

## The Safety of Tofu Food Produced in Klungkung Regency in Terms of Chemical and Microbiological Aspects

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### Abstract

*This research was aimed at investigating the safety of tofu food in terms of chemical (formalin) and microbiological (E. coli and Salmonella sp.) aspects produced in Klungkung Regency that met SNI 01-3142-1998 requirements. The test was conducted in the Laboratory of the Great Hall of Denpasar Veterinary. This research was explorative research that used in-laboratory experiment design. The population of this research was obtained through the survey by conducting a survey, namely the number of tofu producers in Klungkung Regency that was 6 producers located in Klungkung Sub-District and Dawan Sub-District. The safety test of tofu food was conducted against formalin parameters (qualitative), E. coli and Salmonella sp. The result of this research from the six tofu samples showed negative content of formalin. Thus, the safety of tofu food based on the chemical (formalin) aspect had met the requirement by the Decree of the Health Minister of the Republic of Indonesia Number 722/Menkes/Per/IX/88 and the Decree of the Health Minister Number 1168/Menkes/Per/1999. The E. coli Microbe contamination which resulted <3.6 – 9.2 had met the established standard on SNI 01-3142-1998, namely the E. coli requirement which was 10 APM/gram at the highest. The Salmonella sp. test of all samples had a negative result that meant it had met the requirement of SNI 01-3142-1998. Therefore, it can be concluded that the microbiological quality of the tofu produced in Klungkung Regency had been good enough because it had met the requirements of SNI 01-3141-1998 regarding tofu quality requirements.*

*Keyword: Tofu, Formalin, E. coli, Salmonella sp.*

### 1. Introduction

Tofu is a kind of food material that is highly demanded by the Indonesian people. Tofu, which is rich in protein, has been long consumed by the Indonesian people as a dish. Tofu is a protein extract of soybean with high protein levels, low carbohydrate levels, and has very remarkable nutritional value and digestibility [1]. The drawback of tofu is that it has a very limited duration of storability. In normal conditions (room temperature), the storability duration is 1-2 days on average. If it is more than such a limit, it will taste sour and rotten so that it would not be suitable for consumption [1]. The relatively short duration of storability surely inflicts financial loss for the tofu producers. This has triggered tofu producers to abuse chemical materials as additives in the food. One such material that is often abused is formalin.

Formaldehyde or formalin is toxic material and is harmful to human health. The short-term effects of formalin exposure, among others, are irritation in the respiratory and digestive tract, vomiting, dizziness. In the longer-term, it can cause liver, kidney, heart, spleen and pancreas damages as well as premature aging [2]. The threshold of formalin level tolerated in the body is 0.2 milligrams per kilogram of body weight [3] [4].

Aside from food additives, hygiene and sanitation are the other important thing in determining food safety. Microbiological contaminant (Escherichia coli and Salmonella sp.) is an indicator that can cause foodborne diseases. The condition of raw production water, environment hygiene and personals insufficiency in producing tofu can cause cross-contamination in the microbiological

contaminant. Based on the aforementioned argumentation, tofu food safety produced in Klungkung would be analyzed based on different research perspective namely chemical aspect (formalin) qualitatively and microbiological aspect (*Escherichia coli* and *Salmonella sp.*).

## **2. Material and Methods**

### **Location and Time of Research**

This research was conducted on the tofu producers in Klungkung Regency, specifically in Semarapura City and in Gelgel Village, Klungkung Sub-District as well as SampalanKelod Village, Dawan Sub-District. The test was conducted in the Laboratory of the Great Hall of Denpasar Veterinary. The test was conducted from July until November 2018.

