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Preservation of Tofu with Liquid Smoke From Coconut Shell

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Abstract

Tofu is one of the processed soybean products which is processed through the clumping of soy protein extract. Under normal conditions (room temperature) the average shelf life of tofu is 1-2 days. If it is more than this limit, the taste of tofu will become sour and rotten so it is not suitable for consumption. This study aims to determine the effect of coconut shell smoke and storage time on the chemical, microbiological, and organoleptic characteristics of tofu stored at room temperature. This study used a Completely Randomized Design (CRD) with two factors and two replications The first factor was concentrations (0%, 2%, 4%, 6%, and 8%) and the second factor was storage time (1, 2, 3 and 4 days). Observations were made objectively including total phenol, water content, ash content, fat content, and protein content. Microbiological analysis by testing Escherichia coli and Salmonella sp. While organoleptic parameters subjectively observed include color, aroma, texture, taste, and overall acceptance. The results showed that the concentration of 6% coconut shell liquid smoke in three days storage at room temperature resulted in the best tofu characteristics with a subjective assessment of neutral – to moderately like (score 4.40) and total phenol 0.57%, water content 82.34%, ash content 0.34\%, fat content 5.72%, protein content 9.15\%, Escherichia coli <3 APM/g, and negative for Salmonella sp. The shelf life of soaked tofu with a concentration of 6% coconut shell liquid smoke at room temperature is 3 days.

Keywords: Tofu, Liquid Smoke of Coconut Shell, Storage Time

1. Introduction

Tofu is in the form of a soft solid that goes through a soybean processing process by making its protein, or without the addition of other ingredients. Under normal conditions (room temperature) the average shelf life of tofu is 1-2 days. If it is more than this limit, the taste of tofu will become sour and rotten so it is not suitable for consumption. Traders usually use preservatives to make tofu more durable, one of the preservatives that is often used is formalin. Formalin is a chemical that is prohibited for food products but is often misused to preserve food [1].

One solution that can be used is preservation with liquid smoke. Liquid smoke has bioactive compounds such as phenol, carbonyl and organic acids that function as antibacterial [2]. Currently, liquid smoke has been widely used by the food industry as a giver of distinctive aroma, texture, and taste in food products, such as meat, fish, and cheese. Liquid smoke has the ability to preserve food due to the presence of acidic, phenolic and carbonyl compounds [3].

This study aims to determine the effect of liquid smoke concentration and storage time on the chemical, microbiological and organoleptic characteristics of tofu. This research hypothesizes that soaking tofu with coconut shell liquid smoke with a concentration of 6.0% for 20 minutes can extend the shelf life up to 3 days at room temperature and can maintain the characteristics of tofu and accepted by consumers. Concentration of 6% was chosen because it was previously conducted preliminary research.

2. Material and Methods

This research was conducted from February to April 2022 at the Laboratory of Basic Science and Food Processing of the Faculty of Agriculture, Warmadewa University and Balai Besar Veteriner, Denpasar. The design used in this research was a completely randomized design (CRD) with a factorial pattern consisting of two factors. The first factor is the concetration of liquid smoke from coconut shell which consists of five levels (0%, 2%, 4%, 6% and 8%). The second factor is the storage time which consists of four levels (1, 2, 3 and 4 days). Each treatment was repeated 2 times to obtain 40 treatment combinations. Research implementation stage : Preparation of Tools and Materials, Preparation of Coconut Shell Liquid Smoke (The coconut shell liquid smoke was then diluted in 300 ml of water with concentrations of 0%, 2.0%, 4.0%, 6.0%, 8.0%), Preserving Tofu with Coconut Shell Liquid Smoke (In the soaking process, the cleaned tofu is soaked using food - grade liquid smoke with concentrations of 0%, 2.0%, 4.0%, 6.0%, and 8.0% for 20 minutes and then stored for 1 until 4 days).

