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Food Safety Study in Terms of Formaldehyde Content and Bacterial Contamination Levels of Cob Pindang Fish in Simo Gunung and Balong Sari Traditional Markets, West Surabaya

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A B S T R A C T

Formalin is a food additive that has been banned by BPOM but can still be found in food products. This study aims to determine: 1) the food safety of pindang fish sold in traditional markets in West Surabaya in terms of formalin content and bacterial contamination; 2) the relationship between the hygienic conditions of traders and formalin content on the level of bacterial contamination; and 3) the bacterial growth patterns during room temperature storage of formalin-positive and formalin-negative tuna pindang fish. This is a cross-sectional study with a sampling technique of saturation. A qualitative formalin test was conducted using a formalin test instrument. The results indicated that 88.23% (15 of 17 samples) of cob pindang contained formalin and that 64.7% (11 of 17 samples) exceeded the SNI 2717: 2017 maximum limit for total microorganisms. There was a significant correlation between the level of bacterial contamination and the hygienic conditions of traders. There is no correlation between formalin concentration and bacterial contamination level. The pattern of bacterial proliferation did not differ between formalin-positive and negative cob pindang fish.

1. INTRODUCTION

1.1. Research Background

The case of use of formalin as a food preservative became an issue that shocked the community after the Food and Drug Monitoring Agency (BPOM) released the results of its examination of various types of food circulating in the community (one of which was fish and processed products) which proved to contain formaldehyde. This action is inappropriate because formaldehyde is prohibited from being used as a food preservative [1]. The addition of formalin at the trader level aims to preserve or extend the product's shelf life. The results of laboratory testing by BPOM RI in 2016 from 15,758 food samples showed that food products containing hazardous materials were still found to be misused, as many as 221 samples contained formaldehyde [2].

Formalin is a formaldehyde compound in water with an average concentration of 37% and 15% methanol; the rest is water [3]. The molecular formula of formalin is CH_2O and is systematically named methanol. Formaldehyde in free air is a gas but can dissolve in water. Formaldehyde is generally sold under the trademark formalin or formol [4]. In general, formalin is used as a disinfectant, bactericidal/germicidal. Formaldehyde irritates the respiratory tract and immunotoxicology, while methanol is very toxic and can cause death in people.

In 2010, the Indonesian Food and Drug Supervisory Agency (BPOM) found the presence of formaldehyde in fish and marine products in the top ranking, namely 66% of a total of 786 samples. The use of formalin in food ingredients has been prohibited by the Regulation of the Food and Drug Supervisory Agency Number 11 of 2019 the use of formalin food additives in food ingredients to hide food spoilage has been prohibited. However, formalin is still commonly found in food ingredients such as fish, meatballs, tofu, and wet noodles.

Cob (*Euthynnus affinis*) is one of the fish that is popular with the public. Cob meat has a good taste, contains the nutrients needed, and is beneficial for the body. The nutritional content of cob includes 25% protein, 1.50% fat, 69.40% water, and 0.03% carbohydrates [5]. Until now, cob fish processing is still mostly done conventionally, such as boiling, boiling, or frying [6].

In general, business actors produce pindang products with a production capacity of 10 kg in 1 furnace and a cooking time of 4-10 hours. Hence, it requires a lot of fuel when producing in large quantities, the use of boiling water is repeated so that it will affect product quality. Business actors pay little attention to sanitation and hygiene [7].

The decline in the quality of pindang fish is due to the lack of business actors paying attention to sanitation and hygiene at the production site and the boiling equipment used, the use of traditional tools that are easily dirty and rusty can affect the product physically and in terms of quality [7]. One of the most common efforts to prevent fish quality degradation is using formalin because it can extend shelf life at a relatively low cost [8].

Cases of the use of formalin in pindang fish circulating in 6 out of 10 traditional markets in the city of Yogyakarta are positive for formaldehyde [9]. Testing the formalin content of 24 samples (12 fresh fish, 12 pindang) at TPI Tulungagung using the Formalin Kit test showed that all samples turned purple, indicating that the samples contained positive formalin [10].

This research aims to 1). Food safety of pindang fish sold in traditional markets in West Surabaya in terms of formalin content, and level of bacterial contamination; 2) The relationship between the hygienic conditions of traders and the content of formalin on the level of bacterial contamination; 3). Bacterial growth patterns during room temperature storage of formalin positive and negative cob pindang fish.

1.2. Literature Review

1.2.1 Food Safety

Food safety is one of the problems of processed fish products. So far, there have been four main food safety problems in Indonesia, namely (1) food contamination by microbes due to poor sanitation and hygiene practices, (2) food contamination by hazardous chemicals such as pesticide residues, veterinary drug residues, heavy metals, mycotoxins, etc., (3) the use of hazardous materials that are prohibited for food such as formalin, borax, rhodamine B, and methanol yellow, and (4) the use of exceeding the maximum allowable limit of food additives [11].

