Combination of Alginate and Ginger Oil as Edible Coating Formulation for Reducing Pathogenic Bacteria in *Litopenaeus vannamei*

Ikhlasul Amal[1], Ervia Yudiati[1]*, Sri Sedjati[1], Mostafa Imhmed Ighwerb[2]

[1]Department of Marine Science, Faculty of Fisheries and Marine Sciences, Diponegoro University
Jl. Jacub Rais, Tembalang Semarang, Central Java 50275, Indonesia
[2]Department of Fisheries and Marine Sciences, Asmarya Islamic University
PO Box 495-471, Zliten. Libya
Email: erviayudiati@lecturer.undip.ac.id

Abstract

Litopenaeus vannamei is a world trade commodity that has a high economic value but is easily degraded. To maintain the quality, post-harvest peeled shrimp needs to be coated. The research objective was to determine the effectiveness of adding edible coatings with a formulation of alginate and ginger oil combination as a natural preservative for shrimp. The research method used was laboratory experimental with a completely randomized design (CRD). The treatments were additional peeled shrimp with different concentrations of alginate, i.e. 0 g, 1.5 g, and 3.0 g with two repetitions. The addition of 100 ml aquadest, 4 g $CaCl_2$, 15 mL glycerol, and 1.5 mL of ginger oil was administered in every treatment. Total Plate Counts (TPC), Escherichia coli, and Vibrio cholerae were determined in initial condition, 3rd, and 6th days after the storage time in a 4°C refrigerator. The edible film test with 5 repetitions, including thickness, tensile strength, and break elongation. The results on TPC (5.00 x 10^5 CFU.g^{-1}) and TPC of V. cholerae (Negative.25 g^{-1}) show that all the treated shrimp meet the standard value (SNI 3457:2021) requirements. Most Probably Number of E. coli in alginate addition of 0 and 1.5 g i.e. <3 MPN.g^{-1} treatments, do not meet the requirements. The bacterial test and edible film mechanical properties showed that the addition of 3.0 g alginate was the most effective treatment compared to other treatments (α=0.05). A formulation of alginate and ginger oil combination is an effective edible coating for reducing the pathogenic bacteria of peeled shrimp L. vannamei.

Keywords: *Alginate, antimicrobial; Edible film, food safety, Ginger Oil, Shrimp*

Introduction

Litopenaeus vannamei is a world trade commodity due to its high economic value. High demand from consumers has resulted in an increase in the production *of L. vannamei* in the past few years (Novriadi *et al*., 2021). According to data from KKP (2022), Indonesia exports fishery with shrimp being the main commodity with a value of USD 2.16 billion which supports the Indonesian economy. Indonesia's target in exporting shrimp is America, Japan, and the European Union. These countries provide safety criteria for the imported food, which include being free and clean from contamination by pathogenic bacteria including *Vibrio cholerae* and *Escherichia coli*, not containing preservatives that can harm human health, as well as containing high protein (Asikin *et al*., 2014).

The presence of pathogenic bacteria in food is caused by several factors including contamination from the environment and handling of food processing using unhygienic tools that allow pathogenic bacteria such as *E. coli* bacteria to grow (Lina *et al*., 2019). Edible coatings are biopolymer products produced from natural ingredients that are safe to consume, non-toxic, and easily decomposed (Balti *et al*., 2020). The proper formulation of edible coating for *L. vannamei* preservation can extend the shelf life of the product. The technology has to be simple, easy to produce, and able to maintain the physical appearance of shrimp. Edible coatings that contain antibacterial and antioxidant activities have the potential to inhibit the growth of pathogenic bacteria, and maintain the quality of food products (Goma *et al*., 2018).

Alginate is a polysaccharide composed of uronic acids α-L-guluronic and β-D-mannuronic. Alginate is a negative polymer that has good solubility in water. Alginate is widely used in the food sector because it is safe for consumption, non-toxic, and easily decomposed in living bodies (Zhang *et al*., 2023). The use of alginate as an edible coating must have a good composition to create physical and mechanical properties so that it can be used as a food product (Albertos *et al*., 2019). Ginger contains antimicrobial substances that can be used as a natural preservative. Ginger inhibits the growth of pathogenic bacteria *Staphylococcus aureus, Bacillus*

*) Corresponding author

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cereus, Clostridium perfringens, Listeria monocytogenes, Escherichia coli, and *Salmonella sp.* (Akullo *et al*., 2022).

