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Inhibition of Microencapsulated Liquid Smoke on the Foodborne Pathogens and Histamine-Forming Bacterias' Growth in Tuna Loin Sashimi

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Abstract

BACKGROUND: Sashimi, a unique and simple fresh fish dish, is commonly served at a restaurant or as a family dinner in Japan. Because sashimi was created from fresh tuna loin, it is easily ruined by spoilage germs and pathogens, particularly when served without ice. Apart from ice, new preservatives must be investigated to avoid pathogenic and histamine-producing microorganisms. Liquid smoke (LS) contains antimicrobial chemicals including organic acids, carbonyl, and phenols from pyrolyzing coconut shells. However, because the evidence of physicochemical features of LS is scarce, research into liquid smoke microencapsulation is required.

AIM: The researchers wanted to figure out how liquid smoke microencapsulation (LSM) is made, how effective it is against harmful germs, and how much total histamine is present in LSM-coated sashimi maintained at room temperature.

METHODS: Histamine content, antibacterial inhibitory activity, total microbial count (TPC) of Salmonella and E. coli, water content, and pH level were tested.

RESULTS: According to the findings, LSM with maltodextrin: sago flour: 1% LS ratio of 10:1:5 efficiently prevented E. coli and Salmonella development and reduced histamine level in sashimi refrigerated for 6 days in the refrigerator.

CONCLUSION: LSM effectively prevented pathogenic bacterias' growth and reduced histamine level in Tuna sashimi. Consequently, the application of liquid smoke microencapsulation (LSM) in tuna loin sashimi is a novelty.

Highlights

- The application of liquid smoke microencapsulation (LSM) in tuna loin sashimi is a novelty.
- LSM effectively prevented pathogenic bacterias' growth and reduced histamine level in Tuna sashimi.
- A flour created from sago Baruk palm (*Arenga microcarpha* Becc.) is a new ingredient for the LSM.

Introduction

Tuna-based sashimi is a popular dish in Indonesian large cities such as Manado and Bitung. The issue is that because sashimi uses fresh flesh, it must be kept cool before and in the serving process. Both harmful and spoilage bacteria can easily infect fresh meat. As a result, handling fresh meat should be thorough and meticulous. The following should be included in the standards for frozen tuna loin: Raw material classification, ingredients, food additives, handling-processing methods, sanitation-hygiene techniques, food safety aspects, sampling-testing methods, labeling, and packaging criteria. Several standard techniques for the production of tuna loin have been created, including specifications for frozen raw tuna [1], handling and processing processes [2], and sampling methods [3]. However, many industries continue to fail to achieve the criteria in practice. In Maluku, Indonesia, sanitation, and hygiene have failed to support the tuna loin supply chain [4]. Similarly, the total plate count (TPC) for tuna arriving at Pelabuhan Ratu on a ship is 1.0×10^2 CFU/g, while it was 4.7×10^6 CFU/g in the equipment used to prepare tuna loin on board, and the total coliform was 235 MPN/mL [5]. When compared to the coliform standard for tuna loin, which is 3 MPN/g,

this value is rather high [1]. Intracellular bacteria, such as *E. coli*, Salmonella, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Vibrio* cholerae, and Bacillus cereus are dangerous microorganisms that commonly infect fishing products. According to Dien *et al*. 2018 [6], harmful bacteria such as *E. coli*, *Vibrio* cholerae, *Vibrio* parahaemolyticus, and Salmonella were found in fish taken around Manado Bay. However, just two indicator bacteria, *E. coli* and Salmonella, were found in this investigation.

Consumption of fish, including tuna, skipjack, blue marlin, and mackerel causes scombroid-toxin poisoning. Histamine, produced by the degradation of histidine amino acids by histidine decarboxylase, is a poisonous chemical found in the scombroid group of fish. Histamine-producing microbes such as *Pseudomonas* sp., *Morganella morganii*, and *E. coli* generate the histidine decarboxylase enzyme[7], [8].

For the reasons stated above, it's vital to investigate how to preserve tuna by utilizing LS from natural coconut shells (CS). However, because LS characteristics easily change, it must be formulated into microencapsulation to be protected. Maltodextrin and sago starch (MD-SS) and CS-LS were combined to create coconut shell microencapsulated liquid smoke (LSM), which was then applied to tuna sashimi in this investigation. LSM has never been used in tuna loin sashimi before.

