



**ASSESSMENT OF RHODAMINE B, MICROBIOLOGICAL QUALITY AND SANITATION OF STREET-VENDED FOOD-SNACK AROUND ELEMENTARY SCHOOL IN SAMARINDA, INDONESIA: LESSON TO PROMOTE FOOD SAFETY IN SCHOOL ENVIRONMENT  
(KAJIAN RHODAMIN B, KUALITAS MIKROBIOLOGI DAN SANITASI PADA JAJANAN KAKI LIMA SEKITAR SEKOLAH DASAR DI SAMARINDA, INDONESIA: PELAJARAN UNTUK MENINGKATKAN KEAMANAN PANGAN DI LINGKUNGAN SEKOLAH)**

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**ABSTRACT**

*The incidence of foodborne disease caused by street food in Indonesia tends to increase. Meatball served with tomato sauce is popular among elementary school students in Indonesia. This research examined the safety level of the sauce used for meatball sold among the elementary school students in Samarinda. Rhodamine B and microbiological quality of the tomato sauce were examined as the food safety level parameters. Twenty-four vendors participated in this study. Sanitation during the meatball preparation by each vendor was also questioned. Results showed that 79.2 percent of the vendor sold the meatball with Rhodamine B was positively detected in the tomato sauce. All sauce did not meet the requirements of the microbiological quality of the Indonesian standard. It might be caused by cross-contamination of the pathogenic microbes from equipment, poor vendor sanitation while preserving the sauce, or the water used in the preserving processed. Contamination from air pollution also needs serious attention because the vendors sold the meatball in the street or by the roadside.*

**Keywords:** coliform, foodborne disease, rhodamine B, sanitation, tomato sauce

**ABSTRAK**

Kejadian keracunan pangan yang disebabkan oleh jajanan kaki lima di Indonesia cenderung meningkat. Bakso daging (pentol) yang disajikan dengan saus tomat merupakan jajanan yang digemari diantara murid sekolah dasar di Indonesia. Penelitian ini mengkaji tentang keamanan dari saus yang digunakan bersama pentol diantara murid sekolah dasar di Samarinda. Sebanyak 25 responden (penjaja pentol dengan saus tomat) diambil sebagai sampel melalui metode *self-purposive sampling*. Dilakukan pula penggalan informasi tentang sanitasi yang diterapkan oleh penjaja pentol dalam penyiapan saus tomat. Hasil penelitian menunjukkan bahwa 79.2 persen penjaja pentol menggunakan saus tomat yang terindikasi mengandung Rhodamin B. Semua saus yang digunakan oleh penjaja pentol tidak memenuhi standar Indonesia untuk kualitas mikrobiologi. Hal ini mungkin disebabkan oleh kontaminasi silang dari penggunaan peralatan, sanitasi yang buruk dalam penyiapan saus tomat, atau karena rendahnya kualitas air yang digunakan dalam proses penyiapan saus. Kontaminasi dari udara juga menjadi pertimbangan yang serius karena pentol di jajakan di tepi jalan yang berdekatan dengan sekolah. [Penel Gizi Makan 2019, 42(2):49-56]

**Kata kunci:** coliform, keracunan makanan, rhodamin B, sanitasi, saus tomat

## INTRODUCTION

**F**ood safety is one of the critical issues in the world today, especially in developing countries. In 2007, the Indonesian Agency of Drug and Food Control reported that all food groups from the school canteen contained hazardous chemicals and showed an ineligible microbiological quality. In food and drinks, a hazardous chemical such as formalin, borax, Rhodamine B, methanyl yellow, and amaranth was detected at the amount of 16.06, 9.11, 3.11, 2.10, and 3.22 percent, respectively. While the microbiological quality of the food from the school canteen did not meet the standard requirement, i.e., TPC for food and drink, *Staphylococcus aureus* for food and *Escherichia coli* for a drink was determined at the amount of 27.71, 24.09, and 69.03 percent<sup>1</sup>.

In Manado, all samples of tomato sauce from street food did not meet the food quality requirements because they were contaminated by *E. coli*<sup>2</sup>. The problems of food safety affected many aspects, particularly children at the elementary student age. The students in the elementary schools are considered as the biggest snack consumers that often get poisoned<sup>3</sup>.

