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Food Safety of Shrimp Paste Products in Sidoarjo Regency Judging from the Presence of Microbiological Contamination and Chemical Contamination

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A B S T R A C T

Shrimp paste is a processed fishery product made from shrimp, fish or both. Food safety is the conditions and efforts needed to prevent food from three possible contaminations, namely microbiological, chemical and other contamination that can disturb, harm and endanger human health. This research aimed to determine the relationship between hygiene and sanitation and microbiological contamination (*Escherichia coli* and *Salmonella*); and determine the presence of contamination (rhodamine B and lead) qualitatively and quantitatively in shrimp paste circulating in Sidoarjo Regency. This research was carried out by taking 23 samples from 23 traders in the centre of Sidoarjo Regency using the purposive sampling method. This research consisted of an interview process, sanitation hygiene observations, microbiological contamination analysis (*Escherichia coli* and *Salmonella*) and chemical contamination analysis (rhodamine B and lead). The results of microbiological analysis on 23 samples of shrimp paste in Sidoarjo Regency were positive for the presence of *Escherichia coli* bacteria. Two samples of shrimp paste had values < 3.6 MPN/g, and data was obtained that environmental sanitation practices correlated with Escherichia coli contamination in shrimp paste samples in Sidoarjo with ($p<0.05$). The presence of *Salmonella* bacteria and rhodamine B chemical contamination was not detected in all samples of shrimp paste in Sidoarjo Regency. However, 14 samples of shrimp paste were identified as contaminated with the heavy metal lead >1.0 mg/kg. Based on the results of these four parameters, the "I" shrimp paste sample has fulfilled the SNI (2716:2016) criteria well when compared with 22 other types of shrimp paste in Sidoarjo Regency.

1. INTRODUCTION

1.1. *Research Background*

Sidoarjo is a district located in East Java Province. Covering 29.99% of the Sidoarjo Regency area in the eastern part, it is a farming area. The aquaculture sector has great potential for Sidoarjo Regency every year. According to data from the Central Statistics Agency in 2018, it is clear that there are several superior commodities produced, including milkfish, shrimp, tilapia, crab and seaweed [1]. Shrimp paste is one of the processed fishery products that is often found in Sidoarjo Regency. Shrimp paste is a traditional food product obtained from the fermentation process of fishery products. The raw materials used include reborn shrimp, fish or a mixture of both with or without the addition of other food ingredients and permitted food additives [2].

Food safety is the conditions and efforts needed to prevent food from three possible contaminations, namely microbiological, chemical and other contamination that can disturb, harm and endanger human health so that it is safe for consumption. Microorganisms basically influence the quality of a food product. The pathogenic bacteria that most often contaminate fishery products include *Salmonella* and *Escherichia coli* bacteria [3]. Several factors cause bacterial contamination of food products, including the way they are made using unhygienic tools and materials and the place where they are made or sold is not clean.

The quality of fisheries in Sidoarjo has decreased, and this is proven by shrimp samples being identified as being contaminated with heavy metals Pb and Zn [4]. The quality of raw materials will determine the quality of the processed product. Lead contamination of shrimp paste has occurred in Tuban, where there were four samples of shrimp paste containing heavy metal lead

>1.0 mg/kg. The use of contaminated raw materials and air pollution can cause sources of heavy metal lead pollution in food products. They can be contaminated by the tools used during the production process. This lead poisoning causes high levels of lead in the aorta, liver, kidneys, pancreas, lungs, bones, spleen, testes, heart and brain [5].

The characteristics of a good shrimp paste product can usually be seen from the physical product, which has an inconspicuous colour, distinctive aroma and is not rancid. The colour of the product is influenced by the type of raw material and the addition of dyes during production. The Food and Drug Monitoring Agency (BPOM) explains that four types of dangerous additives are often found in food. Namely formalin, borax, metal yellow, and rhodamine B. Rhodamine B is a synthetic dye that is commonly used in the paper and textile industries. It has been determined by the Minister of Health that regulation No.239/Menkes/Per/V/85 should not be applied to food products. The 30% of 20 school snack samples in Sidoarjo contained rhodamine B of 0.3314-0.6521 ppm [6]. The use of rhodamine B in food for a long time (chronic) can cause liver function disorders or cancer. However, if exposed to large amounts of rhodamine B, acute symptoms of rhodamine B poisoning will occur within a short time [7].