Currently, information and research regarding the formulation of the alginate and ginger essential oil as ingredients in the manufacture of *edible coatings* is still limited, so needs to be explored. To extend the shrimp shelf life, Farajzadeh *et al.* (2016) use of chitosan-gelatin as a preservative. However, by the 6th day of storage, the total plate count has exceeded the threshold. So, it is necessary to add essential oils as antibacterial agents to inhibit bacterial growth. Farajzadeh *et al*. (2016) research fact suggested that the main drawback of chitosan is its water vapor permeability, which limits its use as a standalone packaging material. Shrimp preservation using 1% sodium alginate has been performed to inhibit bacterial growth until the 5th day. Moreover, it is necessary to add antimicrobial compounds such as essential oils to inhibit the growth of pathogenic bacteria (Baek *et al*., 2021).

Innovation in edible coating components is still needed. The use of ginger essential oil which contains antibacterial activity and alginate as a biopolymer might be a good formulation for this purpose. Edible coating plays an important role in inhibiting the growth rate of pathogenic bacteria, extending the product's shelf life, and eliminating the fishy smell so that it attracts people to consume. The aim of this research is to identify the potential of alginate and ginger essential oil as an edible coating to inhibit the growth of pathogenic bacteria in peeled *L. vannamei* shrimp products and to identify the physical characteristics of the edible coating.

Materials and Methods

The experimental design used was a completely randomized design with control and two treatments. Each treatment was repeated twice (Rodríguez *et al*., 2006). Pathogenic bacteria tests performed were Total Plate Count, *Escherichia coli*, and *Vibrio cholerae*.

Production and application of edible coating

Production of the edible coating was basically modified research (Duong *et al*., 2023) by preparing 100 ml of aquadest, and heating it up with a hot plate for 25 mins at a temperature of 70ºC. After that, commercial alginate was added with a concentration of 0, 1.5, and 3.0 g, then homogenized with a magnetic stirrer. Four g of $CaCl_2$ and 15 mL glycerol were added and homogenized for 10 mins. Finally, ginger essential oil was added with a concentration of 1.5 mL and mixed. The solution was then cooled to 40°C and ready to be applied as an edible coating for peeled *L. vannamei*. The composition of the edible coating can be seen in Table 1.

L. vannamei peeled shrimp were dipped into the edible coating for 2 mins. Shrimp that have been coated with an edible coating were drained and wait to dry. To determine the antimicrobial activity, the pathogenic bacteria test was carried out before preservation, the third and sixth days after the preservation process in the 4^{o}C refrigerator (Kim *et al*., 2018).

Total Plate Count Test (TPC)

The TPC test was conducted according to SNI 01-2332-3-2006. Twenty-five grams of shrimp samples were added with 225 mL Butterfield's phosphate-buffered solution, homogenized, and diluted by serial concentrations (10^{-1} to 10^{-5}). One milliliter of each dilution is pipetted into a petri dish with agar (15 mL) and incubated at 35ºC for 50 h. Bacterial colony counts are calculated using a colony counter.

Escherichia coli bacteria test

E. coli bacteria test was carried out based on SNI 01-23321-2006 by weighing 25 g of peeled shrimps, which was put into a bottle filled with 225 mL of Butterfield's Phosphate Buffered. Homogenization was done with vortex for 1 min to obtain a serial dilution from 10^{-1} to 10^{-5}. This is followed by taking as much as 1 mL for each dilution, then putting it in a Durham tube containing lauryl tryptose broth. The tubes were incubated for 48 hours at 36˚C. Positive results were indicated by the formation of gas and turbidity in each tube (LTB) Lactose Broth in double and Lactose Broth in single concentration. *Escherichia coli* test was carried out by inoculating the bacteria with an ose needle from the LTB tube to the Durham tube which had been filled with the *Escherichia coli* colonies. Then the media was incubated at 46° C for 26 hrs. Positive bacteria are determined using the Most Probably Number.g^{-1} value.

