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Stability of liquid smoked nano-encapsulated on the foodborne pathogens and histamine-forming bacteria's growth in tuna loin sashimi

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ABSTRACT

Introduction: In Japan, sashimi, a distinctive and straightforward meal of fresh fish, is frequently offered for dinner with family or at restaurants. Since sashimi is made from raw tuna loin, infections and spoiling microorganisms can readily damage it, especially if it is served without refrigeration. In addition to ice, novel preservatives need to be researched to prevent the growth of harmful and histamine-producing microbes. Carbonyl, phenols from burning coconut shells, and organic acids are among the antibacterial substances found in liquid smoke (LS). However, more study on liquid smoke nano-encapsulation is needed because there is a lack of evidence about the physicochemical properties of LS.

Aims: The goal of the study was to determine the production process of liquid smoke nano-encapsulation (LSN), the level of total histamine in LSN-coated sashimi stored at room temperature, and the effectiveness of LSN against pathogenic microorganisms.

Materials and Methods: The following parameters were measured: pH levels, water content, Salmonella, *E. coli*, total microbial count (TPC), histamine concentration, and antibacterial inhibitory action.

Results: The findings demonstrated that LSN with maltodextrin: sago flour: 1% LS ratio of 10: 1: 5 effectively inhibited the growth of *E. coli* and Salmonella and decreased the amount of histamine in sashimi that was refrigerated for ten days.

Conclusions: LSN was effective in preventing the growth of pathogenic bacteria and reducing the histamine content in tuna sashimi.

KEYWORDS

Liquid smoke, tuna loin, nanoencapsulation, pathogenic, histamine.

HIGHLIGHTS

- The use of liquid smoke nanoencapsulation (LSN) in sashimi made with tuna loin is new.
- LSN successfully decreased the amount of histamine in tuna sashimi and stopped the formation of harmful microorganisms.
- A novel component for the LSN is flour made from the sago Baruk palm (*Arenga microcarpha* Becc.).

INTRODUCTION

Tuna-based sashimi stands as a sought-after culinary delight in various Indonesian urban centers, including Jakarta, Surabaya, Manado, and Bitung. The essence of this delectable dish lies in its utilization of fresh tuna flesh. However, the inherent challenge lies in maintaining the freshness and safety of raw meat, given its susceptibility to contamination by harmful bacteria and spoilage during the handling and serving processes[1]. Preserving the integrity of fresh tuna is paramount, demanding a meticulous and thorough approach to handling. Establishing stringent standards for frozen tuna loin becomes imperative to ensure the quality and safety of the end product. These standards encompass a multifaceted framework, addressing raw material classification, ingredients, food additives,

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handling and processing methods, sanitation and hygiene practices, food safety considerations, sampling and testing methodologies, as well as criteria for labeling and packaging[2].

While standardized techniques for tuna loin production exist, the practical implementation of these measures remains a challenge for numerous industries. Notably, in the Maluku region of Indonesia, the tuna loin supply chain grapples with issues of sanitation and hygiene, contributing to lapses in meeting established criteria[3]. The situation is exacerbated by high microbial counts, highlighting a considerable risk to food safety[4]. The presence of intracellular bacteria, including *E. coli*, Salmonella, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Bacillus cereus*, poses a serious threat to the safety of fishing products. Studies, such as that conducted by Dien et al., reveal the prevalence of harmful bacteria like *E. coli*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, and Salmonella in fish sourced from Manado Bay[5]. However, the most commonly detected were *E. coli* and Salmonella, serving as crucial indicators of potential contamination.