1.2. *Literature Review*

Food safety covers all food and food ingredients, starting from harvesting, processing, storage, distribution, handling, preparation and various other activities before consumption. Food safety is basically an effort to maintain food sanitation, nutrition and safety. Food sanitation hygiene, called food hygiene, is an effort to control factors in places, equipment, people and food that can or may cause health problems or food poisoning. Food sanitation hygiene can be determined by several factors, including handler hygiene, equipment sanitation, condition of vending facilities, and selling location.

Almost all food ingredients are contaminated by various microorganisms from the surrounding environment. Several types of microbes found in food are *Salmonella sp*., Staphylococcus aureus, *Escherichia coli*, mould, yeast and other pathogenic microbes. Microbes have certain limitations in food ingredients that affect their sustainability [8]. Shrimp paste circulating in the Surabaya area was found to contain *Escherichia coli* bacteria contamination in 12 samples of shrimp paste above the predetermined limit, namely 3.60 - 4.56 Log CFU/g [9]. *Salmonella* and *Escherichia coli* bacteria are also found in shrimp paste circulating in Medan traditional markets [10].

Rhodamine B is a dye used in the paint, textile and paper industries, so it is not intended for use in food. 60% of 15 shrimp paste samples consisting of branded and unbranded shrimp paste samples in Makassar City contained rhodamine B at levels of 11.81-19.05 ppm [11]. The use of rhodamine B in shrimp paste is partly due to producers' inadequate knowledge regarding the dangers of using synthetic dyes on health and also because the level of public awareness is still low.

Heavy metal content in food that exceeds the threshold can also be one of the causes of poisoning, which makes food unfit to eat. Lead is a heavy metal whose presence is not expected in food because it is very dangerous if consumed. Testing of lead contamination content in marine products in Banyuwangi Regency proved that of the 15 test samples, there was one sample that contained lead, namely shrimp paste products amounting to 2.0 mg/kg [12].

1.3. *Research Objectives*

This research aims to determine the relationship between hygiene and sanitation on microbiological contamination (*Escherichia coli* and *Salmonella*) in shrimp paste and to determine the presence of contamination (rhodamine B and lead) in shrimp paste circulating in Sidoarjo Regency.

2. MATERIALS AND METHODS

2.1. *Materials and Tools*

A total of 23 shrimp paste samples were obtained from 23 traders in the centre of Sidoarjo Regency. Materials for analysis in this study include: Materials for analysis in this study include Lactose Broth (LB), Brilliant Green Lactose Broth (BGLB), EMB Agar (EMBA), Salmonella Shigella Agar (SSA), distilled water, rhodamine B reagent-1 ($SbCl_5$ reagent solution), reagent-2 rhodamine B (toluene reagent solution), lead reagent test kit reagent solution.

The tools needed in this research are analytical balance, hot plate, knife, mortar, dropper pipette, measuring pipette, beaker glass, erlenmeyer, test tube, Durham tube, ose, vortex, incubator, atomic absorption spectrophotometry (AAS).

2.2. *Design Experiment and Analysis*

This research was carried out by taking 23 samples from 23 traders in the centre of Sidoarjo Regency using the purposive sampling method. Several types of samples will be used in this research, namely branded shrimp paste and unbranded shrimp paste. Apart from looking at the packaging of the shrimp paste product, sampling was determined based on the shrimp paste that is most commonly found (homogeneous) in the centre of Sidoarjo Regency and the best-selling value or the shrimp paste that consumers buy the most. The population in this study were shrimp paste traders in traditional wholesale markets, supermarkets and souvenir shops in the centre of Sidoarjo Regency.