Vibrio cholerae bacteria test

V. cholerae bacteria test was carried out based on SNI-01-23324-2006, by weighing 25 g peeled shrimp. After being crushed, 225 ml of Alkaline Peptone Water was added. Homogenization was done with vortex for 3 mins to obtain a serial dilution from 10^{-1} to 10^{-5}. After that, the media was incubated for 8 h at 37°C. Positive bacteria are indicated by a cloudy color on the tube. Alkaline Pepton Water was added into the cup that was filled with Thiosulfate Citrate Bile Salts Sucrose media.

Incubation was done for 24 h at 37ºC. The presence of *V. cholerae* bacteria is characterized by large colonies, smooth surfaces, rather flat, opaque centers, and bright, yellow edges.

Characterization of the edible film

Tests for the characteristics of the edible film include thickness, strength, and elongation at the time of breaking. The edible film was produced on a 20 x 10 cm plastic tray. Thickness measurement on edible film using a digital micrometer mitutoyo at 5 different places as repetitions. Tensile strength and break elongation were tested using Torsee's electronic system universal testing machine (Sinaga *et al*., 2013).

Shrimp physical appearance analysis

L. vannamei peeled shrimp's physical and color appearance were carried out at intervals of 1, 3, and 6 days of exposure. Peeled shrimp that had been coated with an edible coating with the addition of different alginate concentrations were stored in the refrigerator at 4°C, and stored in vacuum plastic boxes. Evaluation of the quality of the physical appearance of the shrimp was carried out by observing the intact color appearance of the shrimp (Liu *et al*., 2020).

Data analysis

Analysis of data characteristics of edible film was tabulated and analyzed descriptively. Data on the effect of using edible coating as preservatives to inhibit the growth of *Escherichia coli*, *Vibrio cholerae,* and TPC bacteria were analyzed using ANOVA analysis, using SPSS software. An independent T-test was carried out on the results of the edible film characteristics (thickness, tensile strength, and break elongation) to see the differences in the influence of alginate on the results obtained.

Results and Discussion

Analysis of Total Plate Count (TPC) bacteria

Based on the bacterial TPC test results, it was shown that the number of bacteria increased with storage time. Table 2. All treatments showed that the TPC value still met the requirements, but in the treatment without the addition of alginate, the increment was highest (5.00 x 10^3 CFU.g^{-1}) on the sixth day of storage. The lowest TPC value was obtained by adding 3.0 g of alginate (1.00 x 10^3 CFU.g^{-1}) on the sixth day of storage.

Microbial contamination analysis needs to be conducted to ensure the safety of shrimp, making it suitable for consumption. BSN SNI 3457:2021 regulates the allowable consumption limit for frozen raw peeled shrimp. The allowable limit for Total Plate Count (TPC) that can be consumed is 5.00 x 10^5 CFU.g^{-1}. Based on the testing conducted on peeled shrimp coated with an edible coating, all treatments remain suitable for consumption.

Table 1. Treatments of edible coating in peeled shrimp with the addition of alginate, ginger essential oils, $CaCl_2$, and glycerol

Treatment	Ginger essential oil (mL)	$CaCl_2$ (g)	Glycerol (mL)
Alginate 0 g	1.5	4	15
Alginate 1.5 g	1.5	4	15
Alginate 3.0 g	1.5	4	15

Table 2. Total plate count (TPC) of bacteria on different additions of alginate treatment in different storage times. Letters behind the results indicate significant differences (α=0.05)

Treatment	Storage time (Days)	TPC (x 10^3 CFU.g^{-1})	Categories*	Standard value (SNI 3457:2021)
Initial condition	0	0.11 ± 0.10	MR	5.00 x 10^5 CFU.g^{-1}
Alginate 0 g	3	1.45 ± 0.35 ^{a}	MR	
	6	5.00 ± 1.00 ^{d}	MR	
Alginate 1.5 g	3	0.31 ± 0.10 ^{b}	MR	
	6	2.85 ± 0.50 ^{e}	MR	
Alginate 3.0 g	3	0.23 ± 0.10 ^{c}	MR	
	6	1.00 ± 0.10 ^{f}	MR	