### **Research Materials**

The materials used in this research included the tofu produced in Klungkung Regency, 0.5% phenylhydrazine solution, 5% sodium nitroprusside solution, 5% sodium hydroxide solution, 10% phomaldehyde standard (Cat PS-2031) with 37 Wt.% purity, distilled water, 0.1% BPW (Buffered Peptone Water), BGLBB (Brilliant Green Lactose Bile Broth), LSTB (Lauryl Sulfate Tryptose Broth), ECB (Escherichia Coli Broth), L-EMBA (Levine Eosin Methylene Blue Agar), MR-VP (Methyl Red-Voges Proskauer), PCA (Plate Count Agar), KCB (Koser Citrate Broth), SCA (Simmons Citrate Agar), Reagen Kovac, Reagen Voges-Proskauer (VP), LB (Lactose Broth), SCB (Selenite Cystine Broth), TTB (Tetra Thionate Broth), RV (Rappaport Vassiliadis), XLDA (Xylose Lysine Deoxycholate Agar), HEA (Hektoen Enteric Agar), BSA (Bismuth Sulfite Agar), TSIA (Triple Sugar Iron Agar), LIA (Lysine Iron Agar), LDB (Lysine Decarboxylase Broth), KCNB (Potassium Cyanide Broth), MR-VP (Methyl Red-Voges Proskauer), SCB (Selenite Cystine Broth), TB (Tryptose Broth), TSTB (Trypticase Soy Tryptose Broth), SIM (Simmons Citrate Agar), BHI (Brain Heart Infusion), Urea Broth, Malonate Broth, Phenol Red Lactose Broth, Phenol Red Sucrose Broth, keratin crystal, 0.2% Bromcresol Purple Dye solution, 0.85% Physiological Saline solution, Formalinized Physiological Saline solution, Salmonella Polyvalent Somatic (O), A-S Antiserum, Salmonella Polyvalent Flagellar (H), Phase 1 and 2 Antiserum, Salmonella Somatic Group (O), Monovalent Antisera : Vi.

The equipment used in this research included: glass funnels, drop pipettes, 100 mL measuring flask, filtering papers, test tubes, Durham tubes, petri dish, test tubes, 1 mL, 2 mL, 5 mL, and 10 mL pipettes, media bottle, scissors, tweezers, inoculation needles, stomacher, Bunsen burner, pH meter, scale, magnetic stirrer, vortex, incubator, water bath, autoclave, clean bench, refrigerator, freezer, as well as 10 x 75 mm serology tubes.

### **Research Implementation**

This research was explorative research using in-laboratory experiment design. The survey was conducted based on the existing population. The population is the overall unit of analysis with characteristics that can be generally observed which would later be used as the goal of the research. The population in this research was obtained through the survey by using the census method, in this case, the six tofu producers in Klungkung Regency located in Klungkung Sub-District and Dawan Sub-District. The said tofu producers were all utilized as respondents and key informants.

In this research, the sampling method used is purposive sampling technic. The reason for using a purposive sampling technic was due to the result of the census-method survey, the writer found six tofu producers registered in the regency in question. Therefore, the overall tofu producers were used as the research samples due to the relatively small number of population. The sampling of tofu was conducted at the end of the production process in each producer. The resampling was

conducted twice at the same time on different days. The test on the tofu was a chemical parameter (formalin) qualitatively and microbiological parameter (*Escherichia coli* and *Salmonella sp.*)

### Data Analysis

A descriptive analysis would be conducted based on the results of the chemical and microbiological tests found. The descriptive analysis conducted would refer to the Decree of the Health Minister of the Republic of Indonesia Number 722/Menkes/Per/IX/88 and the Decree of the Health Minister of the Republic of Indonesia Number 1168/Menkes/Per/1999 regarding Food Mine and SNI 01-3142-1998 regarding tofu quality requirements. The results of the analysis would generate the safety of tofu food produced in Klungkung based on the chemical and microbiological aspects.

### 3. Results and Discussion

The analysis result of the tofu food safety was shown in three parameters namely: formalin content, *E. coli*, and *Salmonella sp.* The data of the test result can be seen in Table 1.

Table 1.  
The Result of Formalin, *E. Coli* and *Salmonella sp.* tests

Test Parameter	Time	Sample					
		A	B	C	D	E	F
Formalin	P1	Negative	Negative	Negative	Negative	Negative	Negative
	P2	Negative	Negative	Negative	Negative	Negative	Negative
<i>E. Coli</i>	P1	<3.6	3.6	3.6	<3.6	<3.6	9.2
	P2	<3.6	<3.6	7.4	<3.6	<3.6	6.1
<i>Salmonella sp.</i>	P1	Negative	Negative	Negative	Negative	Negative	Negative
	P2	negative	Negative	Negative	Negative	Negative	Negative

Legend:

P1: First Test (the samples were taken in the first week)

P2: Second Test (the samples were taken in the second week)

A: NyomanTerang's tofu, B: WayanSuwati's tofu, C: Nengah Sondra's tofu, D: Nur Hisri's tofu, E: Imam Budiarto's tofu, F: NyomanAlit's tofu

Based on the above Table 1, it was known that the six producers of all tofu samples had met the quality standards set in the Decree of the Health Minister of the Republic of Indonesia Number 722/Menkes/Per/IX/88 and the Decree of the Health Minister of the Republic of Indonesia Number 1168/Menkes/Per/1999 which do not permit or forbid the usage in food due to its carcinogenic nature. The formalin content in the six tested tofu samples was negative. This result obtained from *E. coli* bacteria contaminant by using Most Probable Number (MPN) testing showed that the six samples had met the requirements of SNI 01-142-1998 (less than 10 APM/gram samples). The *Salmonella sp.* bacteria content testing showed similar results. The six samples were found negative of *Salmonella sp.* bacteria content, which meant that it had met the requirements of SNI 01-142-1998.