In January-September 2017, there were 93 poisoning incidents in Indonesia from different areas. Food poisoning accounted for 76 outbreaks in which 13 outbreaks were from street foods with 3,026 people exposed, in which six deaths<sup>4</sup>. Foodborne diseases could be caused by chemical substances or microbiological contamination that could harm human health.

Safety and health snacks should be free from physical hazards, harmful chemical agents, and biological hazards. Colour is one of the critical factors for influencing the consumer's acceptability<sup>5</sup>. Therefore, food colorant is added to make food products more attractive. Recently, there are many problems regarding the use of harmful synthetic colorant to food products. Besides the contamination from chemical hazards, food poisoning primarily caused by microbial contamination.

Samarinda is divided into ten sub-districts, with the total area is as much as 71,800 ha<sup>6</sup>. There are 246 elementary schools spread across the city, Samarinda<sup>7</sup>. Many of them might have a risk of foodborne disease cases regarding the condition of food-snack, especially meatball with tomato or peanut sauce sold around by moving vendors at the schools. Ningsih<sup>8</sup> reported that fried and baked meatball sold from the food-snack vendor around the elementary school in Samarinda

contained *E. coli*. In general, the tomato or peanut sauce is a companion to the meatball. The vendors usually put the meatball together with the sauce into 0.5 kg plastic slips. It is assumed that bacteria might contaminate the food-snack either from the meatball or the tomato/peanut sauce. Cross-contamination is one of the risk factors of foodborne illness identified by the U.S. Department of Health and Human Services Centers for Disease Control and Prevention<sup>9</sup>.

This study aimed to assess the safety level of tomato sauce from Street-Vended Food-Snack (SVFS) by detecting the Rhodamine B, evaluating the microbiological quality, and investigating the food safety knowledge and awareness among the vendors.

## METHOD

This research was conducted in April-May 2016. The tomato sauce obtained from SVFS (mobile stalls with bicycle) around the elementary schools in Samarinda. Media for total plate count (PCA) and NaCl were purchased from Merck, chromogenic coliform agar from Scharlau, and the Rhodamine B Test Kit from Easy Test. A questionnaire was prepared to collect sanitation on meatball and tomato sauce preserving by SVFS.

Leveled sampling was conducted to collect the respondents. Firstly, sampling on elementary schools in all ten sub-districts in Samarinda (Sungai Pinang, Samarinda Kota, Samarinda Utara, Samarinda Ulu, Samarinda Seberang, Palaran, Samarinda Ilir, Loa Janan Ilir, Sungai Kunjang, and Sambutan). The required number of schools was estimated by Slovin's sampling technique<sup>10</sup>, resulting in 22 elementary schools from a total of 214 elementary schools in Samarinda. Secondly, purposive sampling was then conducted to choose the SVFS, considering that they sold meatball served companion with tomato sauce. They sold the food-snack at the outdoor area, close to the street, and uncovered. Three SVFS were selected for densely populated sub-districts, while two SVFS were chosen for the less populated sub-districts. A total of 24 respondents were selected in this study.

The observation was conducted for two sub-districts in a day. Tomato sauces were sampled every day when the first break of the school ended (the student bought the meatball with tomato or peanut sauce during the school-break). Respondent was sampled with the criteria of SVFS having the most consumers each elementary school, and two to three elementary schools were selected in each sub-district. The number of sampled elementary

schools was depended on the number of elementary schools in the sub-district. In total, four to six tomato sauce samples were collected per day from different respondents, and they were questioned. Sanitation data on the preserving the food-snack by every Street-Vendors Food Snack, by which the food-snack collected, was carried out by direct observation using a structured questionnaire. Samples of tomato sauce from every SVFS were aseptically collected. They were put into sterile glass bottles and directly analyzed for the Rhodamine B detection and microbiological quality (TPC, *coliform*, and *E. coli*).

Sanitation data of the tomato sauce preparation that served as a companion with meatball sold by every SVFS were analyzed descriptively.