Denote: *MR (Meet the Requirements) based on BSN SNI 3457 (2021); Initial conditions did not carry out an Anova test

The growth of different bacteria in each treatment was influenced by the composition of the edible coating. Based on the results obtained, the addition of alginate concentration can form a better edible coating, to prevent direct microbial disturbance of shrimp that have been coated with *L. vannamei* peeled shrimp. These results are in accordance with research by Liu *et al*. (2016; 2020) who also used *L. vannamei* in their research. Alginate dissolves easily in water and can form a gel, so this will cover the peeled shrimp easily and thoroughly (Gu *et al*., 2023).

E. coli bacteria count was 3 MPN.g^{-1} on the sixth day of storage in 0 g alginate and the addition of 1.5 g alginate. The lowest *E. coli* bacteria value was <3 MPN.g^{-1} on the sixth day of storage with the addition of 3.0 g alginate treatment. The results of the ANOVA test on *E. coli* bacteria showed that there was no significant effect between each treatment on the growth of *E. coli*.

Based on BSN SNI 3457:2021, the maximum threshold value for *E. coli bacteria* for peeped shrimp is <3 MPN.g^{-1}. Results showed that there were differences in the number of *E. coli* bacteria among treatments. Different from other treatments, the addition of alginate at the highest concentration (3.0 g) still meets the requirements, even on the 6th day of storage. The role of *edible coating* in inhibiting bacterial growth is by covering the whole peeled shrimp, so that it is fully protected from bacterial activity (Balti *et al.,* 2020). The concentration of ginger oil is adequate as an antibacterial compound and inhibits bacterial growth optimally. Moreover, the addition of 3.0 g alginate to the edible coating is able for *E. coli* bacteria to grow, though the number is still below the threshold value set (Awanis and Mutmainnah, 2016)

The alginate composition used as an edible coating is relevant to the presence of pathogenic bacteria in *L. vannamei* peeled shrimp. Alginate has the ability to inhibit the growth of pathogenic bacteria (Yudiati *et al.,* 2020). Alginate hydrogel has potential as an antibacterial against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus* by forming an inhibition zone on the growth medium of the tested bacteria. Alginate also has antioxidant activity (Sayed *et al.*, 2023). Research by Yudiati *et al*. (2018) reported that alginate had an antioxidant activity of 17.48 % at a concentration of 50 ppm testing with the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method.

Analysis Vibrio cholerae

Based on the results of the *V. cholerae* test, the bacteria were absent along with the storage time. Table 4. All treatments showed that they still met the requirements for safe consumption of *L. vannamei* peeled shrimp products, which is negative per 25 g.

Table 3. Most Probable Number of *E. coli* on different additions of alginate treatment in different storage time.

Treatment	Storage time (Days)	*E. coli* (MPN.g^{-1})	Categories*	Standard value (SNI 3457:2021)
Initial condition	0	< 3	MR	
Alginate 0 g	3	< 3	MR	
	6	3	DMR	
Alginate 1.5 g	3	< 3	MR	< 3 MPN.g^{-1}
	6	3	DMR	
Alginate 3.0 g	3	< 3	MR	
	6	< 3	MR	

Denote: *MR (Meet the Requirements), DMR (Do Not Meet the Requirements) based on BSN SNI 3457 (2021)

Table 4. The results of *V. cholerae* on different additions of alginate treatment in different storage times

Treatment	Storage time (Days)	*V. cholerae* (CFU/ 25 g)	Categories*	Standard value (SNI 3457:2021)
Initial condition	0	-	MR	
Alginate 0 g	3	-	MR	
	6	-	MR	
Alginate 1.5 g	3	-	MR	Negative.25 g^{-1}
	6	-	MR	
Alginate 3.0 g	3	-	MR	
	6	-	MR	

Denote: *MR (Meet the Requirements) based on BSN SNI 3457 (2021)

Analysis Escherichia coli

The results of *Escherichia coli* bacteria calculations before and after coating on peeled *L. vannamei* shrimp are presented in Table 3. Based on the *E. coli* bacteria test results, it is shown that there is an increase in the bacterial count over the storage time. All treatments comply with the requirements on the third day of storage, but on the sixth day of storage, alginate concentrations of 0 g and 1.5 g, exceed the threshold values.

