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Research article

Preservation of meatballs with edible coating of chitosan dissolved in rice hull-based liquid smoke

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ABSTRACT

The purpose of this study was to determine the effectiveness of edible coatings of chitosan dissolved with liquid smoke in preserving meatballs. The liquid smoke was derived from rice hulls pyrolyzed at 340 °C. The edible coating was made by dissolving 0.5%, 1%, and 1.5% chitosan in 100 ml of liquid smoke at concentrations of 3% and 5%. Preservation was carried out by soaking the meatballs in the edible coating solution for 15 min and storing them at room temperature with observations every 6 h. Food resistance was examined using the Antibacterial Activity Test, Total Plate Count (TPC), and Total Volatile Base Nitrogen (TVB-N).

The results of the antibacterial activity test showed that chitosan-dissolved liquid smoke had inhibition zones ranging from 6.49–7.07 mm against *E. coli* and 6.52–7.26 mm against *Salmonella* bacteria. The use of 5% concentrated liquid smoke reduced the number of bacterial colonies, with TPC values not below the SNI threshold after 48-hour storage. This indicates that liquid smoke has potential as an antibacterial. The TVB-N value doubled after 24 h, but the meatballs still had good freshness. After 54 h of storage time, the TVB-N value in all treatments exceeded the SNI threshold of 0.254 mgN/100 g, and the meatballs were no longer suitable for consumption.

1. Introduction

Liquid smoke contains organic components, including phenols and acetic acid, which have antimicrobial and antioxidant properties. These components can inhibit the activity of spoilage microbes in food to extend the shelf life of food products (Faisal et al., 2018a,b, c). The phenol compounds in liquid smoke serve as an antibacterial, and a low pH can cause damage to bacterial cell walls. Therefore, liquid smoke can be used as a natural preservative. Studies have been carried out on the use of liquid smoke as a preservative for a variety of food materials, including sausage (Adzaly et al., 2016), meat (Lingbeck et al., 2014; Hanafiah et al., 2018), fishball (Faisal and Gani, 2018), salmon (Martinez et al., 2010), and tuna (Saloko et al., 2014).

Food preservation using antimicrobial substances as edible coatings can inhibit bacterial growth. To date, the compound used to dissolve chitosan is acetic acid combined with various natural ingredients including cassava starch (Araujo et al., 2018), tapioca (Vasconez et al., 2009; Pratama et al., 2019), gelatin (Kumar et al., 2018; Yi et al., 2018), spermidine and glycerol (Sabbah et al., 2019), and green tea extracts (Apriyanti et al., 2018). Chitosan dissolves well in acidic compounds but does not dissolve in the neutral pH range. At present, the compounds used to dissolve chitosan consist of organic acids (formic, acetic, lactic, citric, and succinic acids) and inorganic solvents (hydrochloric and nitric acids and phosphorus). The amine group (NH_2) in chitosan, when dissolved in an acid (a dilute acid such as liquid smoke), will protonate into a cationic amino group (NH_3^+), disrupt negatively bacterial cell membranes, and inhibit DNA replication (Atay, 2019). Other cheaper acid compounds can be used as an alternative to chitosan solvent, for example, liquid smoke, which has an acidic pH. Liquid smoke contains acetic acid and phenol compounds that have antibacterial and antioxidant properties (Faisal et al., 2016; Faisal et al., 2017). Therefore, liquid smoke was expected to potentially substitute the acetic acid that has always been used to dissolve chitosan. Yet, few studies on edible coatings using liquid smoke and chitosan for food preservation have been conducted.

Many foods sold in traditional and modern markets around the world are using formalin as a preservative agent. Formalin is a chemical compound made by chemical synthesis, which consists of formaldehyde, known to be a carcinogenic agent linked to cancer. Natural preservative agents as an alternative to substitute the synthetic preservatives are needed. Chitosan-combined liquid smoke as an edible coating may be a natural alternative for a preservative to maintain the quality of food

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products to be safe to consume. Edible coatings from chitosan and liquid smoke have been developed in the food industry, especially in processed meat products (Kanatt et al., 2008). Meatballs are processed meat products with complete nutrients, but their quality can decline due to chemical and microbiological processes. Meatballs can survive well and remain suitable for consumption for 12 h at room temperature. The high protein levels in meat easily undergo lipid oxidation, which causes spoilage due to pathogenic microorganisms.