This research consisted of observing the sanitation hygiene of traders, analysing microbiological contamination (*Escherichia coli* and *Salmonella*) and analysing chemical contamination (rhodamine B and lead). Samples of shrimp paste will be tested at the Microbiology Laboratory and Food Analysis Laboratory, Food Technology Department, Universitas Pembangunan Nasional "Veteran" Jawa Timur and the Food Quality and Safety Testing Laboratory, Universitas Brawijaya, Malang.

2.3. *Implementation of Research*

2.3.1. *Analysis Escherichia coli MPN method [13]*

Take a sample of 1 g and put it in 9 ml of distilled water, then voxex until homogeneous. Dilute to 10^{-3}. Take 1 ml of each sample and put it in a tube containing 9 ml of LB media. Put the tube into the incubator at 37ºC for 24 hours. If there is a colour change and gas is formed in the Durham tube, the sample is declared positive (+) and continues with the confirmation test. Samples that proved positive (+) were put into each tube containing 9 ml. BGLB and adjusted for dilution level. Put the tube into the incubator at 37ºC for 24 hours. Note the number of

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BGLB tubes that are positive (+) gas and the colour change and refer the results to the MPN table. The numbers obtained from the table show the MPN Coliform per 100 ml of test sample. The sample suspension from the tube that was declared positive (+) was taken with a loop and incubated in a petri dish containing EMB Agar. Then incubated at 37ºC for 24 hours. Samples that are positive for *Escherichia coli* are characterised by the presence of bright red (metallic green) colonies.

2.3.2. Testing *Salmonella [8]*

The shrimp paste sample was weighed as much as 10 g, then ground with a mortar, then put into a tube containing 90 ml of distilled water and homogenised using a vortex. Take 1 ml of dilution at a dilution of 10^{-1} and put it in a test tube containing 9 ml of distilled water (dilution 10^{-2}). Take 1 ml of sample solution from both dilutions using the pour method on SSA media. Next, incubate at 37ºC for 24 hours. Observations were made on colonies of *Salmonella* bacteria growing on SSA media, namely that transparent colonies were formed with black spots in the middle.

2.3.3. Rhodamine B testing with *Test Kits [14]*

Weigh a sample of 25 grams, then grind it. Add 50 ml of distilled water, stir, and filter. Next, take 3 ml of the filtrate from the sample and put it into a test tube. Add one drop of Rhodamine-1 reagent, then stir until homogeneous. Add three drops of Rhodamine-2 reagent, then stir until homogeneous. Observe the colour changes that form. If the purple colour appears again or increases in intensity, it means that Rhodamine B is in the test sample. If no colour change occurs, then the sample is negative or not identified as containing Rhodamine B.

2.3.4. Lead Testing with *Test Kits [12]*

Put 25 g of the sample to be tested in a volume of 50 ml of distilled water or hot water, then chop and crush until completely dissolved. Prepare a test tube, add 2-3 ml of sample extract and add three drops of Plumbum reagent - 1. Add three drops of Plumbum reagent - 2. Add three drops of Plumbum reagent - 3. Add three drops of Plumbum reagent - 4, and stir until the solution is completely mixed. If red bubbles form, then the sample is declared positive for containing lead (Pb). Positive results in the qualitative analysis of lead will be continued in quantitative analysis carried out at the Quality and Food Safety Testing Laboratory, Universitas Brawijaya, Malang.

3. RESULTS AND DISCUSSION

3.1. *Trader Observation Results*

The results of this observation were obtained from direct observation to determine the hygiene of the handlers, sanitation of the surrounding environment and sanitation of serving.

a. Handler Hygiene

A handler is someone who is involved in a series of food processing processes starting from preparation, cooking, cooling, storage, reheating and serving food. The results of the implementation of hygiene by handlers of shrimp paste traders in Sidoarjo are presented in Table 1.