The materials used in this research were distilled water, filter paper, Lowry reagent, Folin Ciocalteu reagent, Na2CO3 5% (Sodium Carbonate), Plate Count Agar (PCA), Buffer Peptone Water 1% (BPW), Brilliant Green Lactose Bile Broth 2% (BGLBB), Levine's Eosin Methylene Blue Agar (L-EMBA), Methyl Red-Voges Proskauer (MR-VP), Potassium Cyanide Broth (KCB), Lactose Broth, Tetrahinate Broth (TTB). The tools used in this study were measuring flask, erlenmeyer, round bottom flask, petri dish, analytical balance, drying oven, desiccator, crusher, dropper, incubator, soxhlet apparatus, autoclave, incubator, porcelain dish, measuring handle, reducer, uv-vis spectrophotometer, durham tube, needle inoculation (ose), bunsen burner, vortex, colony counter.

Research parameters include analysis of Total Phenol was read by UV-Vis 765 nm Spectrophotometer [4], Water Content by heating method, Ash content by Gravimetric method, Fat content by Soxhlet method [5], Protein content by Lowry method, *Escherichia coli* test by Most Probably Number method, *Salmonella sp.* [6] and Organoleptic Test which includes a preference test using 7 hedonic scales on the characteristics of color, taste, aroma, texture and overall acceptance.

The data were obtained from the results of the study were analyzed by the method of analysis of variance (ANOVA). For objective data, if a real or very real treatment is obtained, then it is continued with the Least Significant Difference Test (BNT) at a 5% significance level (0.05% BNT) to find out the different pairs. Microbiological data were analyzed descriptively subjective data continued with Dunchan's test.

3. Results and Discussion

3.1 Total Phenol

Phenol is a compound that has a function as an antioxidant that has a role as a taster or flavor in food and extends the shelf life because phenol compounds have antimicrobial and antimicrobial effects. As shown in Table 1, the highest phenol content is found in tofu with 8% liquid smoke with an average value of 0.75 and is still within the safe limits of phenol for consumption and can be accepted by consumers from the sensory, physical, and chemical characters, which is 0.02 - 2.00% [7]. Phenol content decreased during storage. The decrease in phenol content indicates that the rate of damage due to the storage temperature used is room temperature (25 - 30°C) so that it can degrade the phenolic compounds contained in the material. [8]. This is supported by research by Octavia and Leny [9], low temperature storage showed better phenol content than room temperature storage.

	1		0		
Concetration of Liquid	ion of Liquid Storage Time (Days)				
Smoke	1	2	3	4	
0%	0.00	0.00	0.00	0.00	0.00 b
2%	0.55	0.51	0.47	0.38	0.47 ab
4%	0.61	0.57	0.52	0.45	0.53 ab
6%	0.69	0.62	0.57	0.51	0.59 ab
8%	0.84	0.79	0.74	0.60	0.75 a
Average	0.53 a	0.49 ab	0.46 ab	0.38 b	

Table 1 Effect of Liquid Smoke Concentration and Storage Time on Total Phenol Tofu

Descriptions: The same letter next to the average value in the same row and column shows no significant effect (p>0.05).





The Effect of Storage Time on Total Phenol Tofu

3.2 Water Content

Analysis of variance showed the water content of tofu with the total concentration of liquid smoke and storage time ranged from 77.28 to 90.36%. The water content of tofu before being treated was 77.18%. Table 2 and Figure 3 show that tofu with a concentration of 0% liquid smoke has the highest water content on the first day of storage until the fourth day so that it is easier for bacteria to grow and multiply which will accelerate the decay process. Tofu with 8% liquid smoke immersion has a relatively lower water content due to the many components of liquid smoke attached to the tofu, causing the water content to decrease as the concentration of liquid smoke increases [7;10]. According to Herawati [11] the most influential factor in the decline in the quality of food products is changes in the water content in the product. The water content of tofu during storage has increased. The longer the storage, the water content will continue to increase so that it will reduce the quality of the product. According to Herawati [11], the most influential factor in decreasing the quality of food products is changes in water content.