1.2.2 Microbes in Fish

Microbes need nutrients and an appropriate environment for their growth. According to the growth temperature, bacteria are grouped: Psychrophilic bacteria (can grow at cold temperatures of 15 –20°C, with an optimum temperature of 10–15°C); mesophilic bacteria (can grow at room temperature 25–37°C) and thermophilic bacteria (can grow at high temperatures of 40–55°C). Bacterial species that cause human disease grow well at human body temperature (37°C) because they are classified as mesophilic [12].

Bacteria that can grow on cob pindang are bacteria that are classified as halophilic bacteria, namely *Shewanella soehaensis* and *Vibrio alginolyticus* . Bacteria that can produce histamine include *S. nematodiphila* and *Enterobacter cloacae* . *Bacillus cereus* bacteria which are thermophilic (50°C), and *Kurthia gibsonii* are classified as psychotropic bacteria (4°C) [13]. The maximum limit for bacterial contamination in pindang fish is 1 x 10^5 colonies/gram [14].

1.2.3 Pemindangan

Pemindangan is fish processing using a combination of boiling and salting. [15] stated that the basic principles of scanning are killing or reducing bacteria, adding salt, and reducing water content. The addition of salt can kill or inhibit the growth of bacteria. The process of pemindangan is traditional: sorting the raw materials, cleaning them, adding salt, then putting them in a container and boiling them for about 2 hours. Salt solution for boiling can be used repeatedly.

According to [16] the basic principles of screening are: 1. killing or reducing bacteria through heating; 2. Adding salt can kill or inhibit the growth of remaining bacteria in fish; 3. The occurrence of a reduction in water content in fish meat. The success of the pemindangan process is strongly influenced by the freshness of the fish as a raw material, the quality of salt, and environmental conditions [15].

1.2.4 Formalin

Formalin is a saturated solution containing 37% formaldehyde, 6-13% methanol and the remainder is water. In general, formalin is used as a disinfectant, bactericidal/germicidal. The high content of formalin in the body can cause stomach irritation, allergies are carcinogenic (cancer-causing) and mutagenic (causing changes in cell/tissue function), and people who consume it will vomit, diarrhea mixed with blood, urine mixed with blood, and death caused by circulatory failure [17].

Formaldehyde is a substance that can cause the simplest cross-linking and has broad reaction specifications. Formaldehyde can react with the amino acid lysine, it can also react with the side chains of cysteine, tyrosine, histidine, tryptophan, and arginine [18]. Formaldehyde cross-links in protein, nucleic acid, and polysaccharide networks will form stable and *irreversible methylene bonds* [19].

The protein in fish can react with the aldehyde elements found in formalin, the protein elements will be bound by formalin from the surface of the meat so that it continues to seep into it. After the protein is bound to the chemical elements of formalin, when it is pressed it will feel rubbery, besides that the protein will not be attacked by putrefactive bacteria which will produce acidic compounds [20].

1.3. Research Objectives

This research aims to 1). Food safety of pindang fish sold in traditional markets in West Surabaya in terms of formalin content, and level of bacterial contamination; 2) The relationship between the hygienic conditions of traders and the content of formalin on the level of bacterial contamination; 3). Bacterial growth patterns during room temperature storage of formalin positive and negative cob pindang fish.

2. MATERIALS AND METHODS

2.1. Materials and Tools

17 samples of cob pindang were obtained from 17 pindang fish traders at the Simo Gunung and Balong Sari traditional markets, in West Surabaya. The materials used were a formalin test kit, plate count agar (PCA), NaCl (Sodium Chloride) 0.85%, and distilled water.

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The tools used include autoclaves, incubators, laminar flow, vortex, Erlenmeyer, measuring cups, petri dishes, test tubes, micropipette, blue tip, yellow tip, Bunsen, and test tube racks.

2.2. Design Experiment and Analysis

This research is a cross-sectional study with a saturated sampling method. Sampling was taken at the Simo Gunung and Balong Sari traditional markets in West Surabaya and continued with formalin testing at the Food Analysis Laboratory and then continued with total bacterial testing at the Microbiology Laboratory of the Food Technology Study Program at the National Development University "Veteran" East Java.

2.3. Implementation of Research

2.3.1 Testing Formalin with the Formalin Test-Kit

Twenty-five grams of the sample was weighed and homogenized in 50 ml of distilled water. A total of 1-3 ml of sample was taken, put in a test tube, and added 1 drop of formalin-1 reagent and homogenized. Add 3 drops of formalin-2 reagent. Wait and let stand for ± 5-15 minutes, the sample will slowly change from light purple to dark purple, indicating positive formaldehyde.