LS is used as a colorant and flavor in fish items containing protein, but it also possesses antimicrobial and antioxidant qualities [9]. The pH of the LS solution will be lowered by various phenolic chemicals and organic acids, which will destroy the bacterial cell wall [10]. The pH of basic LS is 3.2, whereas the pH of skipjack dipped in a liquid smoked at 0.4% and 0.8% has a pH of 4.8 and 5.5, according to Berhimpon *et al*. 2018 [11]. Many investigations at Sam Ratulangi University have discovered that coconut shells and mangrove wood are the two best types of LS fuel [12], [13], [11], [14]. Mangrove forests, on the other hand, are being protected due to the improved coastal environment. Coconut shells are abundant as a by-product of the copra business in North Sulawesi. Because bioactive substances such as phenol, carbonyl, and organic acids are found in LS coconut shells, LS has a significant potential to increase food goods' shelf life [15], [16]. Higher phenol concentration will result in longer products' shelf life [17]. High phenol content was linked to increased polycyclic aromatic hydrocarbons in traditional smoked meals (PAH). As a result, a high phenol level indicates that the substance is hazardous to human health. The greatest PAH concentration was 5 ppb [11].

During processing and storage, the bioactive components in LS must be protected from degradation and evaporation. The flavor encapsulation technology, which consists of core and wall materials to preserve taste components, is gaining popularity [18]. The regulation and maintenance of microencapsulation composition may protect the material from oxygen during storage. The sensitivity of the main material, the physiochemical characteristics, the dimension of the capsule, the application target, the release mechanism of the material, and the cost are all factors to consider when choosing an encapsulating technology.

Maltodextrin is regularly utilized for bioactive encapsulation regarding its water-soluble and protection from oxidation. In this research, besides maltodextrin, sago flour will be added to stabilize the encapsulation. LSM with MD-SS is a natural antibacterial that can be utilized to improve quality, and safety, and lengthen food storage of foods by altering the growth of spoilage microbes [19]. LS is sensitive to several foodborne pathogens *in vitro* and in food, including Salmonella, *Listeria monocytogenes*, Staphylococcus, and *Escherichia coli*. As a result, LS is a potent natural antimicrobial to be applied commercially, especially if a smoky flavor is wanted [6], [20]. This study strives to: (1) Formulate the LSM, particularly the ratio of MD, SS, and LS; (2) evaluate pathogenic bacteria inhibition by LSM in 6 days of refrigerated sashimi; and (3) evaluate the total histamine in LSM-coated sashimi refrigerated for 6 days.

Materials and Methods

Production of liquid smoke sample

Blue Ocean Grace International enterprise in Bitung sold first-grade frozen tuna loin for sashimi, which required 2 h to transfer to the Manado Laboratory. Tuna loin and ice were put into a cold box with a ratio of 1:2. Using smoke condensation apparatus (patent P00201405308) and coconut shell as fuel, low PAH liquid smoke (benzo(a)pyrene 0.25 ppb) was created in the laboratory of Sam Ratulangi University. A food-grade MD was procured from Lansida Herbal Technology Indonesia, as SS was created from the sago Baruk palm *Arenga microcarpha* Becc.; while analytical grade, high-media brand chemicals, and media were utilized for analysis. Coconut shells were employed as raw materials, and 10 kg yielded 2.8 L of LS with a 60–70% concentration. Coconut LS was created using a condenser in the Fish Processing Laboratory at Sam Ratulangi University. The optimal LS concentration for smoked fish in prior research was 1%. The crude LS was distilled before being filtered using Whatman paper number 40.

Treatments design

Based on the maltodextrin: sago: 1% LS ratio, three alternative formulations were evaluated to get a high yield. 10:1:5, 10:2.5:5, and 10:5:5 were the three formulations. A series of studies utilizing local sago

constituents as variables resulted in the formula ratio. 500 mL distilled water was used to dilute each recipe. Each formula was combined for 30 minutes at room temperature with a magnetic stirrer, then homogenized for 15 min using a Homogenizer Stirrer at 1000 rpm, and finally dried for 8 h in a cabinet drier at 50°C. The crystal powders were collected and separated from the starch granules, then placed in an amber container and kept at room temperature in a desiccator. The treatments included tuna loin coating with LS microencapsulation, as well as un-coating treatments as a control, where all samples were maintained in a refrigerator at 52°C for 6 days, with samples taken every day and tested promptly. The temperature was consistent with that of sashimi storage. A centigrade thermometer was placed on the side of the refrigerator to monitor the temperature, which was checked 2 times a day.