Addressing these challenges demands a concerted effort across the tuna supply chain, from stringent adherence to established standards to comprehensive sanitation and hygiene practices. Such measures are essential not only for safeguarding consumer health but also for sustaining the reputation and viability of the tuna-based sashimi industry in Indonesia. The consumption of Scombroid fish, encompassing varieties like skipjack, tuna, blue marlin, and mackerel, is frequently associated with the risk of scombroid-toxin poisoning[6]. This type of poisoning is attributed to histamine, a toxic compound produced during the degradation of histidine amino acids in fish meat. The enzymatic action of histidine decarboxylase, facilitated by histamine-forming bacteria (HFB), catalyzes this conversion. Notably, histamine is prevalent in the Scombroid group of fish, making it imperative to understand the underlying mechanisms that contribute to its formation[7]. Histamine-producing microbes, including but not limited to *Pseudomonas sp.*, *Morganella morganii*, and *E. coli*, play a crucial role in this process. These microbes are commonly found in environments such as waste, unclean surfaces, and on the hands of workers involved in the handling of fish. The histidine decarboxylase enzyme, produced by these bacteria, is responsible for the transformation of histidine into histamine, thereby rendering the fish toxic when consumed[8].

Given the aforementioned considerations, a critical exploration into the preservation of tuna through the utilization of liquid smoke (LS) derived from natural coconut shells (CS) becomes interesting[9]. The challenge lies in the inherent susceptibility of LS characteristics to alterations over time. To safeguard its effectiveness as a preservative, it is crucial to employ nano-encapsulation techniques. In this context, the combination of maltodextrin and sago starch (MD-SS) with CS-derived liquid smoke (CS-LS) was undertaken to formulate coconut shell nano-encapsulated liquid smoke (LSN). Subsequently, this innovative LSN was applied to tuna sashimi in the course of this research, marking a pioneering endeavor as the application of LSN in tuna loin sashimi has not been previously explored. Nano-encapsulation serves as a sophisticated approach to enhance the stability and efficacy of liquid smoke, particularly when derived from natural sources such as coconut shells. The combination of MD-SS and CS-LS in the creation of LSN provides a protective matrix that not only preserves the essential properties of liquid smoke but also facilitates its controlled release, ensuring optimal application to tuna sashimi.

LS is utilized not only as a colorant and flavor enhancer in protein-rich fish products but also for its inherent antimicrobial and antioxidant properties[10]. The introduction of various phenolic chemicals and organic acids into the LS solution leads to a reduction in pH, resulting in the destruction of bacterial cell walls[11]. The coconut shell-derived LS contains bioactive substances such as phenol, carbonyl, and organic acids, endowing it with significant potential to extend the shelf life of food products. It is noteworthy that a higher concentration of phenol correlates with an extended shelf life for these products. Nevertheless, it is crucial to be mindful of the potential drawbacks, as an elevated phenol level has been associated with an increase in polycyclic aromatic hydrocarbons (PAH) in traditionally smoked products[12]. This is particularly significant considering that a PAH concentration exceeding 5 ppb is deemed hazardous to human health.

In the course of processing and storage, it is imperative to shield the bioactive constituents within LS from degradation and volatilization. An increasingly favored method for accomplishing this is flavor encapsulation technology, encompassing the use of core and wall materials to safeguard the taste components. The careful regulation and maintenance of the nano-encapsulation composition serve to shield the material from exposure to oxygen during storage[13]. Several considerations come into play when opting for encapsulation technology, including the sensitivity of the primary material, its physiochemical attributes, capsule dimensions, the intended application, the mechanism for material release, and the associated costs[14]. These factors collectively contribute to the decision-making process in selecting an appropriate encapsulation technology.

Maltodextrin is commonly employed for encapsulating bioactive substances due to its water solubility and protective qualities against oxidation[15]. In this study, sago flour is introduced as an additional component alongside maltodextrin to enhance the stability of the encapsulation. The combination of liquid smoke (LSM) with maltodextrin-sago starch (MD-SS) forms a naturally antibacterial agent, presenting opportunities to enhance the quality, safety, and shelf life of food products by influencing the growth of spoilage microbes.

Liquid smoke exhibits sensitivity to various foodborne pathogens both in vitro and in food matrices, including

Salmonella, *Listeria monocytogenes*, Staphylococcus, and *Escherichia coli*[16]. Consequently, LSM emerges as a promising natural antimicrobial agent with potential commercial applications, particularly when a smoky flavor profile is desired. The objectives of this study are threefold: (1) to formulate LSM, with a specific emphasis on determining the optimal ratio of maltodextrin, sago starch, and liquid smoke; (2) to assess the inhibitory effects of LSM on pathogenic bacteria during a 6-day refrigerated storage period of sashimi; and (3) to evaluate the total histamine content in LSM-coated sashimi over the same refrigerated duration of 6 days.