Studies have been conducted on the use of liquid smoke from oil palm shells combined with chitosan as an edible coating to preserve beef (Hanafiah et al., 2018). Other studies have found that the addition of 3% liquid smoke from oil palm shells to beef retained the taste, aroma, and texture, remaining acceptable for consumers until the sixth day of storage (Faisal et al., 2018a,b, c), while preservation of tofu and meatballs with a combination of 1.5% liquid smoke and 2.5% chitosan extended their shelf life by three days (Purba et al., 2014). Liquid smoke can be produced from various biomass wastes, including rice hulls. So far, rice hulls have been used for preparation of silica nanoparticles (Ramazani and Mahyari, 2010), activated carbon (Mukoko, 2016) and biochar (Shukla et al., 2019). The combination of chitosan and liquid smoke from rice hulls can be an alternative for meatball preservation. Rice hulls contain cellulose and hemicellulose compounds, making it useful as a raw material for producing liquid smoke. The degrading process of the compound through pyrolysis produces acetic acid and phenols that have antibacterial and antioxidant properties. Liquid smoke from rice hulls contains phenol (0.43%) and acetic acid (15.27 ppm). A gas chromatography–mass spectrometry (GCMS) analysis identified more than 15 compounds, the most prevalent being acetic acid, phenol, and its derivates, and no carcinogenic compounds such as PAH had formed (Faisal, 2019). This research aimed to study the effectiveness of edible coatings from chitosan from rice hulls dissolved in liquid smoke as a natural preservative for meatballs.

2. Methodology

2.1. Preparation and application of edible coatings

The liquid smoke used in this study was produced from the pyrolysis of rice hulls at 340 °C using a pyrolysis reactor (stainless steel, 32 cm diameter × 50 cm height). Crude liquid smoke was purified by distillation at 190 °C. The procedures to prepare the liquid smoke followed previous research (Faisal et al., 2018a,b, c). GCMS was used to analyze the compounds. HPLC (Hitachi L-4200H) and a spectrophotometer (Agilent Cary 60 UV-VIS) were used to analyze the acetic acid and phenol concentrations, respectively. Chitosan from shrimp shells was purchased from the market (medical grade, CV. Chi Multiguna, the deacetylation degree = 94%). The edible coating was made by mixing 100 ml of 3% and 5% liquid smoke with chitosan at three concentrations—0.5%, 1%, and 1.5%—in Erlenmeyer flasks, stirred using a magnetic stirrer (IKA C-Mag HS 7) at a temperature of 50 °C to a homogeneous mixture (±90 min), as detailed in a previous study (Hanafiah et al., 2018). The meatballs were prepared according to a conventional formula: 500 g beef, 50 g ice-cubes, 150 g tapioca flour, 20 g garlic, 1 g salt, and 2 g pepper (purchased from local supermarket). The beef, ice cube, and salt were grounded using a food processor until mixed well and then tapioca flour, garlic, salt, and pepper were added, then formed into meatballs and cooked in boiling water. Finally, the prepared meatballs were soaked in the edible coating solutions for 15 min, then stored at room temperature and observed every 6 h.

2.2. Antibacterial activity test

The antibacterial activity test was carried out using the disk diffusion method (Kirby–Bauer test). A sterile inoculating loop was inserted into a test tube containing bacterial suspension and then applied to the nutrient agar (NA) medium. After the spread of bacteria dried, the paper disk that had been soaked in edible coating extract was drained and placed on the medium containing bacterial spread with a little pressure so that the paper disk stuck to the medium's surface. Further, it was incubated at 37 °C for 24 h. Antibacterial activity is said to be positive if an inhibition zone appears in the form of a clear zone around the paper disk. The Antibacterial Activity Test used *E. coli* ATCC 25992 and *Salmonella cholera suis* ATCC 335345 McFarland 0.5 (The initial concentration is 2 McFarland). The positive control used tetracycline antibiotics, while the negative control used distilled water. For statistical analysis, one-way ANOVA and the Least Significant Difference (LSD) tests were conducted with SPSS version 22 software.

