Bacteria contamination and Cadmium heavy metal content of blood cockle (*Anadara granosa* Linn) satay on street vendors in Surabaya, Indonesia

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Abstract. This study aims to detect the food safety of blood cockle satay sold by street vendors in Surabaya Indonesia, in terms of bacterial contamination and Cadmium heavy metal content. This research is descriptive research with survey method. The sampling technique was carried out randomly from 15 street vendors, for further testing of bacterial contamination which included total bacteria, *Coliform* bacteria, and detection of heavy metal Cadmium (Cd) in blood cockle satay. The results showed that of all (15) satay samples studied have the average total microbes was 7.22 Log CFU/gr, *Coliform* bacterial contamination exceeded the maximum limit determined by the food regulatory agency Food and Drug Supervisory Agency of Republic Indonesia in 2009 (> 3 MPN/gram). As many as 5 (33.33%) of the 15 samples of scallop satay containing heavy metal Cd exceeded the maximum limit determined by Food and Drug Supervisory Agency of Republic Indonesia in 2017 (> 0.1 mg/kg).

Keywords: Safety Food, Bacterial Contamination, Cadmium Heavy Metal

1 Introduction

Food safety is a condition and effort needed to prevent food from being contaminated by biological, chemical, and other objects that can interfere, harm, and endanger human health and do not conflict with religion, belief, and community culture so that it is safe for consumption [1]. There are three hazards that need to be watched out for in relation to food safety, namely microbiological hazards, chemical hazards, and physical hazards. Microbiological hazards include pathogenic bacteria, viruses, and parasites [2].

Surabaya has a special food "Blood cockle satay" made from blood cockle meat, which in the manufacturing process is not baked or grilled like other satays, but boiled and usually served as a complement to "Lontong Balap" and "Lontong Kupang". Blood cockle satay must meet health requirements including adequate nutritional content, free from heavy metals and microorganisms that can interfere with health [3].

Besides having high nutritional content, blood cockle also have the ability to accumulate heavy metals compared to other marine animals. Several studies on

the heavy metal content of Cadmium in blood cockle have been carried out by [4], [5] and [6]. According to [7] the content of Cadmium in blood cockle originating from Kenjeran waters exceeds the threshold set by [8] regarding Cadmium metal contamination (0.1 ppm), while the average Cadmium heavy metal content in blood cockle is 0.301 ppm. Cadmium (Cd) is a heavy metal group with high toxicity. According to [9], due to heavy metal poisoning Cd causes damage to the liver, kidneys, lungs, bones, and is a carcinogen. According to the Regulation of the Food and Drug Supervisory Agency No 5 in 2018 concerning the maximum limit of Cadmium heavy metal contamination in fish and shellfish processed foods that may be consumed, which is 0.10 ppm.

Heavy metals have been shown to suppress the immune system resulting in a decrease in the number of antibody-producing cells, which will increase the body's susceptibility to bacteria, parasites, and viruses as well as susceptibility to cancer [10] and [11]. Heavy metal contamination in food products must be removed or reduced in order to make the product safe for consumption. Processing of foodstuffs before consumption is very important to reduce/eliminate

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levels of heavy metals in foodstuffs. [12], showed that there is a decrease in the content of heavy metals Cd, Cu, Zn and Fe in blood clams (*Anadara granosa*) during the process of making and reheating scallop satay. [13], also suggested that processing by steaming, boiling and sauteing can reduce the levels of heavy metals Pb and Cd in the leaves and fruit of long beans.

Blood cockle satay is traditionally cooked and sold ready-to-eat. Traditional processing causes differences in maturity, hygiene, sanitation and food safety among traders. There is a time lag between the scallop satay when consumed by consumers and processing. Scallop satay is displayed at room temperature before consumption, thus allowing for bacterial contamination from the environment where it is sold. Pathogenic bacteria in scallop satay may come from contamination of scallops that are not destroyed due to processing, cross-contamination of raw scallops due to poor hygiene and sanitation as well as during the scallop satay on display before consumption.