Microbiological count analysis [21]

A magnetic stirrer BPW) was used to homogenize 25 g of material in 225 mL of 0.1% v) buffered peptone water for 5 min. High-media plate count agar (PCA) and the pour plate method were used to determine TPC, which was incubated at 37°C for a day.

Isolation and Identification of E. coli [21]

A method for estimating the concentration of live *E. coli* is the most probable number (MPN). Take one loopful sample from each of the positive Durham tubes, spread it into Eosin Methylene Blue Agar (EMBA), and incubate it under the decided time and temperature similar to Salmonella. The gram stain, IMVIC test, cell morphology and colony, and both oxidase and catalase activity were all performed on such pure cultures.

Isolation and Identification of Salmonella [22]

To test for Salmonella, samples from BPW samples were obtained, and pre-enriched cultures using Lactose broth were performed (LB). Following that, 0.1 of the pre-enriched cultures were moved to RV broth and incubated at 42°C for a full day, followed by one loopful of the broth being spread into Bismuth Sulfite Agar (BSA) plates and incubated at 37°C for a day. If suspected Salmonella colonies emerge on the BSA medium, several biochemical tests must be performed to validate it, including indole synthesis, motility, carbohydrate fermentation, and lysine decarboxylase.

Antibacterial inhibitory activity

The inhibitory activity of Salmonella and *E. coli* isolates was investigated using the disk diffusion technique. SI4 for Salmonella and EcI2 for *E. coli* were the isolation codes. The Kirby–Bauer technique was used to conduct an antibacterial activity susceptibility test on a nutrient agar medium. Salmonella (SI4) and *E. coli* (EcI2) cultures were injected into various Petri plates using the spread plate technique under aseptic circumstances on the solid agar surface. Following that, a saturated filter paper disc containing microencapsulate LS was deposited aseptically over the infected agar surface under aseptic conditions. Finally, the Petri plates were labeled and incubated at 37°C for a full day. In addition, the inhibitory zone was assessed.

Histamine analysis [19]

The sample was macerated, which altered histamine to a hydroxyl form. The histamine level was determined by spectrofluorometric [19]. Furthermore, using o-phthalataldehyde 1 percent as the reagent, histamine was transformed to a derivate, and the fluorescence of the resultant product was observed using a spectrofluorometer at wavelength 444 nm.

pH measurements

An Adwa AD 1000 pH V pH meter was used to determine the pH. Twenty-five grams of the sample was dissolved in 25 mL of distilled water, and 20 mL of the homogenized sample was removed and analyzed using a pH meter.

Moisture content [23]

The gravimetric technique was used to determine the moisture content. 3–5 g samples were dried in an oven at 105°C.

Statistical analysis

Log10 CFU/g values were used to translate microbial data. The mean and standard deviation were computed and graphed. Completely Randomize Design employed a factorial (2 × 4) experimental design (CRD). The data were tested using an ANOVA approach with $p < 0.05$ categorized as significant, followed by the Tukey test for a significant difference [24]. The data were analyzed using Microsoft Excel 2010.

Results and Discussion

Formulation of microencapsulated liquid smoke

Table 1 displays the outcome. The best recipe was formula 1, which consisted of maltodextrin 50 g, sago 5 g,

and 1% LS 25 mL, with a yield of 61.9% and exceptionally fine crystal powders. The more sago there is, the coarser the crystal powder becomes, and the lower the yield.