Table 1. Handler Hygiene Analysis Results

Criteria	Frequency (person)	Percentage (%)
Good	15	65.22
Bad	8	34.78

Table 1 shows that the implementation of hygiene by shrimp paste traders in Sidoarjo has good criteria (65.22%) and poor criteria (34.78%). The observation results in this study are in accordance with [15], which proves that as many as (78.7%) of the 61 respondents were included in the category of actively practising good sanitation and hygiene. 13 people gave negative assessments (21.3%). The bad criteria in this analysis are caused by the lack of optimal hygiene practices of shrimp paste handlers, especially shrimp paste traders who are in traditional wholesale markets. Each handler can carry disease-causing bacteria in splashes of saliva, skin, hair, dirty nails, and clothing and can be a source of food contamination. Handler hygiene is important because it can stop the spread of bacteria from traders who serve food [16].

b. Environment Sanitation

Sanitation of the sales environment is a control effort through monitoring and examining the effects caused by the sales place, which are closely related to the spread of disease. The results of implementing environmental sanitation around the sale of shrimp paste in Sidoarjo are presented in Table 2.

Table 2. Environmental Sanitation Analysis Results

Criteria	Frequency (person)	Percentage (%)
Good	8	34.78
Bad	15	65.22

Table 2 shows that the implementation of environmental sanitation around the sales of shrimp paste traders in Sidoarjo has good criteria (34.78%) and bad criteria (65.21%). The results of this observation are in line with [17], which explains that the sanitation of traders' salesplaces is still not given enough attention. Some locations in traditional markets are close to open sources of waste disposal and unclean environmental conditions. Open dumping of rubbish can attract animals such as rats or flies as well as unpleasant odours. The presence of insects or other animals close to the place of sale can contaminate food through their entire bodies, which carry dirt or disease germs that come from human waste or wastewater [18].

A clean, well-maintained sales area will be a hygienic and pleasant place to work. The cleanliness of the selling place determines the quality and safety of the food produced. Traders need to make efforts to control and improve sanitation at their sales points, including providing special trash bins, sorting the trash and keeping the trash bins in a closed condition. Apart from that, building facilities need to be improved.

c. Serving Sanitation

The results of implementing serving sanitation carried out by shrimp paste traders in Sidoarjo are presented in Table 3.

Table 3. Results of Presentation Sanitation Analysis

Criteria	Frequency (person)	Percentage (%)
Good	17	73.91
Bad	6	26.09

Table 3 shows that the implementation of environmental sanitation around sales has good criteria (73.91%) and bad criteria (26.90%). This proves that the implementation of sanitation in serving shrimp paste products in Sidoarjo Regency is quite good. The results of this observation are in line with [16], which explained that as many as (57.1%) of traders had carried out proper sanitation of the presentation of food products. The poor criteria for serving shrimp paste come from several unlabeled shrimp paste products sold in the traditional wholesale market of Sidoarjo Regency. Basically, every product label must contain information regarding the processed food in the packaging. Several shrimp paste products are considered unclean because there are white spots on the product, such as mould. This can happen due to improper storage or storage that is too long.

3.2. *Results of Microbiological Contamination Analysis*

The results of microbiological contamination analysis were obtained from research conducted at the microbiology laboratory of UPN "Veteran" East Java. There are two types of microbiological contamination observed in shrimp paste samples, namely the presence of bacteria Escherichia coli and Salmonella.

a. Escherichia coli

Test ***results*** of Escherichia coli in shrimp paste samples can be seen in Table 4.

Table 4. Results of Bacterial Analysis Escherichia coli on Shrimp Paste in Sidoarjo Regency

Code	*Escherichia coli*	MPN/g	Status
A	(+)	9.33 ± 0.09	TM
B	(+)	133.33 ± 18.86	TM
C	(+)	9.73 ± 1.79	TM
D	(+)	15.33 ± 0.47	TM
E	(+)	1100	TM
F	(+)	10.8 ± 2.26	TM
G	(+)	153.33 ± 4.71	TM
H	(+)	3	M
I	(+)	3	M
J	(+)	33.67 ± 3.30	TM
K	(+)	105 ± 21.21	TM
L	(+)	>1100	TM
M	(+)	9.8 ± 1.70	TM
O	(+)	36	TM
P	(+)	15.67 ± 0.47	TM
Q	(+)	33.67 ± 3.30	TM
R	(+)	>1100	TM
S	(+)	15.67 ± 0.47	TM
Q	(+)	170 ± 28.28	TM
U	(+)	>1100	TM
V	(+)	64.33 ± 15.08	TM
W	(+)	15.67 ± 0.47	TM
X	(+)	31.33 ± 3.30	TM

Note: (+) identified *Escherichia coli*; TM (Not Fulfilled); M (Fulfilled); Max requirement 3.6 MPN/g.