2.3.2 Testing Total Bacteria with the Drop Plate Method [21]

One gram of the sample was weighed and homogenized in 9 ml of 0.85% NaCl solution to make 10^{-1} to 10^{-5}. A total of 50 µl of samples from each dilution was dripped onto the surface of a sufficiently dry sterile PCA agar medium and incubated at 37°C for 18-24 hours. Growing colonies were calculated in Colony Forming Units per gram (CFU/g) of a sample using the formula:

CFU = number of colonies x 1000/50 x dilution factor

2.4. Analytical methods

The bacterial total was converted into log form before analysis. Data on bacterial contamination levels were expressed in absolute values and percentages using Microsoft Office Excel 2019. The relationship between sanitary hygiene and bacterial contamination levels and the relationship between formalin and bacterial contamination levels were analyzed using the Chi-square test at P<0.05 using SPSS software (version 26). Statistical significance was defined as P<0.05 a 5% error level.

3. RESULTS AND DISCUSSION

3.1. Formalin

Formalin (Table 1) shows that 15 (88.24%) of the 17 samples of cob pindang were positive for formalin. The formalin-positive cob pindang is indicated by a change in color from cloudy white to light purple to purple. This follows [10], which reported on testing the formaldehyde content in 24 samples (12 fresh fish, 12 pindang) using the Formalin Kit test, showed that all samples turned purple.

Table 1. Observation of Formalin on Cob Pindang Fish

Code	Discoloration	Formaldehyde identification
P1	Positive (purple color)	+
P2	Positive (light purple color)	+
P3	Positive (purple color)	+
P4	Negative (color does not change)	-
P5	Positive (light purple color)	+
P6	Negative (color does not change)	-
P7	Positive (purple color)	+
P8	Positive (light purple color)	+
P9	Positive (purple color)	+
P10	Positive (light purple color)	+
P11	Positive (light purple color)	+
P12	Positive (light purple color)	+
P13	Positive (light purple color)	+
P14	Positive (purple color)	+
P15	Positive (light purple color)	+
P16	Positive (light purple color)	+
P17	Positive (light purple color)	+

Description: Positive (discoloration occurs)
Negative (no color change)

3.2. Total Bacterial

The total bacteria (Table 2) shows that (64.7%) of 11 out of 17 samples of cob pindang had a level of bacterial contamination that exceeded the SNI 2717:2017 standard (> 5.00 log CFU/g). In comparison (35.3%) 6 of 17 samples met the SNI 2717:2017 standard (<5.00 log CFU/g).

Table 2. Observation of Bacterial Total in Cob Pindang Fish

Code	TPC (Log CFU/g)	Description	Code	TPC (Log CFU/g)	Description
P1	4.57	E	P10	5.20	NE
P2	4.92	E	P11	5.25	NE
P3	4.85	E	P12	4.90	E
P4	5.18	NE	P13	5.27	NE
P5	5.19	NE	P14	5.36	NE
P6	4.60	E	P15	5.01	NE
P7	5.20	NE	P16	5.28	NE
P8	5.23	NE	P17	5.21	NE
P9	4.81	E			

Notes:
E : (Eligible): Total bacteria does not exceed the standard of SNI Number 2717:2017 (<5.00 Log CFU/g)
NE : (Not Eligible): Total bacteria exceeds the standard of SNI Number 2717:2017 (>5.00 Log CFU/g)

Some factors that influence the high level of bacterial contamination include personal hygiene, sanitary conditions at the point of sale, and the surrounding environment. The highest bacterial contamination of 5.36 Log CFU/g was found in sample code P14 with poor personal hygiene and sanitation criteria at the point of sale and the surrounding environment. This is by [22], high bacterial contamination is influenced by poor personal hygiene and sanitation conditions in the sales environment which can cause health problems such as foodborne disease and cases of food poisoning.

Table 3. Relationship of Trader Hygiene Sanitation to Total Cob Pindang Fish Bacteria

Hygiene and sanitation conditions	Total Bacterial				Total		α	*p-Value*
	Qualify*		note Eligible**					
	N	%	N	%	N	%		
Good	4	23.54	1	5.88	5	29,42	0.05	**0.013**
Poor	2	11.76	10	58,82	12	70.58		
Total	6	35,3	11	64,7	17	100		

Notes:
E : (Eligible): Total bacteria does not exceed the standard of SNI Number 2717:2017 (<5.00 Log CFU/g)
NE : (Not Eligible): Total bacteria exceeds the standard of SNI Number 2717:2017 (>5.00 Log CFU/g)
N: Total

Based on the Chi-square test (Table 3) was obtained (p-value 0.013 <0.05) which showed that there was a significant relationship between the hygiene and sanitation of traders and the level of bacterial contamination in cob pindang. The poor condition of personal hygiene is shown by all traders not using tools to touch the product and rarely washing their hands because they do not provide clean water. The sanitary condition of the selling points and the surrounding environment is poor because all pindang fish are sold open (without cover). This is by [23], which states that not using tools to touch the product, and not using protective equipment such as aprons and pindang fish sold openly without cover can increase bacterial contamination.