2.3. Total Plate Count (TPC)

TPC value is one of the parameters to determine deterioration in the quality of a material. The number of bacterial colonies found in the meatballs determines their edibility. The number of microbes contained in the meatballs after soaking has to be in the safe zone for consumption, that is, 1×10^5 colonies/g, and the testing methods were based on SNI 02-2725-1992 (Standar Nasional Indonesia, 1992). All experiments and analyses have been done for three times. The materials used were nutrient agar (NA) media, buffered peptone water (BFW), distilled water, aluminum foil, and ethanol. Equipment used for microbiological testing were autoclaves, petri dishes, incubators, analytical scales, test tubes, drying ovens, drip pipettes, a spirit lamp, and Erlenmeyer flasks.

2.4. Total Volatile Base Nitrogen (TVB-N) test

TVB-N is a measurement method used to determine the freshness of meatballs based on the accumulation of basic compounds, such as ammonia, trimethylamine, and other volatile compounds that evaporate. Five g of meatballs were added to 15 ml of 7% trichloroacetic acid (TCA) solution, homogenized (±1 min), filtered, and placed in the outer chamber of the Conway dish. In the inner chamber of the Conway dish was one ml boric acid solution. One ml of K_2CO_3 was added to the outer chamber with the Conway dish almost closed. Then the dish was tightly sealed and shaken for 1 min. The sample was then incubated for 2 h at 35 °C. The sample was titrated with HCL 0.1N after incubation. The freshness of the meat was determined by its TVB-N levels, which reflect the quality of meat freshness. The lower the quality of the meatballs, the higher the TVB-N level will be. The maximum value of TVB-N that is still suitable for consumption is 0.20 mgN/100 gr (Pearson, 1968). The TVB-N test was carried out based on the SNI 2354.8-2009 (Standar Nasional Indonesia, 2009).

3. Results and discussion

3.1. Effects of liquid smoke concentration

3.1.1. Formation of inhibition zones on antibacterial activity

The antibacterial activity test of the edible coating was carried out for *Salmonella* and *E. coli* bacteria, which are Gram-negative, using the disk diffusion method (Kirby–Bauer test). An antibacterial activity test was performed to determine the coating's ability to inhibit bacterial growth.

As shown in Table 1, a comparison could be drawn between the concentration of liquid smoke and the inhibition zones formed for *E. coli* and *Salmonella* bacteria. On the control using distilled water, no inhibition zone (0 mm) was formed for either bacterium. The largest inhibition zone was found in the tetracycline control with values ranging from 21.43 mm to 23.38 mm Table 1 show that the diameter inhibitory zone ranging from 6.49 mm–7.75 mm. The antibacterial activity of the liquid smoke for Salmonella was bigger than those of *E. coli* bacteria. Liquid smoke with a concentration of 3% and 5% had different inhibition zones; the zones formed with the use of 5% concentration were larger than those of 3% as shown in Figure 1. This shows that the concentration of the liquid smoke influenced the antibacterial activity. The phenolic

compounds, carbonyl, and acetic acid in liquid smoke lead to a lower pH (acid) and destroy the cell membrane of bacteria. Chitosan has been found to possess antibacterial and antioxidative properties (Saloko et al., 2014; Atay, 2019). Based on the LSD test, the edible coating influenced the inhibition zones of *Salmonella* and *E. coli* bacteria. Previous studies found that the use of liquid smoke from palm shells pyrolyzed at 340 °C could inhibit *Streptococcus* bacteria by 6.6 mm (Faisal et al., 2017). In a study by Milly et al. (2005), the use of liquid smoke from cinnamon inhibited the growth of *Salmonella* at a concentration of 1.5%–9%.

