A combination of *Musa acuminata* peel and clove oil for preserving tuna fillet quality

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Abstract. Tuna (*Thunnus* sp.) is easily oxidized and can often have an unpleasant taste. As a result, post-harvest processing is needed to suppress the deterioration of fish quality. The edible coating can be used as an alternative to prevent tuna fillet deterioration. Therefore, the present study aimed to determine the resistance of tuna fillets coated with edible coating from *Musa acuminata* peel pectin and clove essential oil added as natural antibacterial ingredient. There were 9 preliminary treatments consisting of: P_1S_1 (0.3% pectin + 6% clove oil); P_1S_2 (0.3% pectin + 8% clove oil); P_1S_3 (0.3% pectin + 10% clove oil); P_2S_1 (pectin 0.4% + clove oil 6%); P_2S_2 (pectin 0.4% + clove oil 8%); P_2S_3 (pectin 0.4% + clove oil 10%); P_3S_1 (pectin 0.5% + clove oil 6%); P_3S_2 (pectin 0.5% + clove oil 8%) and P_3S_3 (pectin 0.5% + clove oil 10%), with three replications. Antibacterial activity against *Salmonella* sp. for 24 h was tested. As a result, three treatments (P_3S_1, P_3S_2, and P_3S_3) were chosen for the further step based on their antibacterial activity. For the next step, the study employed a completely randomized design (CRD) with four treatments, including a negative control, a positive control (natrium benzoate), P_3S_1, P_3S_2, and P_3S_3, with three replications for each treatment. The study revealed that the positive control group was superior in inhibiting bacterial development, while the edible coating treatments were the best treatments for the total volatile base nitrogen (TVBN) value. The sensory test for all samples after three days of storage showed that P_3S_3 was considered the best treatment based on the texture parameter, because the aroma and appearance did not show significant differences. The edible coating can compete with the commercial products for preserving tuna fillets.
Key Words: edible coating, microbial analysis, pectin, proximate analysis, total volatile base nitrogen.

Introduction. Tuna (*Thunnus* sp.) is a fish with high economic value, being the most sought fish on Indonesian seas (Hardoko et al 2022). It has high protein, vitamin, and mineral content and an excellent taste, making it a versatile fish for different markets (Nagarajarao 2016). However, fish is generally perishable, highly susceptible to oxidation, and often produces an unpleasant taste due to mishandling or storage (Sampels 2015). According to Mailoa et al (2019) and Yu et al (2020), the fish quality decline is caused by enzyme activity in the fish itself or microbes, so post-harvest processing is needed to suppress this problem.

Another problem is that synthetic preservatives are used to maintain fish products' quality and shelf life (Yu et al 2020). Antioxidants have been employed as preservatives to restrict or delay foods' chemical and biological quality deterioration by avoiding the autoxidation of colors, tastes, fats, and vitamins. Antimicrobials control microorganism-induced degradation (Baptista et al 2020). As a result of their impact on human health, food quality and safety are regarded as the primary concern of food systems (Bensid et al 2022). Therefore, alternative matters that can increase the shelf life of foodstuffs without causing adverse effects on users need to be applied (Senoaji et al 2017). This produces opportunities for the application of food preservation technologies, including packaging with edible coatings (Rojas-Graü et al 2007; Vásconez et al 2009).

Edible layer coating protects the product from damage and is not harmful if consumed with food products (Yu et al 2020). The edible coatings can be made from various ingredients, for example pectin (Maftoonazad & Ramaswamy 2019). Pectin is widely used as a functional component in food because it forms watery gels and stabilizes proteins (Fakhrizal et al 2015). According to Panahirad et al (2021), pectin is the base for edible coatings and current advancements in their utilization for preserving the quality of vegetables and fruits. The development of *Musa acuminata* peel pectin as a primary ingredient for edible films is one of the efforts to increase the utilization of *M. acuminata* peels (Kumar et al 2020; Zaini et al 2022). However, the preservation process using only edible coatings is considered less than optimal without combining essential oils. According to Durmuş et al (2020), citrus oil and olive oil have antibacterial properties that can be used as preservatives. Clove oil can inhibit the growth of gram-negative bacteria, such as *E. coli*, and gram-positive bacteria, such as *B. cereus,* which cause food poisoning (Warsito et al 2017). Previous studies evaluated combining edible coatings and other materials to extend the product's shelf life, for instance, the combination of edible coating from gelatin and essential oil from *Mentha pulegium* (Aitboulahsen et al 2018). Based on previous research, the novelty of this research is that clove oil is combined with *M. acuminata* peels pectin to preserve tuna fillet.