[14], showed that *E. coli* contamination in blood clam satay sold in the Surabaya traditional market area exceeded the SNI threshold. The results of research that researchers have done previously [15], showed that the environmental sanitation conditions of sales in traditional markets in Surabaya affect the level of contamination of pathogenic bacteria in chicken meat. Food safety related to the dangers caused by bacteria is measured by two main parameters, namely: the number of polluting bacteria and the type of pathogenic bacteria. One way to determine the level of food hygiene and sanitation is to use a microbiological analysis method which includes the calculation of total bacteria (Total Plate Count), total Coliform and total *E. coli* which are used as indicators of faecal contamination [16]. This study aims to detect the food safety of blood cockle satay sold by street vendors in Surabaya Indonesia, in terms of bacterial contamination and Cadmium heavy metal content.

2 Research Methods

This is descriptive research with survey method. The sampling technique was done randomly. A total of 15 samples of blood cockle satay were obtained from 15 street vendors in the Kenjeran area of Surabaya. The research methods included observation on hygiene and sanitation from 15 scallop satay traders, microbiological test of blood cockle satay which included total bacteria as an indicator of contamination level and total *Coliform* test with the Most Probable Number (MPN) method and analysis of Cadmium heavy metal content.

2.1 Determination of Total Bacteria (Drop Methods)

A total of 25 g samples were mixed using a stomacher machine for 2 minutes. One gram of the sample was weighed and homogenized in 9 ml of Buffered Peptone Water (BPW, Oxoid, Wesel, Germany) to produce a 10^{-1} dilution, then a 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} and so on with 0.1% Peptone Water solution. For each sample, 50 μ l of each dilution was taken with a micropipette and dripped onto the surface of a fairly dry sterile Plate

Count Agar (PCA) media. PCA was incubated overnight (18-24 hours) at 37° C. Colony growth was counted using the Colony Counting Chamber and calculated in Colony Forming Units per gram (cfu/g) of meat sample using the formula :

$$\text{Cfu} = \text{number of colonies} \times 1000/50 \times \text{dilution factor}$$

Henceforth, it is expressed in colony-forming units on average per gram (average cfu/g) and converted to log 10 (Yulistiani et al., 2019).

2.2 Total Coliform with Most Probable Number (MPN) Methods

MPN is a microorganism enumeration method that uses data from the growth of microorganisms in a specific liquid medium in a series of tubes grown from solid or liquid samples so as to produce a range of the number of microorganisms in the closest approximate number. Coliform bacteria fermented with lactose media will produce gas if incubated for more than 48 hours at 35°C. The basis of the MPN method is by looking at the gas produced in the test tube which is then adjusted to the MPN table [17].

2.2.1 Presumptive Test

Scallop satay samples were placed in a sterile tube containing Lactose Broth. Several tubes were incubated for 48 hours at 35°C, then checked for gas formation, because bacteria will ferment lactose and produce gas. If gas is not formed within 24 hours, incubation is continued for up to 48 hours. The estimator test is said to be positive if there is gas in the tube which is indicated by the floating of the Durham tube. This test detects the fermentative nature of Coliform in the sample and must be confirmed by a confirmatory test to rule out the presence of other organisms that give a positive result in lactose fermentation [17].

2.2.2 Confirmed Test

The positive tube obtained from the estimator test was followed by a confirmatory test. Positive samples showing gas were inoculated on Brilliant Green Lactose Broth media, then incubated at 37°C for 48 hours. After 48 hours, all tubes will be examined for results. If gas is produced, then this confirmatory test is declared positive and recorded to calculate the estimated number of Coliform bacteria according to the MPN table series 3 FDA-BAM tubes [17].