MATERIALS AND METHODS

Material

The high-quality tuna loins were purchased from Blue Ocean Grace International enterprise in Bitung. The tuna loin was put into a plastic bag and sealed, and then together with ice was put into a cold box with a ratio loin: ice of 1: 2 to keep the quality unchanged. It requires two hours to be transported to the Laboratory in the Faculty of Fisheries and Marine Science, Sam Ratulangi University, in Manado.

Production of Liquid Smoke and Nano-Encapsulation

Low PAH liquid smoke (benzo(a)pyrene 0.25 ppb) was produced using smoke condensation equipment and coconut shell as fuel[17]. Since SS was made from the sago Baruk palm *Arenga microcarpha* Becc., a food-grade MD was obtained from Lansida Herbal Technology Indonesia; for analysis, analytical grade, high-media brand chemicals, and media were used. The raw material used was coconut shells; 10 kg produced 2.8 L of LS with a concentration of 60–70%. Whatman paper number 40 was utilized to filter the crude LS after it had undergone distillation. Previous studies found that 1% was the ideal LS concentration for smoked fish[17].

Treatments Design

This research continues the research carried out by Dien et al., which obtained the best microencapsulation formula, namely: maltodextrin 50g, sago 5g, and 1% LS 25 mL [9]. To produce nanoparticles, the same formula was used and the formula was homogenized using a WisetStir-HS30E homogenizer at 2000 rpm rotation (Figure 1).

After being gathered and separated from the starch granules, the crystal powders were put in an amber container and maintained in a desiccator at room temperature. Tuna loin was coated with LS nano-encapsulation as one of the treatments, and as a control, no coating was applied. All samples were kept in a refrigerator at 5 degrees Celsius for 10 days, with samples being removed every 2 days and evaluated right away. It was at a temperature appropriate for storing sashimi. The refrigerator's side was equipped with a centigrade thermometer, which was used to check the temperature twice a day.

Microbiological Count Analysis

For five minutes, 25 g of material was homogenized in 225 mL of buffered peptone water containing 0.1 percent (w/v) using a magnetic stirrer (BPW). TPC was determined using the pour plate technique and high-media plate count agar (PCA), which were incubated at 37 ºC for one day[18].

Isolation and Identification of E. coli

A technique for determining the amount of viable *E. coli* present is the most probable number (MPN). From each of the positive Durham tubes, take one loopful of material, spread it out on Eosin Methylene Blue Agar (EMBA), and incubate it for the predetermined amount of time at a temperature that is comparable to that of Salmonella. Such pure cultures were used for gram stain, the IMVIC test, cell morphology and colony, and both oxidase and catalase activity analyses[18].

Isolation and Identification of Salmonella

Samples from BPW samples were collected, and pre-enriched cultures using lactose broth (LB) were carried out to screen for Salmonella. Then, one loopful of the broth was distributed over Bismuth Sulfite Agar (BSA) plates and incubated at 37 ºC for a day. After that, 0.1 of the pre-enriched cultures were transferred to RV broth and incubated at 42 ºC for an entire day. Indidole synthesis, motility, carbohydrate fermentation, and lysine decarboxylase are among the biochemical assays that must be carried out to authenticate any suspected Salmonella colonies that appear on the BSA medium[19].