Material and Method

Research design and preliminary research. The research was carried out at the Food Technology Laboratory, Faculty of Agriculture and Animal Science, University of Muhammadiyah Malang, Indonesia, for three months, from 1 July to 30 September 2022. Preliminary research had 9 treatments: P_1S_1 (0.3% pectin + 6% clove oil); P_1S_2 (0.3% pectin + 8% clove oil); P_1S_3 (0.3% pectin + 10% clove oil); P_2S_1 (pectin 0.4% + clove oil 6%); P_2S_2 (pectin 0.4% + clove oil 8%); P_2S_3 (pectin 0.4% + clove oil 10%); P_3S_1 (pectin 0.5% + clove oil 6%); P_3S_2 (pectin 0.5% + clove oil 8%) and P_3S_3 (pectin 0.5% + clove oil 10%) with three replications. The treatments were tested for antibacterial activity against *Salmonella* sp., and observations were conducted within 24 hours. As a result, three treatments were chosen for the next step based on their antibacterial activity results.

Table 1 shows the combined treatments in the preliminary research on *Salmonella* sp. growth inhibition represented by the inhibition zone. Overall, only 3 combinations were effective: P_3S_1, P_3S_2, and P_3S_3. Meanwhile, the most negligible concentration of pectin revealed the lowest inhibition zone for *Salmonella* sp. after 24 h. Therefore, the 3 treatments were selected to be applied directly to *Tuna* sp. fillet. For the following step, the study used a completely randomized design (CRD) with 4 treatments consisting of negative control (without treatment), positive control (natrium benzoate), P_3S_1, P_3S_2, and P_3S_3, with 3 replications for each treatment.

Table 1

Analysis of the inhibition of *Salmonella* sp. by various treatments

Musa acuminata peel pectin dose (P)	*Clove oil (S)*	*Inhibitory zone*
P1 (0.3%)	S1 (6%)	6.3 mm
	S2 (8%)	6.8 mm
	S3 (10%)	7.6 mm
P2 (0.4%)	S1 (6%)	9.1 mm
	S2 (8%)	9.2 mm
	S3 (10%)	9.5 mm
P3 (0.5%)	S1 (6%)	10.2 mm
	S2 (8%)	12.2 mm
	S3 (10%)	14.5 mm

Pectin extraction and edible coating preparation. The method for the extraction and preparation of the coating was based on Aprianti (2019). *Musa acuminata* peels were collected from Landung Sari Market, Malang, East Java, Indonesia, and weighed 500 g. The peels were washed in running water and cut to a small size (1 cm^2) before being dried

using a cabinet dryer at 55°C for 48 h. Dried samples were crushed using a blender into powder and sifted through a number 80 mesh. 100 mL of distilled water containing 7% citric acid were added to 15 g of powder. The extraction took place using a water bath at 85°C for 2 h. The extract was filtered using filter paper in a hot state to produce a pectin filtrate solution. The solution was then added to 96% ethanol (1:1.5) and stirred until it settled before being deposited for 24 h. The precipitated pectin was filtered with filter paper and dried in an oven at 50°C for 3 h. Finally, the dry pectin powder obtained was stored for making edible coatings.

100 mL of distilled water was heated at 70°C. In the water, dried pectin powder was added based on each treatment, and stirred for 3 min with a magnetic stirrer at the same temperature. In the following step, 5 mL of 2% (v/v) glycerol were added and homogenized for 1 min before the solution was cooled at room temperature. According to the treatment, clove oil solution was added and mixed until homogeneous. The edible coating was applied to the tuna fillet by soaking it for 30 s twice. The fillet was then drained and aerated until the coating solution partially dried.

Fish sample preparation. *Tuna* sp. was provided by a fish seller in the Landung Sari Market and directly cleaned using running water. Afterward, the tuna was filled and cut into small pieces (3 cm^2) before being treated. The fish fillets were distributed and stored in 15 small containers after being immersed in the edible coating.

Parameter measurements. The data collection was conducted on the 3rd day of post-treatments. The following parameters were determined: moisture, protein, fat, total plate count (TPC), total volatile base nitrogen (TVBN) level, and organoleptic properties (Table 2). Following the treatment, the proximate composition of tuna fillets was determined. The moisture content of the samples was tested in triplicate using AOAC standard method 930.15 (AOAC 1995).

Moisture content (%) = (Initial weight - final weight)/(Sample weight x 1000) x 100

The protein content of tuna fillets was calculated using the Kjeldahl method provided by Purnama et al (2019) and Bremner (1960).