The function of edible coating as a packaging material is to block oxygen and water from adhering to food products, reduce moisture, and inhibit the oxidation process, thereby maintaining the quality of food products from pathogenic bacteria, and maintaining the physical shape of food products (Cen *et al*., 2021). The ability of pathogenic bacteria to develop in *L. vannamei* peeled shrimp was hampered by the presence of an edible coating layer, so the growth in the number of bacteria was still suitable for human consumption.

The addition of ginger oil in the edible coating formulation plays an important role in inhibiting pathogenic bacteria. Ginger oil acts as an antibacterial, a compound that can inhibit the growth of pathogenic bacteria (Malmiri *et al*., 2022). According to Muhialdin *et al*. (2020), ginger oil contains an active chemical compound, namely shogaol which has potency as an antimicrobial. The use of ginger oil as a preservative has begun to be used with the aim of replacing chemical preservatives that have the potency to harm human health (Zhang *et al*., 2023).

Characteristics of edible films

The results on the characteristics of edible film including thickness, tensile strength, and break elongation are presented in Table 5. Characteristic measurements were only carried out on alginate treatment with concentrations of 1.5 and 3.0 g. Based on the results, the tensile strength and break elongation with the addition of 3.0 g alginate treatment were better than 1.5 g alginate. The results of the thickness test indicated that the 1.5 g of alginate concentration was thicker than the 3.0 g of alginate. There was a significant effect on the tensile strength test and break elongation (α=0.05), but there was no significant effect on the thickness (P>0.05).

The thickness of the edible film in this study was determined after the drying process. The thickness can prevent the access of steam, gas, and other compounds and also maintain the physical shape of the product (Ismaya *et al.*, 2021). The thickness is generated from the constituent components. Moreover, the addition of alginate concentration affects the thickness of the edible film.

The higher of the tensile strength value indicates a good edible film. The compounds determine the tensile strength. The high polysaccharide content has a high tensile strength value so the tensile strength value is also high. Polysaccharides can form hydrogen bonds to create strong edible films (Cian *et al*., 2014). The elongation value of breaking is taken from the percentage of elongation when it is given a tensile force until the film breaks. Break elongation is an important mechanical property, which can protect packaged products from outside interference so that packaged products are suitable for human consumption. The break elongation value is determined by the glycerol component used. The greater the amount of glycerol added, the higher the break elongation (Setyaningrum *et al*., 2017).

Shrimp physical appearance

The results of observing the physical appearance in the preservation of *L. vannamei* peeled shrimp using edible coatings are presented in Figure 1. Based on the results, the physical appearance of the shrimp with the addition of 3.0 g was better than 0 g and 1.5 g alginate.

The presence of pathogenic bacteria determines the freshness of the shrimp. On the first day of storage, all the prawns were fresh, translucent, and slightly bright pink in colour. On the third day, the concentration of 0 g alginate treatment began to show black spots, different from the treatments with the addition of 1.5 and 3.0 g

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Table 5. The mechanical characterization of edible film different concentrations of alginate. Letters behind the results indicate a significant difference (P<0.05)

Treatment	Thickness (ụm)	Tensile strength ($N.nm^{-2}$)	Break elongation (%)
Alginate concentration of 1.5 g	916.8 ± 60.00 [a]	0.068 ± 0.007 [a]	1.153 ± 0.465 [a]
Alginate concentration 3.0 g	797 ± 88.55 [a]	0.361 ± 0.150 [b]	44.480 ± 24.689 [b]