Table 1: Formulation and yield of microencapsulated liquid smoke

Materials	F1	F2	F3
Maltodextrin	50 g	50 g	50 g
Sago	5 g	12.5 g	25 g
1% LS Crystal powder	25 ml Very fine	25 ml Fine	25 ml A bit coarse
Yield	61.9%	62.2%	57.7%

Microbial count (total plate count)

TPC will meet all microbes, including harmful and spoiled bacteria. TPC readings in the control (0 min), fresh fillets, and encapsulated-coated samples were 4.5×10^1 CFU/g, 4.5×10^1 to 3.8×10^3 CFU/g, and 1.1×10^1 to 5.1×10^2 CFU/g, respectively, as shown in Figure 1. The quantity of TPC in sashimi grew for 6 days in the refrigerator, as shown in Figure 1, but was still acceptable based on SNI [1]. The coating treatment has a significant impact ($p = 0.05$) according to the analysis of variance. All interactions differ significantly ($p = 0.05$) between uncoated and coated un-stored samples (D0) (Table 2). The highest amount of TPC in food that is safe to eat is 5.0×10^5 CFU/g [25]. According to Buckle *et al*. [26], cell division does not occur during the early stages of microbial development until the ability of a cell to adapt is developed. The bacterial cells will then expand and divide rapidly until they reach their maximum size. The potential of LS to prolong the expiry date of items by reducing damage due to oxidation [27] was explained by a functional phenol and abundance of organic acids composite in LS working synergistically to inhibit and regulate microbial development [10]. After being dipped in LS, vacuum packaged, pasteurized, and refrigerated for 10 days at 5–10°C, the TPC value in fish meatballs is still safe for consumption [28]. Other items, such as *Cakalang pampis* and Roa Abon (floss meat of Roa) packed with MAP and stored at room temperature, can be used up to 30 days after being stored [29]. However, another study found that the number of microorganisms in sardines grew to more than 10^7 CFU/g after 25 h of storage at 30°C [30], [17]. These results were achieved as a result of the optimal storage pH and temperature for microbial development [31].

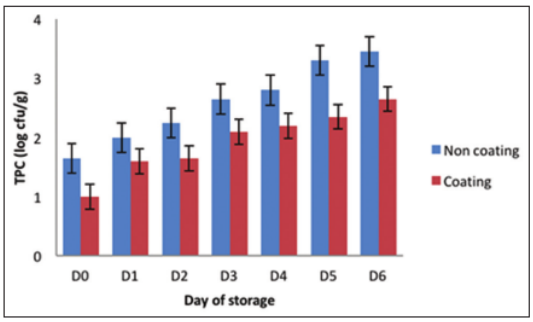

Figure 1: TPC of coated and non-coated sashimi fillets refrigerated for 6 days

Escherichia coli

The amount of *E. coli* in the control fillet ranged from 3.0 to 3.6 MPN/g, then grew during storage, whereas samples coated with encapsulated LS remained at 3.0 MPN/g for 6 days then dropped to 0.3 MPN/g. The presence of organic acids and phenolic compounds in LS made it impossible for *E. coli* to grow. LS is a powerful bactericide that can stop *E. coli* and other pathogens from growing [32]. As shown in the previous research, fish products soaked in LS are still accepted by Indonesian standards and proved negative for microbes [28]. *Coliform, E. coli, Salmonella sp., and Vibrio sp.* were all negative in smoked Abon Roa and *Cakalang pampis* packed by *MAP* [29].

Table 2: *Escherichia coli* in non-coated and coated Sashimi fillets refrigerated for 6 days

E. coli (MPN/g)						
Time (days)	Non-coated			Coated		
	1	2	3	1	2	3
D0	3.6	3.0	3.6	3.0	<3.0	<3.0
D1	3.2	<3.0	<3.0	<3.0	<3.0	<3.0
D2	4.2	<3.0	<3.0	<3.0	<3.0	<3.0
D3	5.4	<3.0	<3.0	<3.0	<3.0	<3.0
D4	6.2	<3.0	<3.0	<3.0	<3.0	<3.0
D5	7.4	<3.0	<3.0	<3.0	<3.0	<3.0
D6	15	36	3.6	<3.0	<3.0	<3.0

Salmonella sp.

Salmonella was found to be negative in both control and LSM samples on the BSA medium. Natural antimicrobials have lately been used to increase the quality and expiry date of food by working antagonistically against the growth of spoilage bacteria and food-borne diseases [19]. According to several studies, the occurrence of Salmonella in goods can be attributed to poor manufacturing hygiene, including cross-contamination [33]. Another study found that all liquid smoke components utilized for Katsuobushi were free of Salmonella sp. and Staphylococcus aureus germs [34]. According to prior research, LS might be used as an all-rounder antibacterial to be applied commercially where a smoke taste is needed. Other benefits include less PAH concentration in LS and improved LS product quality, including taste and flavor [11].