Total nitrogen (%) = [Vol HCl x N(0.2) x x14.008]/Sample weight x 100%

Fat analysis followed the method used by Bontjura et al (2019), in which the fillet samples were boiled for 1 h while maintaining the water volume constant. Afterward, the sample was allowed to cool and transferred into a 600 mL transparent bottle with a long narrow neck. As a result, the fat solution migrated on top of the broth, was collected, and centrifuged for 15 min. The fat was separated and collected for further analysis.

Fat content (%) = [(Flask weight + fat)-Flask weight]/Sample weight x 100%

TVBN was analyzed with the method used by Apriyantono et al (1989). 5 g of grounded sample were weighed. 15 mL of TCA 5% were added. Afterward, the mixture was separated through filtrating or centrifugation for 10 min before 5 mL of the mixture was placed into the Kjeldahl distillation tool with 5 mL of NaOH 2M. The distillation process employed 15 mL of HCl 0.01M to bind the distillate, reaching 30 mL of mixture. Finally, some drops of phenol were introduced into the distillate and titrated with NaOH 0.01M until the color changed to purplish red.

TVBN (mg/100 g) = [14 mg/mol x a (mL HCl) x b (normality HCl) x 300]/Sample weight

Moreover, the TPC calculation followed a mothod provided by Soepranianondo et al (2019) with light modifications. About 25 g of meat was homogenized with buffered peptone water (BPW) (serial dilutions) for 1 min to 2 min before the mixure were spreaded on the nutrient agar and incubated at 37°C for 18 hours to 24 hours.

Microbes = ∑microbes/(1/dilutions)

Finally, an organoleptic test with a score sheet based on the Indonesian National Standard (INS) was conducted. The test method is a sensory test using a scale numbered from 1 to 7. Organoleptic analysis or sensory test employs the human senses (25 panelists) to measure several parameters, such as aroma, texture, and appearance of tuna fish fillets after three-day treatments.

Data analysis. The present study used the SPSS program to conduct the ANOVA (Analysis of Variance) test and continued with Duncan's distance test to determine the differences among treatments.

Results

Proximate analysis. Table 2 shows the proximate composition of *Tuna* sp. fillets after each treatment. Overall, only protein level displayed insignificant differences among treatments ($p>0.05$), while there were significant differences for moisture and fat in three days after treatments. Based on our study, P_3S_2 and P_3S_3 (72.93±0.06% and 72.94±3.01%) displayed the most significant effects in terms of moisture and fat content compared to the control group. All treatments had a higher fat level than the control groups. The tuna fillet qualities could be preserved using *M. acuminata* peels edible coating.

Table 2

Proximate analysis of *Thunnus* sp. fillets each treatment

Treatment	*Proximate analysis (%)*					
	Moisture	*Reference*	*Crude protein*	*Reference*	*Crude fat*	*reference*
Control (-)	71.18±0.52[b]	73.14±0.07 (Hizbullah et al 2020)	24.38±1.06[a]	22.97±0.06 (Hizbullah et al 2020)	0.42±0.84[a]	0.08±0.01 (Hizbullah et al 2020)
Control (+)	67.88±0.88[b]		24.35±0.80[a]		0.70±1.89[a]	
P3S1	70.85±1.52[b]		23.46±0.36[a]		1.4±1.46[b]	
P3S2	72.93±0.06[a]		23.82±0.53[a]		1.37±1.55[b]	
P3S3	72.94±3.01[a]		23.41±0.32[a]		1.45±0.96[b]	

Note: different superscripts in the same column show significant differences ($p<0.05$).

Total plate count (TPC) analysis. The number of microorganisms (cfu mL^{-1}) in each treatment during 3 days of observations is presented in Table 3. Overall, there is a similar tendency for bacteria to grow significantly in the third day after preservation. Moreover, the control (+) showed the best results.

Table 3

Total plate count of *Thunnus* sp. fillets in each treatment

No	*Treatment*	*Observation (day)*			
		0 (cfu mL^{-1})	*1 (cfu mL^{-1})*	*2 (cfu mL^{-1})*	*3 (cfu mL^{-1})*
1	Control (-)	$2x10^4$	$4.5x10^5$	$6.2x10^5$	$6.8x10^6$
2	Control (+)	$1.6x10^4$	$4.7x10^4$	$8.1x10^4$	$1.51x10^5$
3	P3S1	$3.16x10^4$	$3.73x10^5$	$4.53x10^5$	$4.8x10^6$
4	P3S2	$2.44x10^4$	$2.47x10^5$	$4.27x10^5$	$4.04x10^6$
5	P3S3	$2.06x10^4$	$2.17x10^5$	$2.65x10^5$	$3.26x10^6$