3.1.2. Number of bacterial colonies on the TPC test

Based on Tables 2 and 3, the TPC values of the meatballs ranged from 2.80×10^4 to 3.89×10^5 CFU/g. In meatballs without the addition of liquid smoke and chitosan (control), following 12 h storage, the TPC values exceeded SNI standards limit. This indicates that the meatballs' quality deteriorated faster than that of the meatballs with liquid smoke and chitosan coating. During storage, meatballs without chitosan had a higher TPC value than those treated with chitosan 0.5%–1.5%. The liquid smoke concentration of 5% had a lower TPC value than that of 3%. Several studies have shown the effectiveness of antimicrobial properties in liquid smoke due to the presence of phenol, carbonyl, and acetic acid compounds (Saloko et al., 2014;Lingbeck et al., 2014).

3.1.3. Meatballs' freshness level on TVB-N testing

Figure 3 shows that the initial TVB-N values of the samples with liquid smoke 3%, 5%, and without liquid smoke and the chitosan (controls) are almost the same, ranging between 0.011 and 0.017 mgN/100 g. In Figures 2 and 3, it can be seen that the concentration of liquid smoke affected the value of TVB-N. The value of TVB-N with the use of liquid smoke 5% is lower than that of 3%. In the control sample, the TVB-N value is higher than those with the edible coating, and at the 18 h observation, the TVB-N value exceeded the standard limit of 0.212 mgN/100 g. TVB-N results in the samples at storage times of 0–24 h did not change significantly (Figures 2 and 3). After 24 h, however, the TVB-N values increased by up to 50%. This could be due to the increase in trimethylamine and dimethylamine compounds and ammonia (Jinadasa, 2014). Trimethylamine came from the decomposition of bacteria, while ammonia came from the degradation of amino acids that caused protein loss. The results of previous studies showed that the use of liquid smoke from durian skin could maintain the freshness of fish for up to 60 h with liquid smoke concentrations of 0.5%–3% (Faisal et al., 2019). The use of liquid smoke at a concentration of 3% as a preservative was able to maintain the freshness of meatballs up to 15 h of immersion, with a TVB-N value of 12.6 mgN/g (Faisal et al., 2018a,b, c).

3.2. Effect of increased ratio of liquid smoke to chitosan

3.2.1. Formation of inhibition zones on antibacterial activity

In Table 1, it can be seen that the strongest antibacterial activity was from the use of 5% liquid smoke, with inhibition zones of 7.75 mm for *Salmonella* bacteria and 7.07 mm for *E. coli* bacteria. At low chitosan concentration (0.5–1.5%), the diameters inhibitory was not significantly different. The results shows that the diameter inhibitory varying from 6.52 mm to 6.93 mm for Salmonella and from 6.49 to 6.91 mm for *E. coli*. However, as the concentration of chitosan decreased, the inhibition zone formed grew, indicating increasing antibacterial ability. Antibacterial activity in Gram-negative bacteria will increase along with decreasing chitosan concentrations, whereas in Gram-positive bacteria, the antibacterial activity will increase with increasing concentrations of chitosan. For Gram-negative bacteria, at chitosan concentration above 1%, the ability of chitosan antibiotics (antibacterial) decreased. Differences in structure between gram-negative and gram-positive bacteria cause differences in bacterial responses to chitosan (Damayanti et al., 2016). Besides, as the concentration of chitosan decreased, its viscosity decreased, allowing the chitosan diffuse to media and inhibit the growth of *E. coli* and Salmonella bacteria. Chitosan at a low concentration enters the Gram-negative bacterial cells through the spread, while in Gram-positive bacteria, a polymer membrane will be formed on the surface that inhibits nutrients from entering the bacterial cell (Zheng and Zhu, 2003).

3.2.2. Number of bacterial colonies in the TPC test

The addition of chitosan and liquid smoke at higher concentrations resulted in smaller TPC values. Tables 2 and 3 show that the higher the concentration of liquid smoke and chitosan, the lower the number of bacterial colonies. The number of bacteria increased along with longer storage times at room temperature. Meatballs with liquid smoke 3% (Table 2) combined with a chitosan concentration of 1.5% stayed fresh for 48 h, with a TPC value of 4.69×10^4 CFU g, whereas those without chitosan (0%) and with the addition of chitosan 0.5% and 1% returned TPC values exceeding the maximum limit, that is, 3.96×10^5, 3.39×10^5, and 3.13×10^5 CFU/g, respectively.