### Formalin

Formalin is not food mining material and it cannot even be added to any food. According to the Decree of the Health Minister of the Republic of Indonesia number 722/Menkes/Per/IX/88 and the Decree of the Health Minister of the Republic of Indonesia number 1168/Menkes/Per/1999, formalin is one of the food additives in the form of preservative that is banned to be used. Unknowingly, the consumers have slowly been exposed to formalin. It is indeed inevitable due to the frequent usage of formalin in food materials by the producers, one of which is tofu.

Table 2.  
The Result of Formalin Content Testing in Tofu

Testing Parameter	Sample Code	Formalin Level		Maximum Threshold	Notes
		P1	P2		
Formalin	A	Negative	Negative	Negative	Had met the requirements
	B	Negative	Negative		Had met the requirements
	C	Negative	Negative		Had met the requirements
	D	Negative	Negative		Had met the requirements
	E	Negative	Negative		Had met the requirements
	F	Negative	Negative		Had met the requirements

Based on the result of the laboratory testing conducted by using qualitative testing, it was shown in Table 2 that from the six tofu samples that had been analyzed for its formalin content level, it was found that 100% of the tofu samples did not contain formalin. This might be caused due to the good level of producers' awareness, and due to the regional government regulation that sanctions any producers who still use formalin as preservatives. Based on the observation and interview conducted by the writer on the tofu producers, it was found that all producers had been aware that the use of formalin could cause negative effects that could harm the health of the consumers. The producers preserved the tofu for a longer storability up to two days by boiling the tofu and/or by soaking it in salted clean water or in clean water that contains acetic acid.

***E. coli***

The result of *E. coli* testing in Table 3 showed that the overall samples had met the requirements with *E. coli* microbe contaminant up to 9.2 APM/gram. *E. coli* is a group of bacteria contained in a large quantity in human and animal waste so that these bacteria are often used as an indicator for food and water quality, and this bacteria are also used as an indicator for waste contamination [5]. The result of the *E. coli* testing on the tofu samples had met the requirements based on SNI 01-3142-1998 which showed the content level of less than 10 APM/gram.

Table 3  
The Result of *E. coli* Bacteria Content Testing on Tofu

Bacteria Contaminant	Sample Code	<i>E. Coli</i> bacteria Content Level		Maximum Threshold (APM/g) SNI 01-142-1998	Notes
		P1 (APM/g)	P2 (APM/g)		
<i>E. coli</i>	A	<3.6	<3.6	maks. 10	Met the requirements
	B	3.6	<3.6		Met the requirements
	C	3.6	7.4		Met the requirements
	D	<3.6	<3.6		Met the requirements
	E	<3.6	<3.6		Met the requirements
	F	9.2	6.1		Met the requirements

Based on the result of the research, it was found that three tofu samples (B, C, and F) produced in Klungkung Regency showed a high level of *E. coli* contamination. The result of such analysis showed that the tofu that underwent protein deposition and clumping process using acetic acid could reduce *E. coli* contamination. The high level of *E. coli* contamination might also be caused due to the low level of sanitation in the production site, direct contact of food material (soybean) with the uncleanly washed processor's hands. Besides that, the presence of *E. coli* bacteria in food might be caused by insects such as flies since fly can carry waste in its feet to food.

These bacteria could cause indigestion (gastroenteritis). The spreading process is caused due to the waste-contaminated food or water, normally caused by direct (through hands) and indirect

(through water) cross-contamination during the processing [6]. This is because of poor processing and packing. Based on the observation on the sample producers, it was found that the water source used by the tofu producers, which are boreholes and running water. On average, the producers began the production process from 08.00 a.m. until 04.00 p.m. During the production process, the employees did not use a work uniform and only wore plastic as an apron. At the other production sites (Producer C and F) there were found employees who did not wear any shirt during food production. Another example of possible contamination way is through the containers used for the finished products. The containers were in the form of a lidless plastic or aluminum tub that is hardly treated hygienically. The food processors hold a very important role in the effort of food sanitation since they are highly potential in transmitting diseases through food or beverage, i.e. they to the food or beverages that they process or serve to the other people who consume them or simply known as cross-contamination [7]. Therefore, personal hygiene is very important for all food processors.