Antibacterial inhibitory

Table 3 shows the inhibition zone of LSM on *E. coli* and Salmonella strain isolate. A longer incubation period will result in stronger antibacterial properties of LSM, as seen by the increasing width of the inhibitory zone at all treatment dosages. The results of the analysis of variance of the inhibitory zone data on *E. coli* and Salmonella demonstrate that the interaction between LS concentration and storage day has a highly significant impact ($p < 0.01$). Figures 2 and 3 provide inhibition zone data for six treatments, and it can be concluded that *E. coli* and Salmonella are sensitive since the inhibition zone is more than 14 mm. The sensitivity of the tested microorganisms is determined by the diameter of the

Table 3: *E. coli* and Salmonella Inhibition Zone using LS Microencapsulation

Diameter of inhibition zone (mm)						
	1% LS		2% LS		3% LS	
E. coli						
1	15	18	26	28	33	36
2	18	20	27	30	40	41
3	15	17	27	29	39	41
4	19	16	29	28	45	47
5	20	19	35	37	50	46
6	18	17	33	39	47	49
Salmonella						
1	15	16	21	25	20	27
2	14	19	25	33	34	35
3	17	17	24	27	36	35
4	18	18	27	39	28	40
5	20	17	28	30	35	39
6	22	18	29	29	38	34

inhibitory zone. The bigger the antibacterial activity, the larger the zone of inhibition, with a zone of > 14 mm being sensitive and 11 mm being resistant [1]. Phenolic chemicals, which are prevalent in liquid smoke, can harm Gram-negative bacteria's cytoplasmic membrane, and their efficiency increases when the bacteria divide, when the phospholipid layer around the cell is very thin [35], [36]. Skipjack fillet dipped in 0.8% LS with 12.6 mg phenolic chemicals and 0.25ppb benzo(a) pyrene [11]. Skipjack fillets dipped in 2% LS for 30 min had a phenol concentration of 24.21 mg/kg. As an antibiotic, the phenol molecule works by damaging the structure of bacterial cells and inhibiting the production of cell walls, resulting in cell wall lysis [36]. Antibacterial chemicals, according to Silva *et al*. [37] have a variety of mechanisms and actions, including the destruction of the bacterial cell wall. Phenol chemicals can denaturize proteins and induce apoptosis. When the cytoplasmic integrity of bacteria is disrupted, macronutrients and ions escape from the cells. Bacterial cells disintegrate and lyse. The main effects are loss of structure and damage to the cell [38]. The antibacterial mechanism's principal target is the bacterial cell structure, which target the cytoplasmic membrane, loss of stability of ions, and coagulation in the cell's constituent [39]. According to Brooks *et al*. [40], various parameters impacted antibacterial activity, including concentration, antibacterial component content, extract diffusion power and suppressed bacterium kinds. Encapsulation LS, like LS solution, demonstrates that the higher the LS concentration, the larger the inhibitory zone width of *E. coli* and Salmonella. It is reasonable to believe that when the concentration rises, more antibacterial chemicals are produced, enhancing their penetration into bacterial cells.

Histamine content

Figure 2 shows the data on histamine content. The coating treatment has a significant influence ($p < 0.05$) on the results, but the interaction between LS concentration and storage duration has no significant effect ($p > 0.05$). Total histamine in non-coated fillets was 17.26 mg/kg and grew considerably with time, but total histamine in LSM-coated fillets was 16.42 mg/kg and demonstrated stationarity. However, both non-coated and coated samples contained modest levels of histamine, according to the findings. Histamine is permitted up to 40 mg/100 g in fishery products under European legislation. Histamine is produced during the preservation of fresh fish by the activities of histidine decarboxylase enzymes [41] and L-histidine ammonia-lyase, which create glutamate and histamine [42]. Histamine-producing bacteria, such as *Pseudomonas* sp., *Micrococcus luteus*, and *E. coli,* induce decarboxylase proses [43], [44].