In combination with chitosan concentrations of 0.5%–1.5%, liquid smoke 5% (Table 3) had lower TPC values than those of liquid smoke 3%

Table 1. LSD test results of edible coating antibacterial activity.

Bacteria	Liquid smoke (%)	Chitosan (%)	Control (mm)	Aquadest (mm)	Diameter of Inhibitory Region (mm)
E. coli	3	0	21.43	0	6.90 ± 0.37^{Ca}
		0.5			6.91 ± 0.26^{Db}
		1			6.64 ± 0.20^{Bb}
		1.5			6.51 ± 0.04^{Ab}
	5	0	23.38	0	7.07 ± 0.42^{Cb}
		0.5			6.70 ± 0.29^{Da}
		1			6.52 ± 0.25^{Ba}
		1.5			6.49 ± 0.38^{Aa}
Salmonella	3	0	22.25	0	7.26 ± 0.51^{Da}
		0.5			6.93 ± 0.28^{Cb}
		1			6.85 ± 0.04^{Bb}
		1.5			6.80 ± 0.01^{Ab}
	5	0	23.18	0	7.75 ± 0.11^{Db}
		0.5			6.65 ± 0.13^{Ca}
		1			6.56 ± 0.10^{Ba}
		1.5			6.52 ± 0.07^{Aa}

Note: The combination of lowercase (liquid smoke concentration) and uppercase (chitosan concentration) shows the influence between two variables (significantly different: $\alpha < 0.05$).