Table 4. Relationship of the Presence of Formalin to the Total Bacteria of Cob Pindang Fish

Formalin	Total Bacterial				Total		α	*p-Value*
	Qualify*		note Eligible**					
	N	%	N	%	N	%		
Positive	6	35,3	9	52.94	15	88,24	0.05	0.2
Negative	0	0	2	11.76	2	11.76		
Total	6	35,3	11	64,7	17	100		

Notes:
E : (Eligible): Total bacteria does not exceed the standard of SNI Number 2717:2017 (<5.00 Log CFU/g)
NE : (Not Eligible): Total bacteria exceeds the standard of SNI Number 2717:2017 (>5.00 Log CFU/g)
N: Total

Based on the Chi-square test (Table 4) was obtained (p-value 0.2 > 0.05) which showed that there was no significant relationship between formalin and the level of bacterial contamination in cob pindang. It was found that there were 6 samples with total bacteria below the maximum allowable limit and 9 samples with total bacteria exceeding the maximum allowed limit (> 10^5 colonies/g) [14]. Formalin levels affect total bakeries in cob pindang fish, but in this study, a quantitative test was not carried out so the formalin levels in boiled cob fish were not known. The level of bacterial contamination is also influenced by personal hygiene and sanitation at the point of sale and the surrounding environment.

3.3. Relationship between Formaldehyde Content and Bacterial Growth Patterns at Room Temperature Storage

From several research results, formalin can extend shelf life. According to [20], the ability of formalin, which is related to protein, forms methylene compounds so that when high-protein foods are soaked in formalin, the aldehyde group of formaldehyde will bind to protein elements. The bound protein cannot be used by spoilage bacteria so that the food will be preserved.

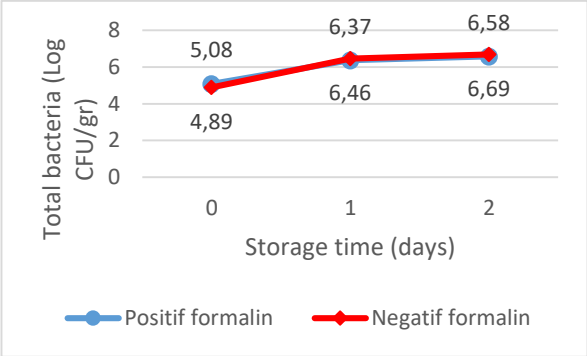

Fig. 1. The relationship between formalin and total bacteria

The graph in (Figure 1), shows the total bacteria on day 1 of storage has increased, both in formalin-negative and formalin-positive fish. On storage on the 2nd day, the total bacteria also increased. There is a difference between formalin-positive and formalin-negative pindang fish, where formalin-negative pindang fish experience a sharper increase in total bacteria on day 1 of storage compared to formalin-positive pindang fish.

Based on the graph in Figure 1, it can be seen that there is no significant difference between the total bacteria in the formalin-positive and formalin-negative cob. Research by Ref. [24] states that formalin levels affect total bacteria; the higher the formalin level, the lower the total bacteria. Still, in this study, no quantitative tests were carried out so the formalin levels in pindang fish were unknown. According to [25], formalin does not affect total bacteria in formalin-positive squid fish having a total bacteria of 26.7x10 ^{5} colonies/gr and formalin-positive rebon fish has a total bacteria of 280x10 ^{5} colonies/gr, this high level of contamination can occur due to contamination by soil. , water dust or air that occurs due to the sale and storage of open fish. The level of initial bacterial contamination varies for each sample and also affects the level of bacterial contamination during storage. The poor personal hygiene and sanitation conditions in the sales area and the environment also affect bacterial contamination. The formalin level of 5.04 ppm in skipjack cob can still be grown by bacteria with a total of 2.3 x 10 ^{5} colonies/gr bacteria [24].

4. CONCLUSION

The results of the qualitative formalin test on 17 samples of cob pindang showed 88.24% positive for formalin and 11.7% negative for formalin. 64.7% (11 of 17 samples) had total bacteria exceeding the maximum limit of SNI 2717:2017 standards and 35.3% (6 of 17 samples) met SNI 2717:2017 standards. There is a significant relationship between traders' hygiene and sanitation conditions and the level of bacterial contamination. There is no significant relationship between formalin and the level of bacterial contamination. There was no difference in the pattern of bacterial growth in positive and negative formalin cob pindang fish during three days of storage at room temperature.

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