On day 0, all samples had a similar number of microorganisms ranging from 1.5 to 3×10^4 cfu mL^{-1} based on the TPC analysis. The numbers increased considerably in control (-), P_3S_1, P_3S_2, and P_3S_3 after 1 day of observation, while the control (+) had a lower increase the following day. Meanwhile, there was a gradual increase in TPC in control (-), P_3S_1, and P_3S_3 by proximately 10%. Finally, on day 3, all microorganisms in all treatments

dramatically increased, with the control (+) having the lowest increase in TPC, reaching $1.51x10^5$ cfu mL^{-1}. The edible coating treatments could not compete with a commercial product in the case of TPC. However, they did produce better results than the control (-). Based on our findings, all treatments could inhibit bacteria growth during observations, although the commercial product showed better results.

Total volatile base nitrogen (TVBN) analysis. Figure 1 presents the TVB level for each treatment measured three days after the trial. The controls and treatment P_3S_1 had the higher number values of TVBN, while P3S3 had a significantly lower value than the 3 values mentioned before ($p<0.05$). Interestingly, P_3S_2 did not show a significant difference with the other treatments or with the control. Based on our study, the edible coating containing 0.5% pectin and 10% clove oil could preserve tuna fillets better for three days, referring to the low rate of TVB.

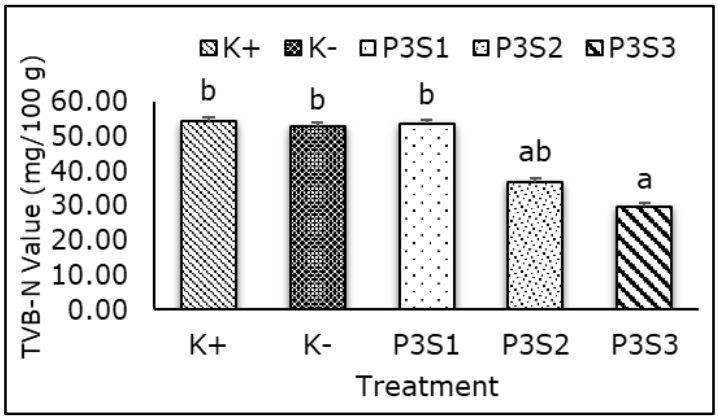

Figure 1. Total volatile base nitrogen (TVBN) of tuna (*Thunnus* sp.) from each treatment; different letters above bars show significant differences ($p<0.05$).

Organoleptic properties. Table 4 displays the sensory analysis of tuna fillets from five treatments with edible coating. Overall, the three treatments revealed good results, equal to or higher than the positive control.

Table 4

Tuna (*Thunnus* sp.) fillet aroma analysis

Day	*Aroma score*				
	K+	*K-*	*P3S1*	*P3S2*	*P3S3*
0	4	4	5	4	4
1	3	3	3	4	4
2	3	2	3	3	3
3	2	1	1	2	2
Score	*Indicator description*				
1	Sour, rancid, rotten, and very fishy				
2	Fishy				
3	A little fishy				
4	Neutral				
5	A little less sweet, fresh				
6	Sweet, fresh				
7	Sweet, very fresh				

The texture determined for each treatment is presented in Table 5. Overall, all treatments showed good results compared to the control groups. On day 0, all treatments shared the same rate of texture grade except P_3S_1, which had one point more. Moreover, there was a

moderate increase in P_3S_2 and P_3S_3 too with one more point from day 0 to day 1. At the end of the period, all treatments had a better texture than the control groups.

Table 5

Tuna (*Thunnus* sp.) fillet texture analysis

Day	*Texture score*				
	K+	*K-*	*P_3S_1*	*P_3S_2*	*P_3S_3*
0	5	5	6	5	5
1	5	4	6	6	6
2	5	4	5	5	5
3	3	3	4	4	4
Score	Indicator description				
1	Very soft				
2	Slightly soft				
3	Soft				
4	Neutral				
5	Slightly dense and elastic				
6	Solid and elastic				
7	Very dense and elastic				

Table 6 provides information on organoleptic analysis from all treatments after three days of observation. In general, the appearance of tuna fillets dropped in all treatments to over 50%. Moreover, the control group (+) had the lowest appearance score. In the initial observation, all samples had the same appearance score (5 points), excepting the sample from P_3S_1, which was identified to have an appearance rate one point higher. On day 1, there was a moderate decline by one point for K+, K-, P_3S_1, and P_3S_2 samples, but P_3S_3 remained the same.