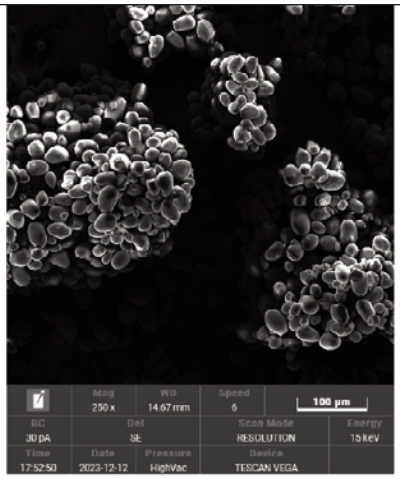
Antibacterial Inhibitory Activity

Disk diffusion was used to test the isolates of *E. coli* and Salmonella for their inhibitory activity. The isolation codes were EcI4 for *E. coli* and SI6 for Salmonella. A susceptibility test for antibacterial activity was performed using the Kirby Bauer method on a nutrient agar medium[20]. Using the spread plate approach, cultures of *E. coli* (EcI4) and Salmonella (SI6) were aseptically injected over the solid agar surface of several Petri plates. Subsequently, the contaminated agar surface was covered aseptically with a saturated filter paper disc that contained nano-encapsulate LS. After labeling and a full day of incubation at 37 ºC, the inhibitory zone was measured on the Petri plates.

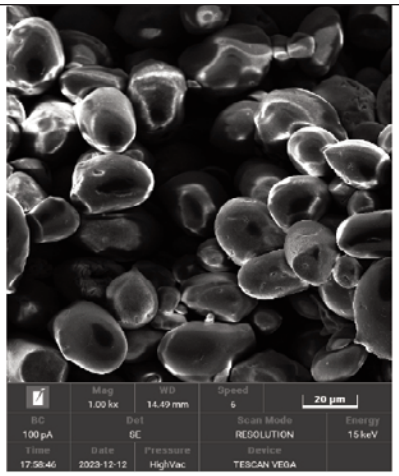
Histamine Analysis

Histamine was changed to a hydroxyl form in the macerated material. Spectrophotometric analysis was used to measure the histamine level[21]. Additionally, histamine was converted to a derivative using o-phthalataldehyde 1 percent as the reagent, and the fluorescence of the end product was measured at a wavelength of 444 nm using a spectrofluorometer.

Sample



Magnification by 1,000 times



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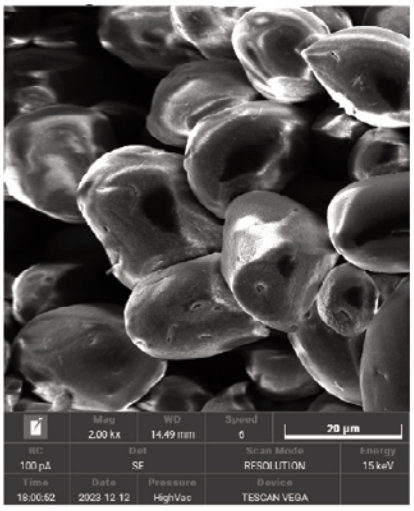
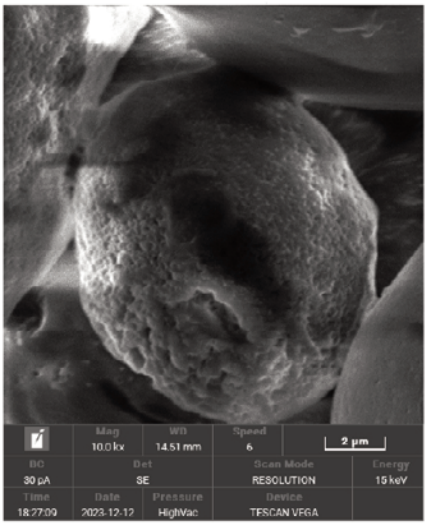


Magnification by 5,000 times

Magnification by 10,000 times



The particle sizes with magnifications of 10,000, 5,000, and 1,000 times respectively are 2 µm, 5 µm, and 20µm so they are still included in nanoparticles. SEM results show a curvature on the surface of particles arranged irregularly and hollowly. The particle size is stable and the same.

Figure 1. Particle Size Resulted from SEM

pH Measurements

To find the pH, an Adwa AD 1000 pH/mV pH meter was utilized. A pH meter was used to assess 20 mL of the homogenized sample after 25 g of the sample had been dissolved in 25 mL of distilled water.