Table 4 explains that 23 samples of shrimp paste in Sidoarjo were identified as positive (+) for *Escherichia coli* bacteria. The results of this study are in accordance with research [9], which proved the existence of *Escherichia coli* bacterial contamination in 12 shrimp paste samples in the Surabaya area. Based on Table 4, 21 samples of shrimp paste in Sidoarjo were declared not to meet SNI standards and the highest MPN value was obtained for the samples (L, R, and U), namely >1,100 MPN/g. There are two samples (H and I) that have an MPN value of 3 MPN/g, and this level is in accordance with the criteria set by SNI (2716:2016) regarding the quality and safety requirements for shrimp paste food that the maximum amount of *Escherichia coli* bacterial contamination in shrimp paste is 3.6 MPN/g.

The presence of *Escherichia coli* bacteria shows a sign that sanitation practices are not good because these bacteria can be transferred by hand-to-mouth activities or by passive transfer through food, water and other products [19]. The spread of these bacteria in food can be through water pollution or from the environment. *Escherichia coli* bacteria can grow at high and low temperatures, with a low temperature of 7°C and a high temperature of up to 44°C, and optimally at a temperature of 35-37°C with a pH of 7-7.5 [20]. According to Ref. [21], this bacteria is relatively easily killed by heating. Namely, it will die at a temperature of 60ºC for 30 minutes. This bacteria can become a pathogen if there is a lot of it in the human body. Besides, this bacteria will produce enterotoxins, which cause diarrhoea. Some types of pathogenic *Escherichia coli* can cause infections of the urinary tract [22].

b. Salmonella

The results of *Salmonella* testing on shrimp paste samples can be seen in Table 5.

Table 5. Results of analysis of *Salmonella* bacteria on Shrimp Paste in Sidoarjo Regency

Code	*Salmonella*	Status
A	(-)	M
B	(-)	M
C	(-)	M
D	(-)	M
E	(-)	M
F	(-)	M
G	(-)	M
H	(-)	M
I	(-)	M
J	(-)	M
K	(-)	M
L	(-)	M
M	(-)	M
O	(-)	M
P	(-)	M
Q	(-)	M
R	(-)	M
S	(-)	M
Q	(-)	M
U	(-)	M
V	(-)	M
W	(-)	M
X	(-)	M

Note: (-) no *Salmonella* identified; M (Fulfilled); Fulfilled if *Salmonella* is not detected in the sample

Table 5 proves that the 23 shrimp paste samples in Sidoarjo Regency were not contaminated with *Salmonella* bacteria. Hence,

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all shrimp paste samples met the requirements of SNI 2716 2016 regarding quality and food safety requirements for shrimp paste. This result is in accordance with [23], which explains that samples of shrimp paste bought and sold at the Makassar City Power Market are not contaminated with *Salmonella* bacteria, but is not in accordance with [10], which explains that samples of shrimp paste at the Medan Traditional Market were identified as containing *Salmonella* bacteria. The presence of *Salmonella* was not detected in shrimp paste samples in Sidoarjo Regency, allegedly because shrimp paste is a food product that uses salt as a fermentation technique. According to [24] in his research, it was explained that the process of making shrimp paste that uses high salt results in the undetectability of *Salmonella* bacteria in the shrimp paste because *Salmonella* cannot tolerate high salt levels and will die if it is in media with salt levels above 9%. Food products can be contaminated with *Salmonella* through infected handlers, pets and pests or cross-contamination due to poor hygiene [25]. The source of transmission is the output (excretion) of animals and humans, both from animals to humans and vice versa. *Salmonella* is often pathogenic for humans or animals if it enters through the mouth. Besides, this bacteria is a bacteria that causes food-borne disease (Salmonellosis). This can occur due to consuming food contaminated by these bacteria [26].