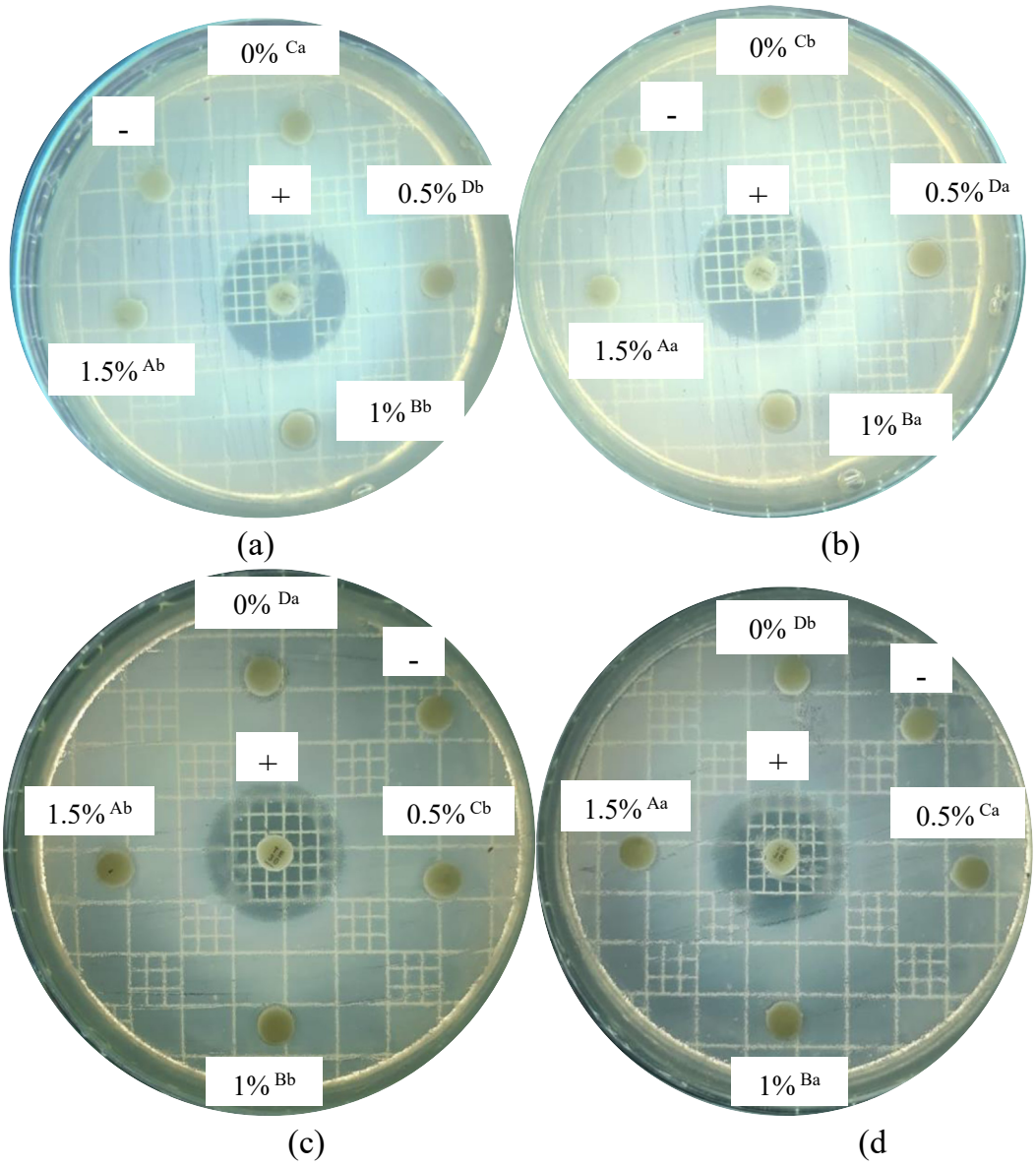

Figure 1. (a). *E. coli* with liquid smoke 3%, (b). *E. coli* with liquid smoke 5%, (c). Salmonella with liquid smoke 3%, and (d). Salmonella with liquid smoke 5%.

Table 2. TPC test results on the edible coating with liquid smoke 3%.

Observation time (Hours)	Total Colony (CFU/gram)				
	Control	Percentage Chitosan			
		0%	0.5%	1%	1.5%
6	4.21×10^4	3.36×10^4	3.32×10^4	3.20×10^4	2.90×10^4
12	2.16×10^5	3.45×10^4	3.38×10^4	3.25×10^4	3.2×10^4
18	5.74×10^5	3.37×10^4	3.65×10^4	3.46×10^4	3.35×10^4
24	3.34×10^6	4.59×10^4	4.52×10^4	4.36×10^4	4.18×10^4
30	3.68×10^6	4.71×10^4	4.43×10^4	4.29×10^4	4.12×10^4
36	4.75×10^6	4.80×10^4	4.63×10^4	4.38×10^4	4.27×10^4
42	2.76×10^7	4.82×10^4	4.78×10^4	4.66×10^4	4.37×10^4
48	4.34×10^7	3.96×10^5	3.39×10^5	3.13×10^5	4.69×10^4
54	4.69×10^7	3.42×10^5	3.89×10^5	3.22×10^5	3.15×10^5

(Table 2). This shows that the concentration of liquid smoke affected bacterial growth in the meatballs. At 54 h, the TPC value in meatballs soaked with chitosan concentration of 0%–1% had exceeded the standard limit, while with the use of chitosan 1.5%, the TPC value had not surpassed the threshold, that is, 4.91×10^4. Chitosan with a concentration of 1.5% and liquid smoke 5% has the greatest ability to reduce the number

Table 3. TPC test results at 5% liquid smoke concentration.

Observation time (Hours)	Total Colony (CFU/gram)			
	Percentage Chitosan			
	0%	0.5%	1%	1.5%
6	2.80×10^4	2.52×10^4	3.15×10^4	2.85×10^4
12	2.98×10^4	3.20×10^4	3.18×10^4	3.21×10^4
18	3.51×10^4	3.48×10^4	3.64×10^4	3.35×10^4
24	3.79×10^4	3.63×10^4	3.23×10^4	3.2×10^4
30	3.45×10^4	3.6×10^4	3.53×10^4	3.48×10^4
36	4.59×10^4	4.44×10^4	4.33×10^4	4.15×10^4
42	4.86×10^4	4.56×10^4	4.38×10^4	4.29×10^4
48	4.92×10^4	5.11×10^4	4.38×10^4	4.32×10^4
54	3.56×10^5	3.24×10^5	3.05×10^5	4.91×10^4

