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Effect of Clove Oil Addition on Edible Coating and Film on Bacterial Activity in Skipjack Fish Fillets

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ABSTRACT Using bioactive compounds in environmentally friendly food packaging can extend the shelf life of food. This study aimed to determine how adding clove oil in manufacturing edible coatings and chitosan films affects bacterial activity in skipjack tuna fillets. Edible coatings and films made from 1% chitosan were prepared by adding clove oil with a concentration of 1; 2; 3; 4; and 5%. The edible solution was applied to the skipjack tuna fillets using two packaging techniques: edible coating by immersing the sample for two minutes and edible film by wrapping the sample. The microbial activity test was carried out using the total plate count (TPC) method a microbiological test to count the number of live cells or colony-forming units in food. The maximum number of bacterial colonies using the TPC test based on SNI 01-2332.3-2006 for fishery products is $1.0x10^5$ colonies/g. The number of bacterial colonies in the edible coating sample was $1.9x10^3$2.4x10^4 CFU/g, while the edible film sample was $1.8x10^4$-$2.4x10^5$ CFU/g. Adding clove oil affected the TPC value compared to the untreated chitosan edible. Using bioactive compounds in environmentally friendly food packaging can extend the shelf life of food. The number of edible coating colonies on adding 1% clove oil was $5.8x10^3$ CFU/g, lower than edible film $4.1x10^4$ CFU/g and complying with SNI. Applying an edible coating on skipjack tuna fillets with 1-5% clove oil is better than using edible film regarding the TPC value.

Keywords: Clove oil; edible coating/film; tuna; TPC

INTRODUCTION

Fish is a very nutritious food product and is in great demand. However, its durability is still the biggest challenge in storage (Prabhakar *et al*., 2020). Meat and fish products are easily damaged due to the entry of spoilage microorganisms in the processing, packaging, transportation and storage processes (Umaraw *et al*., 2020). Strategies to increase the shelf life of fresh fish with minimal impact on quality, especially texture, by extending shelf life compared to frozen preservation. This strategy relies on conventional pre-storage applications, namely using edible films and coatings or non-thermal preservatives, such as hyperbaric storage, to slow down microbial proliferation and reduce degradation reactions to increase the shelf life of fresh fish (Tsironi *et al*., 2020).

Edible films and coatings are innovative food preservation strategies that effectively protect foods' sensory and nutritional properties while enhancing their safety and extending shelf life by reducing or inhibiting microbial growth throughout the supply chain (Valdés *et al*., 2017; Jancikova *et al*., 2020). Edible coatings and films are defined as films of edible materials. Edible coatings are formed directly on the surface of food products by dipping (used on fish and fishery products), spraying, or fluidized bed. The edible film is produced as a solid sheet and placed on the surface of or between food products as a wrapping or separating layer. The application of edible coatings and films is selected according to the characteristics of the food product (Valdés *et al*., 2017; Tsironi *et al*., 2018; Yu *et al*., 2019; Tsironi *et al*., 2020). Films and coatings are bio-based materials. It is called biopolymer because its source is sustainable and environmentally friendly, such as residues from the food industry and components of proteins, lipids, and polysaccharides (starch, chitosan, and carrageenan) or their combinations (Valdés *et al*., 2017; Tsironi *et al*., 2018; Jancikova *et al*., 2020; Tsironi *et al*., 2020). In addition to its high biodegradability, this biopolymer can be eaten, washed, or crushed for further processing (Valdés *et al*., 2017; Ganiari *et al*., 2017). The most common natural polymers in fishery products are chitosan, alginate, whey protein, gelatin, or combinations (Valdés *et al*., 2017).