2.2.3 Completed test

Completed test was carried out by inoculating bacterial colonies on agar medium by scratching and incubating for 24 hours at 35°C. The media used were Endo agar and Eosin Methylene Blue (EMB). Germination on this agar media causes the agar medium to become dark purple in color with a metallic copper sheen and form colonies with dark centers. The result of this MPN method is the MPN value, which is an estimate of the

number of growth units (Growth Units) or colony forming units (Colony Forming Units) in the sample. The unit used is generally per 100 cc, the smaller the MPN value, the higher the quality of food products for consumption.

2.3 Analysis of Cadmium (Cd)

Samples were first weighed with a weight of about 300 grams of wet weight each (labeled), and put in a refrigerator and analyzed in the laboratory. Determination of the heavy metal content in the scallop satay samples using the Nitric Acid-Perchloric Acid Digestion method, where the sample is oxidized by acid so that the metal is dissolved. This process is also known as destruction. Standard and calibrated solutions were prepared according to the Nitric Acid-Perchloric Acid Digestion method. Analysis of the heavy metal content of Cd in samples was carried out in the laboratory using AAS (Atomic Absorption Spectro-photometer) in accordance with the Direct Air-Acetylene Flame Method with a wavelength of 228.8 nm (for Cd) [18].

3 Data Analysis

Data analysis was carried out descriptively, namely providing a description of the sample studied through the research data obtained. The total number of bacteria was transformed in logarithmic form (Log CFU/gr). Data is expressed in absolute values and in percentages using Microsoft Office Excel 2010 software and presented in tabular form.

4 Results and Discussion

4.1 Sanitary Hygiene of Traders

Based on the sanitation hygiene of blood cockle satay traders analysis (Table 1), it shows that the poor condition of personal hygiene is 46.67 %, the poor condition of equipment sanitation is 73.33 % and the bad condition of the sales place is 80.00 %.

Table 1. Hygiene and sanitation of traders

Criteria	Personal Hygiene	Equipment sanitation	The sales place sanitations
Good	7(46,67%)	4(26,67%)	3(20,00%)
Poor	8(53,33%)	11(73,33%)	12(80,00%)
Total	15	15	15

Causing factor of poor sanitation hygiene, among others, influenced by personal hygiene traders and food sanitation. Indicators of hand and nail hygiene are one of the causes of poor personal hygiene of traders [19]. From the observations, it was found that many traders did not wash their hands using soap and clean running water, traders only washed their hands with water in ordinary buckets and it was found that some traders only used rags to clean the dirt on their hands, even traders who did not wash their hands at all. Likewise, the poor environmental conditions at the place of sale where a lot of garbage is scattered and is inundated with

washing water. These conditions can cause contamination of the shellfish satay products sold.

4.2 Total Bacteria

The level of bacterial contamination based on the average value of total microbes in blood cockle satay (Table 2), which is 7.22 Log CFU/gr, far exceeds the maximum limit of microbial contamination in processed foods that has been set based on [20] (5.00 Log CFU/g). This study indicated that poor hygiene and sanitation conditions of traders greatly affect the level of microbial contamination of blood cockle satay products

Table 2. Total bacteria of blood cockle satay

No. Sample	Total bacteria (Log CFU/gr)
1	8.64
2	6.89
3	7.34
4	8.82
5	5.24
6	5.43
7	7.25
8	4.84
9	7.69
10	8.64
11	8.34
12	8.92
13	8.54
14	7.34
15	5.24
Average	7.22

The high total microbe in blood cockle satay is mostly caused by traders not implementing proper and proper hygiene and sanitation requirements. The state of poor sanitation and hygiene condition affects the quality of food served to consumers, so it will also affect the health of consumers. If the food sanitation hygiene is poor, it can lead to health problems such as foodborne disease and cases of food poisoning [21].