Qualitative detection of Rhodamine B was conducted using a test kit from Easy Test<sup>11</sup>. The sample was analyzed in liquid form. The tomato sauce was diluted for the thick sauce in two-fold of volume by hot water and waited until reaching the room temperature before the analysis. Three drops of the reagent A (CCl<sub>4</sub>) was added to a 3 mL sample in a test tube and stirred until homogeneous, then five drops of reagent B (oxidation agent in dilute HCl) and three drops of reagent B2 were added and

briefly vortexed. The sample containing Rhodamine B was indicated with the appearance of a purple circle on the solution surface and the mixture changing into purplish red.

TPC analysis was determined by the pour plate agar method<sup>2</sup>. Samples were diluted 10<sup>-3</sup> to 10<sup>-5</sup>. The sample was taken using a 1 mL pipette then was put into a sterilized petri dish. The Plate Count Agar (PCA) was poured as much as 15-20 mL and incubated for ± 24 hours at 37°C.

Analysis of total *Coliform* and *E. coli* were done by the pour agar method<sup>2,12</sup>. Samples were diluted 10<sup>-3</sup> to 10<sup>-5</sup> and put into sterile petri-dish, then Chromogenic Coliform Agar was poured as much as 15-20 mL as the media. The plate was incubated at 37°C for ±18-24 h. The total *Coliform* was determined by the pink/red color, while the total *E. coli* colonies were in the dark blue/purple color.

## RESULTS

The male (87.5%) were the dominant SVFS respondents (n=24) in the period of this study, while the Junior High School (9<sup>th</sup> class) was the dominant education level (45.8%) among the respondents (Table1).

**Table 1**  
**The Level of Education and Knowledge of SFSV Respondents (n=24) to the Application of Sanitation and Food Security Aspects**

Statement	Male	Female	
Gender	87.5%	12.5%	
Statement	Elementary school	Junior high school	High school
Level of education	25%	45.8%	29.2%
Statement	Yes	Sometimes	Not
Washing raw materials	8.3%	-	-
Self-process of the tomato sauce	4.2%	-	95.8%
Add other ingredients to the sauce purchased	65.2%	-	34.8%
Add water to the sauce purchased	47.8%	-	16.7%
Wash hands before and after processing	100%	-	-
Wash hands with soap	100%	-	-
Wash equipment with city running water	87.5%	-	12.5%
Using synthetic dyes	-	-	100%
Statement	Traditional market	Store	Agent
Place to buy sauce	54.2%	25.0%	20.8%
Statement	<500 mL	500-2000 mL	> 2000 mL
Amount of sauce once bought	20.8%	79.2%	-
Statement	Refrigerator	Open place	Closed place
Sauce Storage	41.7%	12.5%	45.8%
Statement	Yes / agree	Sometimes	No/Disagree
Knowing the importance of cleanliness in processing	50%	-	50%
Know about the dangers of non-food colorant	100%	-	-
Wash the sauce place after selling	100%	-	-
Dip the meatball directly into the sauce	62.5%	-	37.5%
The sauce runs out every day	50.0%	45.8%	4.2%
Reuse the remaining sauce that does not run out	33.3%	8.4%	58.3%

**Table 2**  
**Microbiological and chemical quality of tomato sauce, meatball snacks from Street Food Sold Amongst Elementary Student in Samarinda**

Sub-District	Sampling Location	Rhodamine B	TPC (log cfu.g <sup>-1</sup> )	Coliform (log cfu.g <sup>-1</sup> )	<i>E. coli</i> (log cfu.g <sup>-1</sup> )
Sungai Pinang	A & B	-	6.76	5.32	*
	C	-	6.82	4.91	3.00
Samarinda Kota	D	-	6.32	4.91	4.56
	E	-	6.83	4.80	3.43
Samarinda Utara	F	-	6.68	5.18	3.73
	G	-	6.28	5.32	3.43
	G	+	7.57	5.08	3.00
Samarinda Ulu	H	-	7.69	4.86	3.80
	I	-	6.57	4.92	3.00
	J	-	7.46	5.30	3.26
Samarinda Seberang	K	-	7.43	5.73	4.04
	L	-	6.45	5.04	3.30
Palaran	M	+	5.64	4.59	*
	N	+	6.76	4.23	5.15
	O	-	6.57	7.45	7.45
Samarinda Ilir	P	-	6.26	6.65	6.59
	Q	-	7.67	4.74	4.11
Loa Janan Ilir	R	-	6.54	5.54	4.86
	S	-	6.58	5.71	5.66
Sungai Kunjang	T	+	6.11	5.23	4.73
	U	+	6.11	5.23	*
	V	-	6.40	5.00	5.00
Sambutan	X	-	6.43	7.08	*
	Y	-	6.57	5.00	4.99