Chitosan, a cationic polysaccharide derived from the deacetylation of chitin, has been used for antimicrobial packaging films. The antibacterial activity of chitosan is related to the interaction of positively charged polysaccharides with biological membranes, which causes membrane disruption, permeabilization, and cell death (Abdulkareem *et al*., 2019). Chitosan coating is a non-toxic, attractive, and natural coating agent used in the food industry to inhibit the proliferation of microorganisms and lipid oxidation (Reesha *et al*., 2015; Cardoso *et al*., 2019). Additives in edible coatings further enhance their preservation activity by releasing antioxidants and antimicrobial substances (Zhang *et al*., 2020).

In addition to chitosan, natural product extracts and essential oils are reported to have antibacterial properties, so they are used as additives in manufacturing edible coatings and films. Adding essential oils into edible films can improve physical and mechanical properties. Essential oils extracted from plants such as clove, curcumin, lemongrass, ginger, thyme, cinnamon, turmeric, and garlic have been studied for their antimicrobial, antifungal, and antiviral activities (Zhang *et al*., 2019). More than 67 extracts of spice essential oils show in vitro antibacterial activity against pathogenic bacteria (Ahmed *et al*., 2019). Clove essential oil has antimicrobial, insecticidal, antifungal, and antioxidant activities (Mulla *et al*., 2017).

Clove oil is one of the typical Maluku essential oils but is rarely used as an antimicrobial. Clove oil is obtained from the extraction and steam distillation of clove flowers and leaves of Syzygium aromaticum, which contains various bioactive compounds (Hasheminejad *et al*., 2019).

Therefore, a study was conducted on the effect of adding clove oil on manufacturing edible coatings and chitosan-based films as antibacterial on skipjack tuna fillets. In this study, the TPC test was carried out on samples smeared with clove oil with various concentrations of 1; 2; 3; and 5%. As a comparison, edible coatings and films were made without adding clove oil and samples without treatment (control).

MATERIALS AND METHODS

Materials

The materials used in this research were clove oil, chitosan, acetic acid, glycerol, skipjack tuna, agar bacto, aqua dest, and filter paper. All the chemicals were purchased from Merck with pro-analysis grade. The equipment used in the research is a knife, a set of glassware (Pyrex), pH meter (Hanna), GC-MS (Shimadzu), analytical balance (Denver Instrument XP-300), hot plate (Cimarec 2), glass plate, incubator, digital colony bvb counter.

Methods

This study used clove oil as an additive in manufacturing edible coatings and chitosan-based films on skipjack tuna fillets. The clove oil used was tested for its chemical components using GC-MS. Edible coatings and films made are tested for microbial activity using the TPC method.

Analysis of the chemical components of clove oil

Clove oil used to manufacture edible coatings and films were analyzed for its components using GC-MS.

Manufacture of edible solution

100 mL edible solution was made from 1% chitosan solution (1 g, w/v) dissolved in 1% acetic acid using a hot plate and magnetic stirrer at 130 °C for 60 minutes until the solution was homogeneous and thick. Then glycerol with a concentration of 0.5% (v/v) was added gradually into the coating solution while continuously stirring for 10 minutes until homogeneous. The same method is used to make an edible solution with clove oil using a concentration variation of 1; 2; 3; and 5% before adding glycerol.

Application of edible coating on skipjack tuna fillet

Skipjack tuna is sliced with 3x5x1 cm, dipped in each edible solution for 2 minutes, and drained. Samples were stored for 24 hours at room temperature, then the TPC was determined.

Edible film application on skipjack fish fillet

Each edible solution was shaken with an incubator shaker for 15 minutes to remove dissolved gas and then poured on a glass plate. The film was allowed to dry for 5 hours in a sterile box at room temperature, then placed in an oven at 50 °C for 7 hours. The edible film obtained was applied to the skipjack tuna. Skipjack tuna, size 3x5x1 cm, wrapped in each edible film. The samples were stored for 24 hours at room temperature, and then the TPC was determined.

pH measurement

The pH value of the sample was measured using a pH meter. The pH meter was calibrated, rinsed with distilled water, and dried. Each sample was weighed as much as 10 g; then, each was mashed using a blender by adding 90 mL of distilled water until homogeneous. After that, the pH meter was dipped into the sample solution to determine the pH value.