Personal hygiene is important for contamination prevention because human is a reservoir for any kinds of disease agents. Workers with poor personal hygiene are vulnerable to the transmission of any kind of bacteria such as *E. coli* bacteria [6]. The contamination of pathogenic bacteria such as this enterotoxigenic *Escherichia coli* in food can cause serious problems [8]. The presence of *E. coli* bacteria in the hands of the food processors happens because after defecation the food processors do not wash their hands cleanly [9]. Personal hygiene of food processors is highly required in food processing to prevent disease transmission through food. The knowledge of the tofu producers regarding personal hygiene needed to be improved. One of the ways to do so is by giving counseling to all workers about the importance of personal hygiene, such as: always wash hands using soap in running water before processing food, after holding raw food and after coming back from the toilet, do not wear any jewelry at work, always cover the processed food to steer it clear of fly and other insects, use plastic gloves, food tongs, spoon, and fork when taking the finished food, wear apron at work, do not eat or chew during work, cover any wound with bandage or any waterproof materials. Besides individual hygiene, the employees also needed to be informed of the effects that can be caused due to poor food processing.

Further effort is the quality improvement of the food processing site. A food processing site is a place where the food is processed into processed food/finished food, which is usually referred to as the kitchen. This food processing site requires sanitation, both in terms of its construction, the available equipment and the setting of the available equipment. The requirements of suitable food processing site/kitchen areas such: sufficient water supply is available and meets the health requirements because water is one of the transmission media of some diseases transmitted through water. The health requirements in question include available ever-clean processing site, protected from insects and other rodent animals [10].

### ***Salmonella sp.***

The microbiological test was also conducted to investigate the content of *Salmonella sp.* bacteria in the samples. The result of the test showed that the overall samples found to contain negative content of *Salmonella sp.* bacteria (Table 4.4). In this case, microorganism contaminant in the tofu that met the requirements could be caused by the perfect cooking process, since the raw materials had undergone the boiling process up to 70°C. This perfect cooking process could kill the microorganism of *Salmonella sp.* since such bacteria are not heat resistant.

Table 4  
The Result of *Salmonella sp.* Bacteria Content Testing on Tofu

Bacteria Contaminant	Sample Code	<i>Salmonella sp.</i> Bacteria Content Level		Maximum Threshold (/25g) SNI 01-142-1998	Notes
		P1 (/25g)	P2 (/25g)		
<i>Salmonella sp.</i>	A	Negative	Negative	Negative	Met the requirements
	B	Negative	Negative		Met the requirements
	C	Negative	Negative		Met the requirements
	D	Negative	Negative		Met the requirements
	E	Negative	Negative		Met the requirements
	F	Negative	Negative		Met the requirements

Other important stages are the food storage and packaging stage because in these stages the microorganism can reproduce and food contamination may happen. Practically speaking, tofu producers have comprehended good storage and packaging method, but it has not been done consistently using plastic containers/tub which is only cleaned by using plain cloth rag and they usually only store the finished products in lidless containers. Therefore, the efforts of knowledge improvement and behavior refinement of the tofu producers are highly necessary. It is suggested to the government to conduct quality control on the tofu produced by conducting regular tofu sample testing to ensure the safety of tofu food consumed by the people.

#### 4. Conclusion

Based on the result of the research conducted on the safety of tofu food produced in Klungkung Regency in terms of chemical and microbiological aspects, it can be concluded that the overall tofu samples which had been tested for their formalin content, it was found the negative amount of formalin in the six tofu samples. Therefore, the safety of tofu food in terms of chemical aspect (formalin) had met the requirements in accordance to the Decree of the Health Minister of the Republic of Indonesia Number 722/Menkes/Per/IX/88 and the Decree of the Health Minister of the Republic of Indonesia Number 1168/Menkes/Per/1999.

The safety of tofu food in terms of microbiological aspect on microbe contaminant of *E. coli* with the result of <3.6 – 9.2 APM/gram had met the standard set by SNI 01-3142-1998, namely the requirements for the maximum amount of *E. coli* of 10 APM/gram. Based on the *Salmonella sp.* all samples showed the negative result of *Salmonella sp.* which met the requirements of SNI 01-3142-1998. Therefore, it can be concluded that the microbiological quality of the tofu produced in Klungkung Regency had been fairly good since it has met the requirements of SNI 01-3141-1998 regarding tofu quality requirements.

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