Moisture Content

The moisture content was determined by the gravimetric method, adapted from research by Fardisi et al.[22]. Samples weighing three to five grams were dried in an oven set at 105 degrees Celcius.

Statistical Analysis

Microbiological data were transformed using log10 CFU/g values. We calculated and plotted the mean and standard deviation. A factorial (2 x 4) experimental design was used in the completely randomized design. ANOVA was used to assess the data, and a p-value of less than 0.05 was considered significant. A significant difference was then determined using the Tukey test. Excel 2010 was used to examine the data.

RESULTS

Formulation of Nano-Encapsulated Liquid Smoke

This investigation represents a continuation of the work initiated by Dien et al., where they successfully identified the optimal microencapsulation formula[9]. The established formula, consisting of 50g of maltodextrin, 5g of sago, and 25 ml of 1% LS, served as the foundation for the present study. To ensure uniformity, the components were subjected to homogenization using the WisetStir-HS30E homogenizer, operating at a rotation speed of 2000 rpm. Building upon the groundwork laid by Dien et al., this research sought to replicate and further validate the efficacy of the established microencapsulation formula. The homogenization process, facilitated by the advanced WisetStir-HS30E homogenizer, played a crucial role in achieving a consistent and well-blended formulation. Upon completion of the research, a notable yield of 85.6% was attained, signifying a successful and efficient encapsulation process.

Microbial Count (Total Plate Count)

TPC represents the cumulative count of all microorganisms present in a sample, encompassing both harmful microbes and those responsible for spoilage. The TPC readings in various samples, including fresh fillets, non-coated control samples, and encapsulated-coated samples, were documented as 4.0×10^1 CFU/g, ranging from 4.0×10^1 to 4.3×10^4 CFU/g, and from 4.4×10^0 to 8.0×10^0 CFU/g, respectively, as detailed in Table 1. Notably, the TPC in uncoated sashimi fillets demonstrated a significant increase; however, it remained within acceptable limits according to the standards outlined in SNI[23]. The application of coating treatments exhibited a substantial impact ($p<0.05$), as indicated by the analysis of variance. Significant differences ($p<0.05$) were observed in all interactions between uncoated and coated samples. The highest TPC value considered safe for consumption is 5.0×10^5 CFU/g [24].

Escherichia coli

The *E. coli* levels in fillets, whether coated or non-coated with nano-encapsulated LS, over a 10-day storage period are detailed in Table 2. Initially, the non-coated fillets exhibited *E. coli* levels ranging from 3.0 to 3.2 MPN/g, which increased significantly during storage. Conversely, the fillets coated with nano-encapsulated LS maintained levels below 3.0 MPN/g throughout the 10-day storage period. This signifies a noteworthy inhibitory effect on the growth of *E. coli* due to the application of nano-encapsulation, showcasing the potency of this method.

Table 1. TPC of Fresh Sashimi Fillet Coated, and Non-Coated by Nano-Encapsulation, Stored Up to 10 Days in Refrigerated Condition

Day of Storage	TPC (CFU/g – CFU/mL)					
	Non-Coated			Coated		
	1	2	3	1	2	3
Day 0	4.0×10^1	4.7×10^1	3.3×10^1	5.0×10^0	4.1×10^0	4.0×10^0
Day 2	8.7×10^1	6.9×10^1	6.5×10^1	1.2×10^0	5.0×10^0	2.7×10^1
Day 4	2.9×10^2	1.5×10^2	1.9×10^2	2.4×10^0	6.7×10^0	1.3×10^0
Day 6	3.2×10^3	6.0×10^3	5.0×10^3	3.8×10^1	8.5×10^0	7.5×10^0
Day 8	3.5×10^3	4.7×10^3	4.9×10^3	4.0×10^0	2.1×10^1	2.0×10^1
Day 10	4.0×10^4	2.0×10^4	6.8×10^4	5.0×10^0	3.9×10^0	1.5×10^1

Table 2. *Escherichia coli* of Nano-Encapsulation-Coated and Non-Coated Fillet, Up to 10 Days of Storage