Number of bacterial colonies in the TPC test

A total of 10 g of the sample was crushed, put into a test tube, then dissolved with 90 mL of 0.85% NaCl (physiological/graphic salt solution) to obtain a dilution of 10^{-1}. A total of 1 mL of the solution was pipetted, then put into a test tube containing 9 mL of 0.85% NaCl solution to obtain a 10^{-2} dilution. The dilution was carried out until 10^{-5} was obtained. It was adjusted to estimate the fish spoilage level at the observation time. From each dilution test tube, 1 mL was pipetted and then put into a sterilized petri dish. Each dilution was carried out in triples. The agar medium (sodium agar) was put into a 10 mL petri dish and shaken until the surface was evenly distributed (pour cup method), then cooled and hardened. The Petri dish, which already contained sodium agar and a sample, was put in an incubator for 48 hours at 30 °C in an inverted position; the cup's lid was placed at the bottom of the petri dish. After incubation, the colonies growing on the Petri dishes were counted with sufficient colonies from 30 to 300.

RESULTS AND DISCUSSION

Analysis of the chemical components of clove oil

The results of the GC-MS analysis showed that clove oil contains four components, namely 3-allyl guaiacol, eugenol, trans-β-caryophyllene, and α-Humulene compounds (Table 1).

Clove essential oil, containing several active components such as eugenol and caryophyllene with excellent antimicrobial and antioxidant properties, is a safe, efficient, nontoxic natural food preservative (Hasheminejad *et al*., 2019). Eugenol is an allylbenzene family of phenol derivative compounds used as a quality parameter of clove oil. The higher the eugenol content, the higher the quality of clove oil. The antimicrobial activity of essential oils generally depends on their chemical composition and the

Table 1. The results of the GC-MS analysis of clove oil.

Peak	Retention time (Minute)	% Area	MW	Compound
1	10.268	5.38	164	3-Allyl guaiacol
2	10.705	65.85	164	Eugenol
3	11.142	24.22	204	trans-β-caryophyllene
4	11.458	4.55	204	α-Humulene

quantities of each active component, a feature common to all-natural plant extracts. The main polyphenol constituents isolated from the clove are hydroxyphenyl propenes, phenolic acids, and flavonols. Of these, the major bioactive component is eugenol (Marchese *et al*., 2017).

The analysis and identification of GC-MS showed that eugenol was the main component of clove oil, as much as 65.85%. The antimicrobial activity of eugenol can be ascribed to the presence of a free hydroxyl group in the molecule (Nazzaro *et al*., 2013). The hydroxyl group on eugenol is thought to bind to proteins, preventing enzyme action. Therefore, the eugenol content in clove oil was used as an antimicrobial applied to edible coatings and films on skipjack fillets.

Analysis application of edible coating and film on skipjack tuna fillet

In general terms, fish products are susceptible to fast degradation mainly due to their high content of lipids, moisture losses, and deterioration of their muscles' sensory and chemical quality. As a result, coatings based on proteins, polysaccharides, and lipid materials have been recently applied as antimicrobial active packaging systems to extend the shelf-life of seafood by reducing pathogenic microorganisms at their surface (Alishahi & Aïder, 2012). This study used chitosan and clove oil to make an edible coating and film solution applied to skipjack tuna fillets.

The edible solution was applied to the skipjack tuna fillets with two packaging techniques: edible coating and edible film. Edible coatings and films are thin layers made from edible materials but differ in foodstuff application. The edible coating is applied directly to the surface of food ingredients, while the edible film is applied after being printed in sheet form. Edible solutions can be directly applied by immersion to coat fish fillets. As for the edible film, the edible solution is shaken with an incubator shaker and then printed on a glass plate. The solution was allowed to stand for 5 hours to dry, then dried in an oven at 50°C for 7 hours to form an edible sheet. The edible film obtained was applied to the fish fillet by wrapping it.