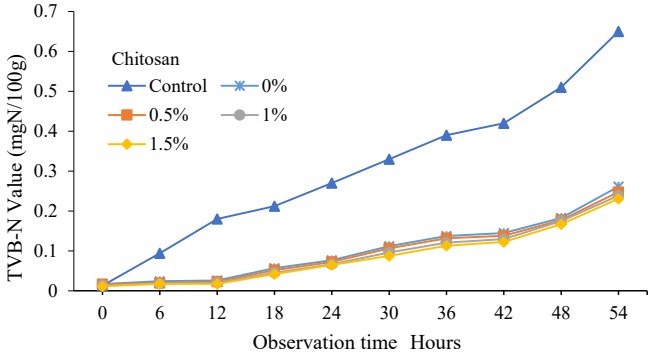

Figure 2. TVB-N values at various chitosan concentration and liquid smoke 3%.

of microbial colonies compared to that with concentrations of 0%–1% and liquid smoke 3%. This confirms that chitosan has antibacterial properties that inhibit microbial growth. Chitosan has good film-forming and antimicrobial properties against fungi and bacteria (Rabea et al., 2003). The chitosan layer becomes a barrier for oxygen, thus inhibiting the growth of aerobic bacteria (Devlieghere et al., 2004).

3.2.3. Freshness level of meatballs on TVB-N test

As shown in Figures 2 and 3, at 24 h storage, the TVB-N values doubled, reaching almost 0.05 mgN/100 g. At 54 h storage, the meatballs had lost their freshness as the TVB-N values exceeded the maximum SNI threshold. This proves that the concentrations of liquid smoke and chitosan influence each other in the meatballs' freshness. Based on Figures 2 and 3, meatballs coated with various concentrations of liquid smoke and chitosan and stored for 54 h lost their freshness. In general, the TVB-N value of meatballs stored for 48 h is around 0.1, indicating that the meatballs still had good freshness.

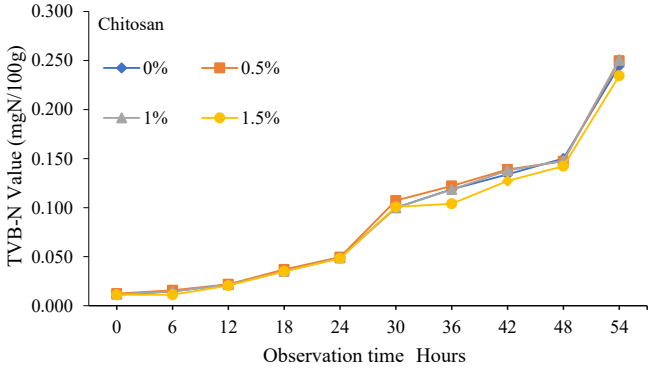

Figure 3. TVB-N values at various chitosan concentration and liquid smoke 5%.

The TVB-N value can be reduced by adding liquid smoke and chitosan. The addition of chitosan can reduce the ability of bacteria to perform oxidative deamination of non-protein nitrogen compounds (Retamal-Valdes et al., 2017). Edible coating with a combination of chitosan and liquid smoke had a good effect on fish preservation, reducing the TVB-N value by 50% compared to preservation with only liquid smoke (da Silva Santos et al., 2017). The results obtained were almost the same as those of Souza et al. (2010) using chitosan-coated salmon, with a reduction in the TVB-N value by 33%–50%.

4. Conclusions

Edible coating with chitosan from rice hulls dissolved in liquid smoke can be used as a preservative in meatballs. Liquid smoke with concentrations of 3% and 5% could replace acetic acid, which has generally been used as a chitosan solvent. The best conditions for preserving meatballs were achieved through the use of chitosan with a concentration of 1.5% and liquid smoke 5%, which was able to maintain the quality of the meatballs up to 54 h of observation time with a TPC value of 4.91×10^4 CFU/g. In general, the TVB-N value of meatballs stored for 48 h is around 0.1 mgN/100 g, indicating that the meatballs were still suitable for consumption. After 54 h, however, the meatballs were no longer suitable for consumption, as both TPC and TVB-N values exceeded edibility standards.

Declarations

Author contribution statement

Hera Desvita: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Muhammad Faisal: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mahidin, Suhendrayatna: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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