Note: Tomato sauce was collected from one respondent (Street-Vendors Food-Snack) at each Elementary School location. TPC<sup>14</sup> and total Coliform<sup>15</sup> Maximum Limit (ML) are  $2.0 \times 10^2$  and  $1.0 \times 10^2$  cfu.g<sup>-1</sup>, respectively. The data with grey and black background shows a number of  $2\text{ML} < \bar{x} \leq 3\text{ML}$  and  $3\text{ML} < \bar{x} \leq 4\text{ML}$ , respectively. \*) No *E. coli* detected.

More than 95 percent (23 people) of the respondents did not produce tomato sauce by themselves. They purchased in the form of ready tomato sauce in the form of 0.5-2.0 L plastic jar from the traditional market, store/mini market, or agents at the number of 54.2, 25.0, and 20.8 percent, respectively. Some of the respondents re-processed the tomato sauce by adding hot water prior they used as a companion for the meatball food-snack. Eighty percent of SVFS bought the tomato sauce directly through agents, while the other 20 percent bought tomato sauce on traditional markets.

As much as 62.5 percent of respondents agreed that the way consumers directly dip the meatball into the sauce, increasing the possibility of contamination. Another factor was the reuse of tomato sauce the next day. As

many as 8.4 percent of respondents said that they occasionally reused the unfinished tomato sauce, while 33.3 percent of respondents always used leftover sauce and mixed it with new sauce for the next day.

In Indonesia, food producers should obey the use of food additives according to government regulation<sup>13</sup>, in which Rhodamine B is not in the list of food colorants allowed. However, five from 24 samples (20.8%) positively contained Rhodamine B. The five samples were from SVFS, i.e., one at elementary school in Samarinda Ulu Sub-district, two at elementary school in Palaran Sub-district, and two at elementary school in Sungai Kunjang Sub-district (Table 2). The tomato sauces used by the SVFS at the Schools location were not fit to the Government Regulation No. 28 of 2004, and the Minister of

Health of Republic of Indonesia Regulation, Permenkes RI No. 033 of 2012, regarding the use of food additive.

All tomato sauce samples collected from 24 SVFS around the elementary school in Samarinda exceeded the standard of microbiological quality from SNI No. 01-3546-2004 regarding tomato sauce products (Table 2). Coliform bacteria and *E. coli* were also detected in the samples from SVFS of an elementary school around Samarinda. The number of total coliform bacteria was over the limit set by the Regulation of the Head of Indonesia Drug and Food Control Agency Number HK.00.006.1.52.4011<sup>15</sup>. Most of the tomato sauce from the SVFS contained *E. coli*. This condition suggests that all the school-food sold at the elementary school were ineligible to be consumed.

## DISCUSSION

### Sanitation

The possibility of cross-contamination during the treatment process was substantial, considering that all samples had an ineligible result for microbiology analysis. Cross-contamination can occur due to pathogenic bacteria originating from unclean kitchen equipment, other contaminated materials, and poor sanitation of processing workers. Many types of harmful pathogenic bacteria might contaminate during the tomato sauce process, including *E. coli*, *S. aureus*, and *Salmonella* spp<sup>16</sup>. The existence of animals that roam in the house can also contaminate food, such as insects or pets<sup>17</sup>.

All respondents had an awareness of hygiene on processing and serving of tomato sauce. However, the samples were not eligible to be consumed due to the microbiology analysis. This fact indicates that the hygiene knowledge practice of the respondents needs to be improved. Personal hygiene and the environment are two essential factors in good practice food processing<sup>8</sup>.

To promote the safety of the school food-snack, adopting a school meals program model, which is already well implemented in many countries, is necessary and urgent the<sup>18</sup>, with some modifications for the Indonesian school environment. The SVFS should be actively involved in this program in the form of the healthy school canteen, with the fix or mobile model. The canteen could be developed by coordinating with City Health Agency for routine controlling to ensure the school food-snack variety, quality, and safety. By this program, a healthy eating behavior could be

developed by offering a variety of food-snack with standard nutrition<sup>19,20</sup>.