Day of Storage	*Escherichia coli* (MPN/g)					
	Non-Coated					
	1	2	3	1	2	3
Day 0	3.2	<3.0	<3.0	<3.0	<3.0	<3.0
Day 2	<3.0	3.2	3.6	<3.0	<3.0	<3.0
Day 4	4.2	4.2	3.6	<3.0	<3.0	<3.0
Day 6	5.2	5.4	6.2	<3.0	<3.0	<3.0
Day 8	7.4	7.4	6.8	<3.0	<3.0	<3.0
Day 10	6.2	7.8	7.0	<3.0	<3.0	<3.0

Salmonella sp.

The presence of Salmonella was determined to be negative in both the control and LSN samples when cultured on the BSA medium. This result aligns with the current trend of utilizing natural antimicrobials to enhance the quality and prolong the shelf life of food products by actively hindering the growth of spoilage bacteria and mitigating the risk of foodborne diseases.

Antibacterial Inhibitory

Table 3 illustrates the inhibition zones of LSN on *E. coli* and Salmonella strains, providing insightful data on the impact of LS concentration and incubation period on the antibacterial properties of LSN. A clear trend emerges from the table, indicating that higher LS concentrations and prolonged incubation periods result in stronger antibacterial effects, evident from the increasing width of the inhibitory zones across all treatment dosages.

Table 3. *E. coli* and Salmonella Inhibition Zone using LS Nano-Encapsulation

	Diameter of Inhibition Zone (mm)					
	1% LS Nano-Encapsulation		2% LS Nano-Encapsulation		3% LS Nano-Encapsulation	
E. coli						
1	24	22	30	31	41	46
2	24	21	33	30	44	42
3	25	22	30	34	39	41
4	25	24	36	30	46	48
5	20	20	35	37	48	50
6	24	27	33	39	49	49
Salmonella						
1	23	24	34	35	37	36
2	24	26	36	33	36	37
3	25	25	37	37	37	35
4	26	27	37	39	38	41
5	23	27	32	30	39	40
6	25	27	35	36	39	44

A detailed analysis of the inhibitory zone data for *E. coli* and Salmonella, employing a highly significant impact assessment ($p<0.01$), reveals the interactive influence of LS concentration and storage day. The table outlines inhibition zone data for three treatments and six storage day variables, leading to the conclusion that LSN, irrespective of LS concentrations (1%, 2%, and 3%), exhibits robust antibacterial properties against *E. coli* and Salmonella. All inhibition zones surpassed 14 mm, indicating a high sensitivity of the tested microorganisms.

Histamine Content

The data on histamine content are displayed in Table 4. The results are significantly impacted ($p<0.05$) by the coating procedure and significantly impacted ($p<0.05$) by the interaction between LS concentration and storage time. Total histamine in non-coated fillets after 10 days of storage was 35.0 mg/kg and grew considerably with time, but total histamine in LSN-coated fillets after 10 days of storage was only 6.3 mg/kg and during 1 day of storage demonstrated stationarity.

pH Level

Table 5 presents the pH data, and the subsequent data analysis underscores the significant influence of the coating treatment as well as the interaction between LS concentration and storage period, both of which exhibited a noteworthy significance level of $p<0.01$. Upon scrutinizing the pH values of fresh fillets coated with LSN and stored in the refrigerator for 10 days, it is evident that they remained consistently within the range of 5.0 to 6.0. Specifically, after 8 to 10 days of storage, the average pH for LSN-coated fillets stabilized at 5.7.

