of total microbe, *Salmonella sp*., *Eschericia coli*

Sample of *mie lethek*	Population (cfu/g)		
	Total microbe	*Salmonella sp.*	*Eschericia coli*
Wet *mie lethek*	1.14×10^4	0	0
Dried *mie lethek*	2.01×10^5	0	1.00×10^1
Standard SNI (total plate count/ TPC)	$\leq 10^6$	0	< 11

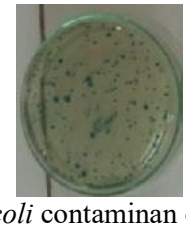

Figure 4. Presence of *mie lethek-E. coli* contaminan on chromogenic media as blue colony.

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Table 1 showed that microbial contaminant on wet and dried noodles were respectively 10^4 cfu/g, and10^5 cfu/g. Spontaneous fermentation of cassava starch and flour resulted no *E. coli,* which likely due to low pH of slurry around pH 2-3. Steaming process reduced bacterial contamination especially negative gram bacteria. *E. coli* and *Salmonella* can be destructed at 90° C for 2 minutes. This heat treatment was more effective (thermal adequate) to destroy *Salmonella* (around 75%) and *E. coli* (more than 80%). The coefficient of destructions (k value) at that conditions were 0.89 for *E. coli* and 0.69 for *Salmonella*[4].

E. coli was detected on dried *mie lethek*. Poor sanitation during drying process seems to enable cross-contamination from environment, equipment, and workers. Bacterial growth generally occurs at room temperature and most bacterial contamination populations are mesophilic bacteria. An unhygienic environment facilitates rapid microbial growth [7].

Indonesian National Standard (SNI) of noodles No. 7388: 2009[8] and Indonesia National Agency of Drug and Food Control (BPOM) No. 13: 2019[9] concerning microbiological requirements of noodles quality including total plate numbers, number of coliform bacteria, and identification of pathogenic bacteria. Maximum limit for total microbial contamination is less than 10^6 cfu/g, the contamination of *E. coli* bacteria is less than 11 NLM/g (most likely number per gram) and there should be no pathogenic bacteria like *Salmonella* sp[8,9]. Wet noodles of *mie lethek* contain 10^6 cfu/g of total microbial cells, 1 cfu/g of *E. coli* and no *Salmobella sp*.

4. CONCLUSION

The most likely number (MLM) of microbial contamination was 38% by workers, 31% by tools and the 15% for auxilary material or environment. Quality of *mie lethek* still meets SNI and BPOM standards, i.e less than 10^6 cfu/g for microbial population, 10^1 cfu/g for *E. coli* and no *Salmonella*. RTU media is able to identify *E. coli* in *mie lethek* by producing a blue-coloured colony.

5. ACKNOWLEDGMENTS

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