### Rhodamine B in Tomato Sauce

Rhodamine B in tomato sauce can harm the consumers because the substance that is in the body can cause damage to the vital organ and increase the risk of exposure to mutagenic and carcinogenic compounds. Martony *et al.*<sup>3</sup> reported that Rhodamine B was detected in tomato sauce sold as a companion with meatball among student food-snack in Deli Serdang District, Indonesia. The consumption of foods containing artificial colorants and preservatives in children can trigger the hyperactive condition<sup>21,22</sup>.

Almost all respondents (95.8%) purchased the tomato sauce from the local market. Rhodamine B was detected in about 21 percent of the tomato sauce served by SVFS, in which 60 percent of the detected tomato sauce was sold by high school graduated respondents. All of the respondents know about synthetic food colorant and its negative impact. These findings show that there is a lack of knowledge among the respondents on the appearance and characteristics of food suspected containing Rhodamine B, or they did not pay proper attention to this issue.

### Microbiological Quality of Tomato Sauce

The high amount of microbial contamination might be caused by cross-contamination originating from various factors such as unclean kitchen equipment, other contaminated materials, dirty water sources, and poor sanitation. As much as 65.2 percent of tomato sauce purchased was added with other ingredients such as water, soy sauce, salt, and onions. The water added to tomato sauce is cooked water. However, the increase of water content could be a good media growth and proliferation for bacteria. Besides, bacteria quickly grow in the water with poor hygiene. Moreover, 12.5 percent of respondents did not use water from the municipal water supply, but they bought from local water suppliers<sup>23,24</sup>.

Inadequate knowledge of workers had improper food practices that they tend to ignore the hygiene and sanitation<sup>16,23,25</sup>. The hygiene knowledge level of SVFS affected insignificantly on the microbial quality of the food-snack as all of the tomato sauce collected from SVFS showed a TPC above the maximal limit of the standard<sup>14,15</sup>. A well-organized food hygiene training is needed to improve the SVFS as also reported for food premises in the UK<sup>26</sup>. Moreover, the tomato sauces as the sample were left open that could be possibly

contaminated by poor hygiene environment<sup>27</sup>. This issue highlighted that improving food quality requires shared responsibility, and the Ministry of Health must integrate food safety as an essential public health function into its program<sup>28</sup>.

Samples of tomato sauce containing *E. coli* could be caused by inappropriate hygiene practices and insufficient cleaning of cooking equipment. Considering the food sold close to the street, which might cause air pollution, may also induce the contamination from water used in the tools washing process<sup>29,30</sup>. *E. coli* can grow well at a temperature of 20-40°C, which allows the bacteria to multiply during the storage period. One of the characteristics of microorganisms is that they quickly grow in room temperature<sup>31</sup>. Thus, the longer the sauce would be used, the more microbial contamination would occur.

The presence of *Coliform* bacteria in food indicated the existence of enteropathogenic and toxigenic microorganisms that can be harmful to human health. The bacteria can cause food poisoning through two mechanisms that are intoxication and infection<sup>2</sup>. From January to September 2017, there were 13 cases of foodborne disease caused by street food in Indonesia or 14 percent of the total outbreaks<sup>4</sup>.

## CONCLUSION

Education level (elementary to the high school level) and knowledge of respondents did not associate to the sanitation and food safety of tomato sauce sold as a companion with meatball by the food-snack vendors around elementary schools in Samarinda. The food safety level of SVFS around elementary schools in Samarinda was classified to a low level. Almost 80 percent of tomato sauce samples met the chemical quality standards (no Rhodamine B detected). All tomato sauce used by 24 SVFS around elementary schools in Samarinda did not meet the microbiological status requirements according to Indonesian Standard. This study highlights that routine surveillance of tomato sauce agents on the Rhodamine B test in tomato sauce available in the market is needed. It is very urgent to obligate the Schools to provide internal space for SVFS so that the school and City Health Agency could control the food safety of food-snack sold in their school area.

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