Edible coatings and films with biopolymers enriched with active compounds have increased the food industry's interest and technology for application to fish, meat, and their derivative products. Edible coatings and films prevent oxidation of lipids/proteins/pigments, off-odors, off-flavors, moisture and loss of color, penetration of oxygen into the food matrix, and transport of solutes out of the food, and, therefore, improve the preservation, quality, and sensory properties of the product. In addition, coatings and films add value to food products as they increase their shelf life by reducing/inhibiting the growth of spoilage and pathogenic microorganisms (Alishahi & Aïder, 2012; Umaraw *et al*., 2020).

Food products are usually coated by a dipping technique, in which a thin film is formed on the surface that acts as a semipermeable membrane to control moisture loss and gas transfer. The dipping process is generally concise; therefore, the evaporation from solvents in the coating and crosslinking solutions is neglected. The duration of the dipping and draining times differs from each study, but it is generally between the 30s to 5 min. The method's main advantage is its total coating, even around complex and rough surfaces (Andrade *et al*., 2012).

pH measurement

The pH of skipjack tuna fillet samples that were applied with an edible coating (EC) and edible film (EF) is presented in Table 2.

Table 2. pH edible coating and film test results.

Sample code	Sample code	pH
EC-Chitosan	EF-Chitosan	6.0
EC-1%	EF-1%	5.9
EC-2%	EF-2%	5.8
EC-3%	EF-3%	5.7
EC-5%	EF-5%	5.5
Control	Control	6.4

The pH value of samples using edible coating and film ranged between 5.5 and 6.0 was lower than the control, namely 6.2-6.4. Fish spoilage occurs more easily when pH> 6. Adding 1-5% clove oil to the sample reduces the pH value, where the pH decreases with increasing clove oil concentration so that it can slow down the process of fish spoilage by microbes. The pH value was lower in the sample that used edible coating and film than in the control after being stored for two days at room temperature.

Edible coatings and films avoid increasing pH by preventing protein and nucleotide degradation and releasing alkaline byproducts during the first days of storage (Mexis *et al*., 2009). The addition of clove oil resulted in a lower pH in the samples using edible coatings and films made from chitosan. Clove oil's antimicrobial activity controls samples' microbiological and oxidative processes. Skipjack tuna fillet was only coated with chitosan and showed a moderate value. Due to the activity of spoilage bacteria, the accumulation of alkaline compounds such as ammonia and trimethylamine promotes an increase in the pH values. A coating can protect the surface of the fillet from the spoiling effect of oxygen, which leads to a pH increase, probably due to essential amine production (Rico *et al*., 2020).

Number of bacterial colonies in the TPC test

The TPC method was used to determine the microbiological quality of the skipjack fillet after being given an edible coating and chitosan-based film with clove oil. The speed of microbiological damage to fishery products depends on the growth of existing microbes, especially spoilage bacteria. The working principle of TPC analysis is to calculate the number of bacterial colonies in the sample by dilution as needed and tripled.

This study also provided control, namely skipjack tuna fillet that was not treated with edible coating and film. As a comparison, edible coatings and films were made without the addition of clove oil. Each sample was stored for two days at room temperature, and then the TPC test was performed. The number of bacterial colonies from the TPC edible coating and film test is presented in Table 3.

The maximum number of bacterial colonies using the TPC test based on the quality standard of SNI 01-2332.3-2006 on fishery products is $1.0x10^5$ colonies/g. The

Table 3. Total plate count (TPC) edible coating and film test results.