Moisture Content

The water content of fresh fillets, coated and uncoated, is displayed in Table 6. Coated fillets have a highly significant ($p<0.01$) effect on moisture, according to the data analysis. However, there is no significant effect ($p\geq0.05$) from the interaction between the LS concentration and storage period. The water content of non-coated fresh fillets dropped after be-

Table 4. Histamine Content of Fillet Coated and Non-Coated by Nano-Encapsulated, Up to 10 Days of Storage

Day of Storage	Histamine (mg/kg)					
	Non-Coated			Coated		
	1	2	3	1	2	3
Day 0	10.0	12.0	9.5	5.0	10.0	7.5
Day 2	15.5	18.8	20.0	5.7	6.0	6.5
Day 4	20.2	19.5	22.2	5.0	5.7	5.5
Day 6	25.6	30.7	32.2	6.2	6.5	7.1
Day 8	29.3	29.5	30.1	5.0	5.5	6.5
Day 10	35.7	38.8	30.5	6.5	7.0	5.4

Table 5. The pH of Fillet Coated and Non-Coated by Nano-Encapsulated, Up to 10 Days of Storage

Day of Storage	pH					
	Non-Coated			Coated		
	1	2	3	1	2	3
Day 0	5.5	5.2	6.0	5.6	5.6	5.7
Day 2	5.9	6.1	6.0	6.0	5.0	5.5
Day 4	6.2	6.5	6.7	5.9	5.8	5.8
Day 6	6.5	6.8	6.9	6.0	5.8	5.7
Day 8	7.0	6.5	7.5	5.5	5.7	5.9
Day 10	7.0	6.9	7.2	5.5	6.0	5.7

ing refrigerated for 6 days but then increased to a similar water content of 10 days of storage. This is probably caused by an increase in the amount of TPC during storage, from an average of 7.4 x 10^1 on day 0 to 4.3 x 10^4 on day 10 (Table 1). The samples are also nearly unwrapped. However, the water content of LSN samples decreased significantly. The average water content on day 0 (which was 76.2%) decreased to an average of 59.1% on day 8, and then 58.5% on day 10.

DISCUSSION

The comprehension of microbial development intricacies has unfolded, shedding light on the early stages characterized by the absence of cell division, followed by a phase of rapid expansion until cells attain their maximum size. Within this context, the potential of liquid smoke (LS) to prolong product shelf life by counteracting oxidative damage is underscored. This remarkable attribute is attributed to the combined effects of functional phenols and an abundance of organic acids inherent in LS, which effectively curtail and modulate microbial growth[25]. However, it's essential to note that the diverse outcomes observed are intricately tied to optimal storage conditions, particularly pH and temperature, exerting notable influence over microbial development[26]. The findings underscore the multifaceted nature of microbial interactions and the impact of preservation methods on ensuring the safety and shelf life of food products.

The observed effect is attributed to the presence of organic acids and phenolic compounds in LS, which, in combination with nanoparticle encapsulation, demonstrated efficacy in impeding the growth of *E. coli*. Notably, when comparing nano-encapsulation with microencapsulation LS, the former proves to be more potent, as indicated by the results presented by Dien et al.[9]. LS, recognized as a potent bactericide, exhibits the capability to halt the growth of *E. coli* and other pathogens effectively[27]. Building on prior research, fish products immersed in LS have consistently met Indonesian standards and demonstrated negativity for microbial presence. These findings underscore the robust antimicrobial properties of liquid smoke and its potential to serve as an effective safeguard against bacterial contamination in various fish products.

Studies have highlighted that the occurrence of Salmonella in food items is often linked to inadequate manufacturing hygiene, including factors such as cross-contamination[28,29]. Conversely, an investigation specific to the components of liquid smoke used in Katsuobushi revealed the absence of *Salmonella* sp. and *Staphylococcus aureus* germs, reinforcing the safety of liquid smoke in this context. Furthermore, liquid smoke emerges as a versatile antibacterial agent suitable for commercial applications where a smoky flavor is desired. Beyond its antimicrobial properties, liquid smoke offers additional benefits such as lower concentrations of PAH and improved product quality, including enhanced taste and flavor[30]. This underscores the potential of liquid smoke not only as a preservative but also as a flavor-enhancing agent with broader implications for the food industry.