4.3 Total Coliform

Table 3. Total Coliform in blood cockle satay

No. Sample	Total Coliform (MPN/g)	Status
1	>1600	Not eligible
2	>1600	Not eligible
3	>1600	Not eligible
4	>1600	Not eligible
5	13	Not eligible
6	11	Not eligible
7	>1600	Not eligible
8	21	Not eligible
9	240	Not eligible
10	13	Not eligible
11	1600	Not eligible
12	>1600	Not eligible
13	>1600	Not eligible
14	540	Not eligible
15	11	Not eligible

Description: Meets the requirements < 3 MPN/g
Not eligible ≥ 3 MPN/g

Coliform is a group of bacteria that is used as an indicator of sewage pollution and poor sanitation conditions for water, food, milk and dairy products. The presence of Coliform bacteria in food indicates the possibility of enteropathogenic and toxigenic microbes that are harmful to health [21].

The results (Table 3), showed that the contamination of *Coliform* bacteria in all (15) samples of blood cockle satay exceeded the maximum limit determined by [22] which was >3 MPN/gram. The highest contamination (total Coliform >1600 MPN/gram) was at 1, 2, 3, 4, 7, 12, 13 traders; while the lowest contamination was at 11 and 15 traders (total Coliform 11 MPN/gram). The high total coliform in scallop satay is mostly caused by traders not implementing proper and proper hygiene and sanitation requirements. Coliform is a group of bacteria that is used as an indicator of sewage pollution and poor sanitation conditions for water, food, milk and dairy products.

4.4 Cadmium (Cd)

Tabel 4. Cadmium (Cd) content in blood cockle satay

No Sample	Cd content (mg/kg)
1	<0.0028
2	<0.0028
3	<0.0028
4	0.73*
5	0.35*
6	<0.0028
7	<0.0028
8	<0.0028
9	<0.0028
10	0.72*
11	<0.0028
12	0.02
13	0.46*
14	0.24*
15	0.03

Note : * heavy metal content exceeds the threshold
The limit set by BPOM in 2017

Table 9, showed that 33.33 % (5 of 15 samples) of blood cockle satay samples contained Cadmium above the maximum allowable limit. The highest level of Cd in scallop satay was 0.73 mg/kg, and the lowest level was <0.0028 mg/kg. The threshold set by [8] regarding Cd heavy metal contamination in processed food is allowed to be <0.1 mg/kg, so it is necessary to re-examine the source of the contaminant and need to be vigilant in its consumption patterns.

The presence of heavy metal content of Cd in blood cockle satay can be influenced by several factors, including the Cd content in fresh scallops as raw material for making scallop satay, as well as improper processing of scallop satay. [23] reported that the level of accumulation of heavy metals in fish body tissues was found in the liver, kidneys, gills and mussel meat.

The presence of Cadmium in raw shellfish is not only influenced by the location of catching mussels which are at the source of heavy metal pollution, but also by the length of time exposed to shellfish by waste containing Cd metal. The longer exposure to heavy metals, the more bioaccumulation that occurs in the

shellfish body will increase. The length of contact with heavy metal contamination can be seen through the increasing size of the shells. The location of catching shellfish in the waters is one of the most supportive factors for the presence of heavy metal content in shellfish. The further away the oyster fishing location is from the pollutant location, the lower the accumulated pollutant content [24]. Cadmium in the human body can cause damage to the liver, kidneys, lungs, bones, and is a carcinogen

5 Conclusion

Total bacteria average of blood cockles satay is 7.22 Log CFU/gr far exceeds the maximum limit of microbial contamination in processed foods that has been set based on Food and Drug Supervisory Agency Regulation No. 13 of 2019 (5.00 Log CFU/g). Coliform bacterial contamination exceeds the maximum limit set by the Food and Drug Supervisory Agency of the Republic of Indonesia in 2009 (>3 MPN/gram). As many as of 33.33% samples contain Cadmium exceeded the maximum limit set by the Food and Drug Supervisory Agency of the Republic of Indonesia in 2017 (>0.1 mg/kg). The high total bacteria and total Coliform was caused because most of the traders did not apply proper and proper hygiene and sanitation requirements.

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