Table 6

Appearance score of tuna (*Thunnus* sp.) fillets for three days of observations

Day	*Appearance score*				
	K+	*K-*	*P3S1*	*P3S2*	*P3S3*
0	5	5	6	5	5
1	4	4	5	4	5
2	3	2	4	3	4
3	1	2	3	2	2
Score	Indicator description				
1	The flesh is light brown, and very loose				
2	Light brown flesh, and loose				
3	The flesh is light brown, and somewhat loose				
4	Faint red flesh, less dense				
5	Pink flesh, less dense tissue				
6	Red meat, dense meat tissue				
7	Bright red meat, tight and dense meat tissue				

Discussion

Proximate analysis. A previous study reported the proximate composition of tuna fillets presented in Table 2 (Hizbullah et al 2020), indicating some variation from the one obtained in the present study, especially for the content of lipids, which was higher in the present study. The compositional variations were hypothesized to induce various sensory changes and influence bacterial development and the acceptability of preserved fish (Sallam 2007).

Total plate count (TPC) analysis. This study found that combining essential oils and pectin as edible coating treatments did not have the same effect as the commercial product (a synthetic chemical). However, they did have better results than the negative control for the three days of observations. The TPC values obtained are below the maximum acceptable limit of 5×10^5 cfu mL^{-1} in the first day (SNI 2009; Martoyo et al 2014). On the second day, the negative control exceeded the established threshold, whereas, on the third day, all treatments remained at a consistent level, except for the positive control, which remained below the threshold.

M. acuminata peels and clove essential oil enrichment in the edible coating solution at sufficient concentrations can inhibit microbial growth. Cinnamon oil on an alginate-calcium coating solution inhibited bacterial development on fresh northern snakehead (*Channa argus*) fillets during refrigeration storage. The total bacteria in the treated sample were 5.27 log^{10}CFU g^{-1} and 8.10 log^{10}CFU g^{-1} in control (Lu et al 2010). Bacterial activity of cod (*Gadus morhua*) fillets was considerably ($p<0.05$) inhibited by the addition of clove oil to a gelatin-chitosan film (Gómez-Estaca et al 2010).

Total volatile base nitrogen (TVBN). TVBN is one of the measurements of fish quality decline (Rasulu et al 2020) and has a direct relationship with the proliferation of microorganisms generating volatile compounds from their metabolisms (Jasour et al 2015; Moosavi-Nasab et al 2021). According to Hamzeh & Rezaei (2012), bacterial spoilage and endogenous enzyme activity are likely responsible for the TVBN values rising as storage time increases. In addition, Fadıloğlu & Çoban (2018) suggested the following categories for describing the quality of fish products by TVBN values: "excellent quality", up to 25 mg 100 g^{-1}; "good quality", up to 30 mg 100 g^{-1}; "limit of acceptability", up to 35 mg 100 g^{-1};, and "spoiled", over 35 mg 100 g^{-1}. Based on those categories, P_3S_3 could preserve the tuna fillet in good quality for three days of storage at room temperature. Our result was also supported by the observations of Nie et al (2020), who determined that pectin coating infused with gallic acid delayed the TVBN value increase of Japanese sea bass (*Lateolabrax japonicus*) fillets for the 20 days of study. In another study, the TVBN level of catfish (*Pangasius* sp.) fillets stored for four to eight days with a coating enriched with essential oil from *Kaempferia rotunda* was considerably lower ($p<0.05$) than the value of the control samples (Utami et al 2014).

Organoleptic analysis. Sensory attributes intuitively reflect fillet quality during storage (Yu et al 2017). Based on aroma, texture, and appearance, the sensory scores of the tuna fillets dropped across all groups during storage. The study of sensory qualities for control and treatments showed that most coated samples might have "unacceptable" sensorial characteristics (score below 4) up to the end of the three days of storage, according to the limit of fish acceptance for human consuming (Fan et al 2008; Alsaggaf et al 2017). However, in the case of tune fillet texture observations, all treatments had the value included in the "acceptable" category, above the control values. The present study assumed that the panelists' evaluations of sensory qualities were highly connected to microbial counts and chemical compositions for coated and control samples. Several studies have concluded that fish loses much of its flavor and texture (Ameur et al 2022) and has a shorter shelf life because of the autolytic enzymatic spoilage (Tavares et al 2021) and the proliferation of microorganisms (Olatunde & Benjakul 2018) while it is being preserved. Pectin extracted from *M. acuminata* peels and clove essential oil could only preserve tuna fillets for two days at room temperature.

Conclusions. The study results concluded that the edible coating constructed with *M. acuminata* peels and clove essential oil positively affected tuna fillets compared to the negative control group, with similar results as commercial products, but only for some of the parameters determined. Further study might be needed to evaluate the optimum dosage of edible coating for preserving tuna fillets.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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