3.3. *The Relationship between Sanitary Hygiene and Microbiological Contamination*

This test was carried out to determine the relationship between sanitary hygiene practices and bacterial contamination in shrimp paste products in Sidoarjo. Chi-square testing of the relationship between sanitation and hygiene was not carried out on *Salmonella* because the research results showed that all shrimp paste samples in Sidoarjo Regency were not identified as containing *Salmonella*, so there was no correlation between sanitation hygiene and the presence of *Salmonella*, which was not detected in shrimp paste in Sidoarjo. Based on the results of hygiene and sanitation observations of traders in (Table 1-3) as well as observations of *Escherichia coli* (Table 4), the Chi-square test results were obtained regarding the relationship between hygiene and sanitation practices and *Escherichia coli*, which can be seen in Table 5.

Table 6. Chi-Square Test Results of The Relationship Between Sanitation Hygiene Practices and *Escherichia coli* Bacteria on Shrimp Paste in Sidoarjo Regency

Category	Criteria	*Escherichia coli*				*p-value*
		Fulfilled		Not Fulfilled		
		N	%	N	%	
Handler Hygiene	Good	2	8.70	13	56.52	0.280
	Bad	0	0	8	34.78	
Environment sanitation	Good	2	8.70	6	26.08	0.043
	Bad	0	0	15	65.22	
Serving Sanitation	Good	2	8.70	15	65.22	0.379
	Bad	0	0	6	26.08	

Based on the results of the chi-square statistical test for each category of sanitation hygiene, shrimp paste traders in Sidoarjo produced different p-values. In the handler hygiene category, the p-value was obtained at 0.280 and the p-value (0.280) > α (0.05), so there was no significant relationship between handler hygiene and *Escherichia coli* contamination on shrimp paste in Sidoarjo. This can be interpreted as handler hygiene not correlated with *Escherichia coli* contamination in shrimp paste samples in Sidoarjo. In the environmental sanitation category, the p-value was obtained at 0.043 and the p-value (0.043) < α (0.05), so there is a significant relationship between environmental sanitation and *Escherichia coli* contamination in shrimp paste in Sidoarjo. It can be interpreted that environmental sanitation practices correlate with the presence of *Escherichia coli* contamination in shrimp paste samples in Sidoarjo. In the serving sanitation category, the p-value was obtained at 0.379 and the p-value (0.379) > α (0.05), so there was no significant relationship and no correlation between serving sanitation and *Escherichia coli* contamination in shrimp paste in Sidoarjo.

This is in line with [27], which explains that there is a real relationship between the sanitation of selling places and the presence of *Escherichia coli* bacteria on products. Poor environmental sanitation practices for selling shrimp paste in Sidoarjo can actually affect the safety quality of shrimp paste products. Poor environmental sanitation conditions can affect the quality of food served to consumers [28]. This will clearly also affect the health level of consumers who consume this food.

3.4. *Results of Chemical Contamination Analysis*

There were two types of chemical contamination observed in the shrimp paste samples, namely the presence of the compound rhodamine B and the heavy metal lead.

a. Rhodamine B

This test was carried out to determine the food safety of shrimp paste in Sidoarjo Regency from the dangerous compound rhodamine B. The rhodamine B test was carried out qualitatively with a rhodamine B test kit. The results of the rhodamine shrimp paste test can be seen in Table 7.

Table 7. Results of Rhodamine B Testing on Shrimp Paste in Sidoarjo Regency

Code	Rhodamine B	Discolouration	Status
A	(-)	Chocolate	M
B	(-)	Pink	M
C	(-)	Orange	M
D	(-)	Yellow	M
E	(-)	Yellow	M
F	(-)	Yellow	M
G	(-)	Chocolate	M
H	(-)	Chocolate	M
I	(-)	Chocolate	M
J	(-)	Chocolate	M
K	(-)	Chocolate	M
L	(-)	Chocolate	M
M	(-)	Orange	M
O	(-)	Yellow	M
P	(-)	Orange	M
Q	(-)	Yellow	M
R	(-)	Pink	M
S	(-)	Brick red	M
Q	(-)	Orange	M
U	(-)	Orange	M
V	(-)	Chocolate	M
W	(-)	Orange	M
X	(-)	Chocolate	M