Figure 3 The Effect of Liquid Smoke Concentration and Storage Time on Water Content Tofu

Concentration of Liquid		Storage Time (Days)							
Smoke	1	2	3	4					
0%	81.49 a	81.98 a	87.79 a	90.36 a					
	с	С	b	а					
2%	78.19 b	80.14 b	84.74 b	85.85 b					
	d	С	b	а					
4%	77.90 b	78.91 c	83.13 c	84.47 c					
	d	С	b	а					
6%	77.70 bc	78.56 cd	82.34 c	84.00 c					
	d	С	b	а					
8%	77.28 c	77.90 d	80.98 d	82.84 d					
	с	с	b	а					
BNT 0.05	BNT 0.73								

Table 2	
Effect of Liquid Smoke Concentration and Storage Time on	Water Content Tofu

Descriptions:

1. The same letter on the same line next to the mean shows no significant effect (p>0.05).

2. Different letters in the same column below the average indicate a significant effect (p<0.05) to very significant (p<0.01).

3.3 Ash Content

During storage, the highest ash content value was obtained in 1 - day storage treatment with an average of 0.56%. According to the Indonesian National Standard Number 01-3142-1998, the maximum ash content in tofu is 1.0%. Ash content is a parameter of the nutritional value of a product material produced by the components of organic substances contained in the product. According to Winarno [12], most of the food ingredients, which is around 96%, consist of organic matter and water. The rest consists of mineral elements. Ash content decreased during storage. The longer storage at room temperature, the ash content of tofu decreased due to microbial activity that requires minerals to reproduce. Putri [13] state that microbes need minerals to sustain life. During storage, an increase in water activity will generally be followed by microbial growth, both bacteria, and molds.

Concentration of		Average			
Liquid Smoke	1	2	3	4	
0%	0.56	0.41	0.33	0.24	0.39 a
2%	0.56	0.41	0.35	0.23	0.39 a
4%	0.54	0.40	0.34	0.24	0.38 a
6%	0.57	0.42	0.34	0.25	0.40 a
8%	0.57	0.40	0.31	0.25	0.38 a
Average	0.56 a	0.41 ab	0.33 bc	0.24 c	

Table 3 Effect of Liquid Smoke Concentration and Storage Time on Ash Content Tofu

Descriptions: The same letter next to the average value in the same row and column shows no significant effect (p>0.05).



Figure 4 The Effect of Storage Time on Ash Content Tofu

3.4 Fat Content

Fats are organic compounds consisting of the elements Carbon (C), Hydrogen (H) and Oxygen (O) which can be dissolved in certain solvents. Fat is one of the important elements in food that serves as a source of energy. The breakdown of fats in foodstuffs can occur during processing and storage. Fat damage causes food to smell and have an unpleasant taste so that its quality and nutritional value can decrease [14]. Tofu fat content during storage decreased due to the activity of microbial enzymes that can break down fat into glycerol and fatty acids [14]. Fat damage can be caused by the oxidation process of unsaturated fatty acids.

		I dole 1			
Effect of 1	Liquid Smoke Con	centration and Sto	rage Time on Fat	Content Tofu	
Concentration of		Average			
Liquid Smoke	1	2	3	4	_
0%	6.34	6.15	5.67	5.07	5.80 a
2%	6.34	6.15	5.67	5.08	5.81 a
4%	6.36	6.17	5.72	5.08	5.83 a
6%	6.36	6.17	5.72	5.08	5.83 a
8%	636	6.18	5.73	5.08	5.83 a
Average	6.35 a	6.16 a	5.70 ab	5.08 b	

Table /

Descriptions:	The same	letter next	to the av	/erage	value i	n the	same	row a	nd col	umn	shows	no s	ignifica	nt
	effect (p>	>0.05).												