Notably, this aligns with previous research by Dien et al., which utilized LS microencapsulation[9]. The antibacterial efficacy of LSN can be attributed to the prevalence of phenolic chemicals in liquid smoke. Phenolic chemicals, found abundantly in LS, have been observed to damage the cytoplasmic membrane of gram-negative bacteria. Their effectiveness is heightened during bacterial division when the phospholipid layer around the cell is thin[31]. For instance, skipjack fillets dipped in 0.8% LS exhibited a phenolic chemical concentration of 12.6 mg and 0.25 ppb benzo(a) pyrene[17]. A higher LS concentration, such as 2%, led to a phenol concentration of 24.21 mg/kg. The antibacterial mechanism of phenol involves disrupting bacterial cell structure, inhibiting cell wall production, and inducing cell wall lysis. Phenol denatures proteins,

Table 6. Water Content of Fillet Coated and Non-Coated by Nano-Encapsulated, Up to 10 Days of Storage

Day of Storage	Water Content					
	Non-Coated			Coated		
	1	2	3	1	2	3
Day 0	76.1	78.2	75.3	76.2	76.5	76.0
Day 2	74.2	75.4	75.5	75.1	74.8	74.5
Day 4	72.1	69.7	70.5	72.2	72.4	73.5
Day 6	60.5	60.7	65.2	65.2	66.0	68.3
Day 8	74.0	73.9	74.5	60.2	60.1	57.0
Day 10	75.0	76.2	75.8	57.5	60.0	58.0

induces apoptosis, and disrupts cytoplasmic integrity, leading to the escape of macronutrients and ions from bacterial cells, ultimately causing disintegration and lysis. The primary target of the antibacterial mechanism is the bacterial cell structure, impacting the cytoplasmic membrane, ion stability, and coagulation within the cell constituents[32].

Several parameters influence antibacterial activity, including concentration, antibacterial component content, extract diffusion power, and the type of suppressed bacteria. Encapsulated LS, akin to LS solution, demonstrates that higher LS concentrations result in larger inhibitory zone widths for *E. coli* and Salmonella[33]. This supports the rationale that increased concentration leads to the production of more antibacterial chemicals, and reducing particle size to the nanoscale enhances their penetration into bacterial cells[34], further fortifying the antibacterial effects of LSN.

The result showed that the effectiveness of LSN in preventing histamine production was higher than the results obtained from microencapsulation in previous work[9]. Until 10 days of storage, the quality of the fillets was still excellent, according to histamine content, because it was maintained lower than 10 mg/kg. According to European regulations, fisheries products may contain up to 40 mg/100 g of histamine[35]. During the process of preserving fresh fish, glutamate and histamine are generated by the enzymes histidine decarboxylase and L-histidine ammonia-lyase. Bacteria that produce histamine, such as *E. coli*, *Micrococcus luteus*, and *Pseudomonas* sp., cause the decarboxylase process[8].

The low pH in LSN-coated fillets is attributed to the presence of organic acids in the LS, and these organic acids also contribute to the preservation of fish and meat products. Notably, liquid smoke itself has a pH in the range of 2.8 to 3.1, qualifying it for use as a preservative. In a related study, when LS was diluted from 0.4 percent to 0.8 percent and applied to skipjack fillets, the resulting pH ranged from 4.8 to 5.5[17]. This inherent acidic nature of LS imparts a preservative impact, inhibiting the growth of pathogens and spoilage microbes. The acidic environment created by LS, with its organic acid content, serves as a deterrent to microbial proliferation, thereby enhancing the safety and extending the shelf life of coated fish fillets. This dual role of pH regulation and antimicrobial action further accentuates the potential of LS coatings in preserving the quality and safety of fish products during storage[36]. To sum up, nanoencapsulation can help prevent the growth of bacteria.

CONCLUSIONS

Total microbiological count (TPC), Salmonella, *E. coli*, antibacterial inhibitory, histamine content, pH, and moisture content are all significantly to highly significantly impacted by the LSN. The results showed that LSN with maltodextrin: sago flour: 1% LS ratio of 10: 1: 5 effectively inhibited the growth of *E. coli* and Salmonella and decreased the level of histamine in sashimi that was refrigerated for up to ten days. In tuna sashimi, LSN successfully inhibited the development of harmful germs and lowered the histamine content. As a result, it is unique to apply LSN to tuna loin sashimi.

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