Sample code	ALT (CFU/g)	Sample code	ALT (CFU/g)
EC-Chitosan	$1.9x10^3$	EF-Chitosan	$1.8x10^4$
EC-1%	$5.8x10^3$	EF-1%	$4.1x10^4$
EC-2%	$1.5x10^4$	EF-2%	$1.4x10^5$
EC-3%	$1.7x10^4$	EF-3%	$2.1x10^5$
EC-5%	$2.4x10^4$	EF-5%	$2.4x10^5$
Control	too many to count	Control	too many to count

test results showed that the value of TPC edible coating and film differed between those given clove oil and those not treated. Microbial growth is the primary cause of fish spoilage and its metabolism, resulting in amines, sulfides, alcohols, ketones, and organic acids with unpleasant and unacceptable off-flavors. The antimicrobial mechanism of chitosan is due to its positive charge, which may compete with Ca^{2+} ions for the negatively charged bacterial membrane.

Edible coating and films with clove oil did not significantly improve the antimicrobial activity compared with chitosan-only. Samples that only used chitosan-based edibles had the lowest TPC values ($1.9x10^3$-$1.8x10^4$). In this study, the addition of clove oil varied with a concentration of 1-5%. The higher the concentration of clove oil added, the higher the TPC value. Applying the edible coating on tuna fillet samples with clove oil was better than using edible film regarding the TPC value. Even in the edible film with clove oil at a concentration of 2-5%, it has passed the maximum threshold for the number of SNI bacterial colonies (1.4-$2.4x10^5$). The coating application on the sample is better than the film because it coats the sample more thoroughly by immersing the edible solution. In samples wrapped with film, it is possible to form spaces or gaps that cause microbiological activity more quickly than in samples coated with an edible coating.

There are some disadvantages of chitosan-essential oil composite film. Hydrophilic chitosan and hydrophobic essential oil tend to form microheterogeneity, disrupting the continuity of the chitosan matrix phase and resulting in lower physical and antimicrobial properties of the composite film. Clove essential oil has shown an excellent inhibitory effect against pathogenic and spoilage microorganisms (Prasetyaningrum *et al*., 2021). The antimicrobial activity of clove oil was maintained when incorporated into the edible film, but differences were found as a function of the biopolymer matrix used.

The polymer structure can affect the release of the active components. Clove oil molecules are only trapped between the polymer matrix (Prasetyaningrum *et al*., 2021). As a result, it reduces the release of essential oils, so the antimicrobial activity obtained is low. This effect can occur because chitosan is a highly reactive molecule forming ionic and hydrogen bonds, reducing the resulting film's solubility. The interaction of chitosan in the coating matrix and the film can interfere with releasing the active compounds in the essential oil, thereby reducing its effectiveness. Therefore, further research is needed on the interaction of matrix components (Gómez-Estaca, *et al*., 2009).

CONCLUSION AND RECOMMENDATION

Conclusion

The addition of 1-5% clove oil to edible coatings and films affected bacterial activity in skipjack tuna fillets. The number of bacterial colonies (TPC) on edible coating with the addition of 1% clove oil ($5.8x10^3$ CFU/g) lower than when using edible film ($4.1x10^4$ CFU/g) for two days of storage at room temperature. The number of bacterial colonies in edible coating samples ranged from $1.9x10^3$-$2.4x10^4$ CFU/g, while in edible film samples, it was $1.8x10^4$-$2.4x10^5$ CFU/g. The research results show that the two TPC values still meet the SNI quality standards: maximum $1.0x10^5$ colonies/g. Applying edible coating on tuna fillet samples with clove oil is better than using edible film regarding the TPC value. Thus, an edible coating made from chitosan with clove oil can be used as active packaging for fish products.

Recommendation

It is necessary to vary the concentration of clove oil and test the quality of the physical, chemical, and biological properties of edible coatings and films according to SNI.

AUTHORS' CONTRIBUTIONS

All authors have contributed to the final manuscript. The contributions of each author are as follows, IBD; compiled main conceptual ideas. FAS; compiled manuscripts, processed and analyzed data, and critically revised articles. RAL; conducted research and data collection. All authors discussed the results and contributed to the final manuscript.

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