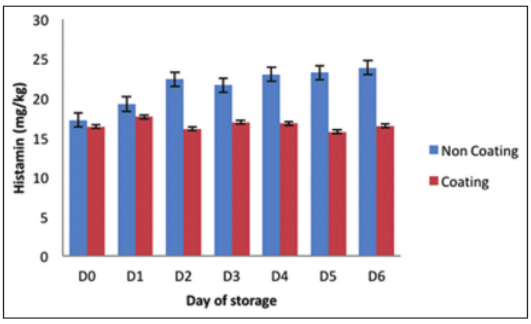

Figure 2: Histamine level of coated and non-coated sashimi fillets during refrigeration

pH

Figure 3 shows pH information. The data analysis reveals that coating treatment has a highly significant ($p<0.01$) influence on pH; however, the interaction between LS concentration and storage period has no significant effect ($p > 0.05$). The pH value of fresh fillets after being stored in the refrigerator for 6 days was practically the same, ranging from 4.8 to 5.6. Organic acids in the LS create the low pH of LSM. Organic acids also have a preservative impact on fish and meat products because liquid smoke has a pH of 2.8–3.1, it may be employed as a preservative. After being diluted from 0.4% to 0.8% and applied to the skipjack fillet, the pH ranged from 4.8 to 5.5 [11], [24], [5]. As a result, LS can inhibit the growth of pathogens and spoilage microbes [18], [6].

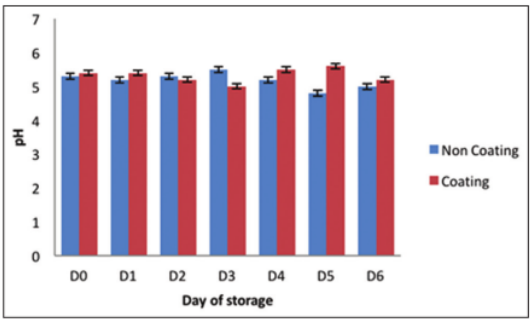

Figure 3: pH of coated and non-coated sashimi fillets during refrigeration

Moisture content

Figure 4 shows the water content of both coated and non-coated fresh fillets. The data analysis reveals that coated fillets have a highly significant ($p < 0.01$) influence on moisture, but the interaction between the concentration of LS and storage duration has no significant effect ($p \geq 0.05$). The water content of fresh fillets dropped after being refrigerated, which may be caused by the refrigerator's relative humidity which is lower than the ambient temperature, and the samples are neatly unwrapped. However, the water content of LS microencapsulated samples is much higher than that of non-coated samples. Microencapsulation can help minimize water evaporation in fresh fillets, according to the article. Non-coated fillets' water content decreased from 77.3% to 56.5% after 6 days in the refrigerator, but fillets coated with LSM only decreased from 72.8% to 64.8% after 6 days. Fresh tuna loin skin-on has 73.14% water, whereas fresh tuna loin skin-less contains 75.05% [45].

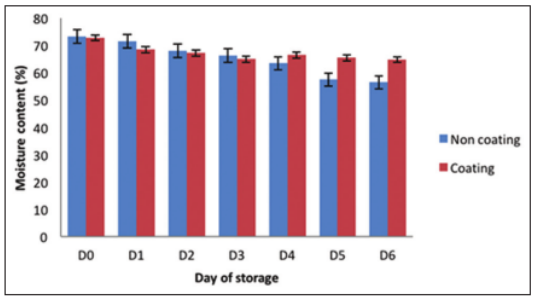

Figure 4: Moisture content of coated and non-coated sashimi fillets during refrigeration

Conclusions

Histamine content, antibacterial inhibitory activity, total microbial count (TPC) of Salmonella and *E. coli*, water content, and pH level were tested. According to the findings, LSM with maltodextrin: Sago flour: 1% LS ratio of 10:1:5 efficiently prevented *E. coli* and Salmonella development and reduced histamine level in sashimi refrigerated for 6 days in the refrigerator. LSM effectively prevented pathogenic bacterias' growth and reduced histamine level in Tuna sashimi. Consequently, the application of liquid smoke microencapsulation (LSM) in tuna loin sashimi is a novelty.

Availability of Data and Material

Data will be available by sending an application email to the corresponding author.

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