Figure 5 The Effect of Concentratrion of Liquid Smoke on Fat Content Tofu

3.5 Protein Content

Protein content in tofu with liquid smoke concentration treatment and storage time ranged from 5.41 to 10.65%. The protein content in the control tofu was 10.69%. Table 5 shows that the addition of 8% liquid smoke concentration resulted in tofu protein content on the first day of 10.65%, while tofu with 0% liquid smoke concentration produced 10.16% protein content. The decrease in protein content can be caused by the presence of protein-decomposing microbes. With the addition of the highest concentration of liquid smoke, acetic acid and phenol compounds in liquid smoke can inhibit bacterial activity so that not too many amino acids are decomposed into ammonia [15].

Protein content decreased during storage. This decrease in protein levels occurs due to microbial activity that breaks down protein [16]. Protein is food for microbes, protein will be broken down by microbes so that an ammonia smell will appear [17]. According to Arini [18], protein foods that have been damaged produce a distinctive rotten smell of protein, the texture is softer and slimy.

Concentration of	_	Storage Time (Days)							
Liquid Smoke	1	2	3	4					
0%	10.16 a	7.94 c	6.34 d	5.41 d					
	a	b	с	d					
2%	10.19 a	8.88 b	8.06 c	7.97 c					
	а	b	с	с					
4%	10.53 a	9.14 b	9.07 b	8.97 b					
	a	b	b	b					
6%	10.64 a	9.27 b	9.15 b	9.05 b					
	a	b	b	b					
8%	10.65 a	10.15 a	10.01 a	9.83 a					
	a	ab	ab	b					
BNT 0.05	BNT 0.73								

Table 5 Effect of Liquid Smoke Concentration and Storage Time on Protein Content Tofu

Descriptions:

- 1. The same letter on the same line next to the mean shows no significant effect (p>0.05).
- 2. Different letters in the same column below the average indicate a significant effect (p<0.05) to very significant (p<0.01).



Figure 6

The Effect of Concentratrion of Liquid Smoke on Proteint Content Tofu

3.6 Escherichia coli

Soaking tofu with 8% coconut shell liquid smoke with a storage time of 4 days had the lowest total *Escherichia coli* at 3.6 APM/g. The substances present in liquid smoke are bacteriostatic and bactericidal. Compounds that play an important role as antimicrobials are phenol compounds and acetic acid [19]. Coliforms are present in places of food preparation through raw materials and then in cooked food through hands, surfaces of utensils, cooking utensils, and other utensils. Treatment of coconut shell liquid smoke at low concentrations has not been able to interfere with the metabolism of *Escherichia. coli* bacteria [20. This is presumably because the content of compounds that have the potential as antibacterial in the liquid smoke is only small, so the ability to inhibit bacterial growth is still very weak. The low effectiveness is because at that concentration the amount of liquid smoke used is small so that the active compounds contained do not work optimally. The low effectiveness indicates that the level of active compounds as antibacterial in the coconut shell liquid smoke used is still low [21].

Concentration of Liquid		Meaning			
Smoke	1	2	3	4	-
0%	Negative	Negative	Negative	Negative	Q
2%	Negative	Negative	Negative	Negative	Q
4%	Negative	Negative	Negative	Negative	Q
6%	Negative	Negative	Negative	Negative	Q
8%	Negative	Negative	Negative	Negative	Q

Table 6 Effect of Liquid Smoke Concentration and Storage Time on Escherichia coli Tofu

Descriptions : Q = Qualify, NE = Not Eligible

3.7 Salmonella sp.

Based on the results of the analysis as shown in Table 7, Salmonella sp. in all treatments still meet the standards set by SNI 01-3142-1998 which is negative. Food processing factors, hygiene factors raw material selection factors and food sales environmental factors can cause Salmonella bacteria contamination, therefore the need for hygiene sanitation in food processing. The quality of food that must be consumed must meet several criteria, namely nutritious, quality, safe from biological, microbiological, chemical and heavy metal pollution [22]. Salmonella sp. bacteria are pathogenic bacteria in humans and contamination of Salmonella sp. in food causes typhoid fever. The maximum microbial limit in food for Salmonella sp. must be negative or must not contain Salmonella sp. and when food is contaminated by Salmonella sp bacteria, the food does not meet the requirements for consumption. Contamination can also occur through vectors such as flies, insects, and others. In addition, contamination can occur during the handling of raw materials, processing, roasting, workers, or lack of sanitation in processing [22].