Description: (-) does not contain rhodamine B; M (Fulfilled); Fulfilled if the sample does not contain rhodamine B

The sample will be declared positive (+) for containing rhodamine B if the colour changes to purple after being dropped reagent. Based on the test results in Table 7, the rhodamine B test was carried out on 23 types of shrimp paste. All shrimp paste samples (100%) tested were declared negative (-) rhodamine B so that all shrimp paste samples met SNI requirements and were not continued with quantitative analysis. This result is in accordance with [29], which stated that the shrimp paste in Sumbawa City Market was not identified as rhodamine B, but is not in line with [30], which stated that ten samples of shrimp paste in Probolinggo were identified as containing the dangerous compound rhodamine B. Rhodamine B is a compound which is generally used in industry. Textiles are very dangerous if used as a colouring in food products. The presence of heavy metal residues in these dyes is very dangerous for health because the accumulation of these dyes can cause liver cancer [31]. The reddish colour of shrimp paste comes from the astaxanthin pigment in the shrimp shell, so the pigment forms a red colour [32]. Astaxanthin at high salt concentrations (15%) is less than at low salt concentrations (2-8%), so that the shrimp paste becomes black [33].

b. Lead

The lead test was carried out qualitatively using a lead test kit. The sample is said to be positive (+) for the qualitative test if an orange-to-pink solution is formed with red bubbles below. Suppose the sample is declared positive for lead. In that case, it will continue with quantitative analysis to determine the lead level in the sample using atomic absorption spectrophotometry (AAS), which is carried out at the Food Safety Quality Testing Laboratory, Universitas Brawijaya, Malang.

Table 8. Results of Lead Testing on Shrimp Paste in Sidoarjo Regency

Code	Lead	Discolouration	Concentration (mg/kg)	Status
A	(+)	Chocolate	1.35 ± 0.025	TM
B	(+)	Pink	1.57 ± 0.005	TM
C	(+)	Orange	1.73 ± 0.025	TM
D	(+)	Yellow	0.74 ± 0.015	M
E	(-)	Yellow	-	M
F	(+)	Yellow	1.97 ± 0.02	TM
G	(+)	Chocolate	1.46 ± 0.011	TM
H	(+)	Chocolate	1.79 ± 0.011	TM
I	(-)	Chocolate	-	M
J	(+)	Chocolate	1.47 ± 0.006	TM
K	(-)	Chocolate	-	M
L	(-)	Chocolate	-	M
M	(+)	Orange	1.54 ± 0.01	TM
O	(+)	Yellow	2.04 ± 0.01	TM
P	(+)	Orange	1.82 ± 0.01	TM
Q	(-)	Yellow	-	M
R	(+)	Pink	2.1 ± 0.006	TM
S	(-)	Brick red	-	M
Q	(+)	Orange	1.81 ± 0.006	TM
U	(+)	Orange	1.04 ± 0.006	TM
V	(+)	Chocolate	1.12 ± 0.006	TM
W	(-)	Orange	-	M
X	(-)	Chocolate	-	M

Description: (+) contains lead, (-) does not contain lead; TM = Does not fulfil, M = Fulfilled; Fulfilled the max requirements. 1.0 mg/kg.

Based on Table 8, 9 shrimp paste samples have met the requirements of SNI (2716:2016), but 14 samples contain lead >1.0 mg/kg, so these samples do not meet the requirements of SNI (2716:2016) when viewed from the metal lead content in the sample. Based on the test table, the highest lead content was obtained at 2.1 mg/kg ± 0.006 in sample (R), and the lowest lead content was 0.74 mg/kg ± in sample (D). This is in line with [34], which stated that two samples of shrimp paste from several traditional markets in Medan identified metals > 1.0 mg/kg where this figure had exceeded the specified limit, in addition to that [35] proved that there was 1 sample out of 16 samples Food in Surabaya was identified as lead, namely in shrimp paste samples originating from the Gersikan Market in Surabaya at 0.005-0.01 ppm.