Effect of Liquid	Smoke Concentra	tion and Storage	Time on Salm	nonella sp. To	fu
Concentration of Liquid		Meaning			
Smoke	1	2	3	4	
0%	<3	<3	3,6	9.2	Q
2%	<3	<3	<3	9.2	Q
4%	<3	<3	<3	9.2	Q
6%	<3	<3	<3	9.2	Q
8%	<3	<3	<3	3.6	Q

Table 7

Descriptions : Q = Qualify, NE = Not Eligible

3.8 Subjective Variables

Colour

The highest average preference for color was found in the treatment with 4% liquid smoke concentration and 1 day storage time. While the average preference for the lowest color is found in the treatment of 0% liquid smoke concentration on the fourth day of storage. On the fourth day of storage, all of the tofu was damaged so that the tofu was overgrown with mold and the color of the tofu was brownish-yellow. Tofu that has been damaged by microbial activity will cause a change in color around the tofu due to the breakdown of protein compounds in the tofu, causing the color of the tofu to turn greenish-yellow or brownishyellow [23].

Aroma

The average preference for the highest aroma was found in the treatment with 2% liquid smoke concentration and 1 day storage time. While the average preference for the lowest aroma is found in the treatment of 0% liquid smoke concentration on the fourth day of storage because the tofu emits a rotten smell. spoilage microorganisms will damage the compounds of protein, the results of the overhaul of these compounds will produce a rotten aroma which will affect the aroma of the food itself [24].

Texture

The highest average texture preference was found in the treatment of 4% liquid smoke concentration and 1 day storage time. Meanwhile, the lowest average texture preference was found in the treatment with 0% liquid smoke concentration on the fourth day of storage because tofu has a slimy texture. Mucus on the tofu indicates that the tofu is damaged. Nur (2009) stated that the activity of microorganisms that cause the ability of protein to bind water will decrease so that the decrease in the water binding capacity of the protein causes mucus in the tofu. [25]. texture value is inversely proportional to value of water content, it means that if the total water content of smoked skipjack tuna decreases, the value of the texture will improve. As well on the other hand, if the amount of water content increases then the texture value will decrease [26]. **Flavour**

The average preference for the highest taste was found in the treatment with 4% liquid smoke concentration and one day storage time. While the average preference for the lowest taste was found in the treatment with a concentration of 8% liquid smoke on the third day of storage. Tofu that has been damaged is not subjected to organoleptic testing for taste.

According to Refilda [27], the concentration of liquid smoke in tofu has a significant effect on panelists' preference because it gives a distinctive flavor. This is due to the presence of carbonyl compounds which give a specific taste effect on the tofu. According to Darmadji [28], the components in liquid smoke that can cause a delicious taste in the product are formaldehyde and furaldehyde. The lowest preference value for taste is due to the more concentrated the liquid smoke used, the more the components of smoke contained in it seep into the tofu, so that to a certain extent it will cause a slightly bitter taste.

Overall Acceptance

Based on the results of the analysis, The highest average overall acceptance was obtained from the treatment of 6% liquid smoke concentration on the first day of storage. While the lowest overall acceptance average was found in the treatment of 0% liquid smoke concentration on the fourth day of storage.

4. Conclusion

The concentration of 6% liquid smoke from coconut shell can extend the shelf life of tofu up to 3 days at room temperature and produce the best tofu characteristics with a neutral – to moderately liking (4.40) and a total phenol of 0.57%, 82.34 levels. water, ash content 0.34, fat content 5.72, protein content 9.15, *Escherichia coli* <3 APM/g, and negative for *Salmonella* sp. The shelf life of tofu soaked with a concentratrion of 6% coconut shell liquid smoke is three days.

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