The source of lead (Pb) contamination in shrimp paste is thought to come from the raw material for making shrimp paste, namely shrimp. Shrimp can contain Pb because they live in an environment that is polluted by lead. Pb levels in the shrimp's body can be influenced by how long the shrimp's body is in contact with a polluted environment [36]. High Pb levels in shrimp will increase the risk of disease if you consume shrimp or its processed products. The next source of heavy metal Pb pollution is from the equipment used. Lead (Pb) enters the human body through the consumption of food, air, water and dust contaminated with lead (Pb). Lead (Pb) is a heavy metal that is harmful to organisms and has carcinogenic properties that can cause changes in toxicity, decomposition for a long duration, and mutation [37].

3.5. *Results of Analysis of Shrimp Paste in Sidoarjo Regency*

The results of the analysis of 23 shrimp paste samples in Sidoarjo Regency in terms of microbiological contamination and chemical contamination are presented in the following table.

Table 9. Results of Analysis of Shrimp Paste in Sidoarjo Regency

Code	*E. coli* (MPN/g)	*Salmonella*	Rhodamine B	Lead (mg/kg)	Status
A	9.33 ± 0.09	(-)	(-)	1.35 ± 0.025	TM
B	133.33 ± 18.86	(-)	(-)	1.57 ± 0.005	TM
C	9.73 ± 1.79	(-)	(-)	1.73 ± 0.025	TM
D	15.33 ± 0.47	(-)	(-)	0.74 ± 0.015	TM
E	1100	(-)	(-)	-	TM
F	10.8 ± 2.26	(-)	(-)	1.97 ± 0.02	TM
G	153.33 ± 4.71	(-)	(-)	1.46 ± 0.011	TM
H	3	(-)	(-)	1.79 ± 0.011	TM
I	3	(-)	(-)	-	M
J	33.67 ± 3.30	(-)	(-)	1.47 ± 0.006	TM
K	105 ± 21.21	(-)	(-)	-	TM
L	>1100	(-)	(-)	-	TM
M	9.8 ± 1.70	(-)	(-)	1.54 ± 0.01	TM
O	36	(-)	(-)	2.04 ± 0.01	TM
P	15.67 ± 0.47	(-)	(-)	1.82 ± 0.01	TM
Q	33.67 ± 3.30	(-)	(-)	-	TM
R	>1100	(-)	(-)	2.1 ± 0.006	TM
S	15.67 ± 0.47	(-)	(-)	-	TM
Q	170 ± 28.28	(-)	(-)	1.81 ± 0.006	TM
U	>1100	(-)	(-)	1.04 ± 0.006	TM
V	64.33 ± 15.08	(-)	(-)	1.12 ± 0.006	TM
W	15.67 ± 0.47	(-)	(-)	-	TM
X	31.33 ± 3.30	(-)	(-)	-	TM

Notes: (-) not identified; (TM) is not fulfilled; (M) fulfilled; Fulfilled SNI requirements (2716:2016).

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Based on Table 9, data shows that the shrimp paste sample "I" is a shrimp paste sample that has met the SNI (2716:2016) criteria well when compared with 22 shrimp paste samples in Sidoarjo Regency, this is proven by the amount of *Escherichia coli* contamination of 3 MPN/g and no Identified the content of *Salmonella* bacteria, rhodamine B and also the heavy metal lead.

4. CONCLUSION

A total of 23 shrimp paste samples in Sidoarjo Regency were positive for *Escherichia coli* bacteria. Two shrimp paste samples had values < 3.6 MPN/g, and data was obtained that environmental sanitation practices correlated with *Escherichia coli* contamination in shrimp paste samples in Sidoarjo with ($p< 0.05$). The presence of *Salmonella* bacteria and Rhodamine B compounds was not detected in all samples of shrimp paste in Sidoarjo Regency. However, 14 samples of shrimp paste were identified as containing lead >1.0 mg/kg. Based on the results of these four parameters, the "I" shrimp paste sample has fulfilled the SNI (2716:2016) criteria well when compared with 22 other types of shrimp paste in Sidoarjo Regency.

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