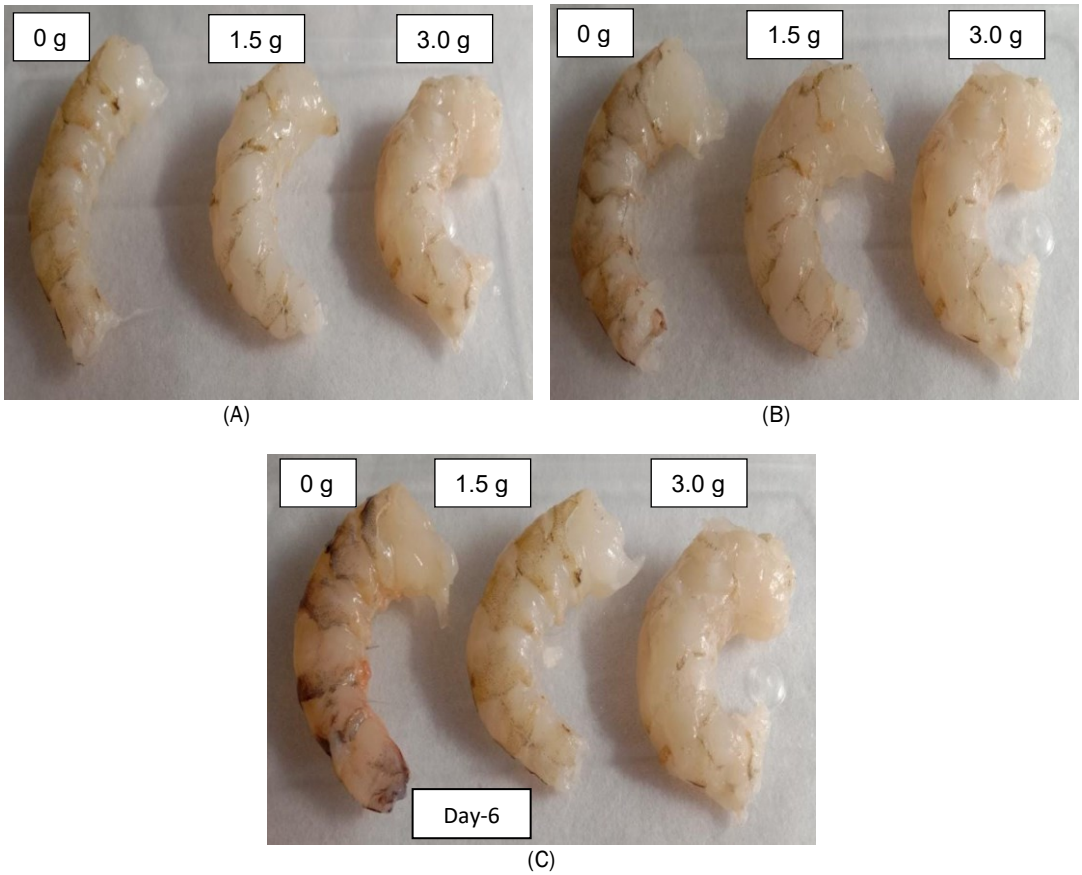

Figure 1. The appearance of shrimp during 6 days of storage was coated with edible coatings with different concentrations of alginate: A) Day 1, B) Day 3, C) Day 6

alginate. This indicates the formation of melanosis in shrimp due to the activity of polyphenol oxidases which oxidize phenol in natural amino acids into quinones. Quinones produce black spots that are exposed in peeled shrimp *L. vannamei* (Baek *et al*., 2021). Furthermore, melanosis in shrimp increased during the storage time of peeled shrimp. The treatment of edible coating with the addition of alginate is different from the treatment without the addition of alginate. Alginate managed to extend the shelf life of the product and maintain the quality of the shrimp with the presence of antioxidant and antibacterial activity so that it can inhibit the growth of pathogenic bacteria (Liu *et al.*, 2016).

Conclusion

The concentration of alginate in edible coating yields better results compared to edible coatings without alginate. Edible coating treatment formulation with 3.0 g alginate, 100 mL aquadest, 4 g $CaCl_2$, 15 mL glycerol, and 1.5 mL ginger oil was the most effective composition to inhibit TPC, *E. coli*, and *V. cholerae* bacteria with high-quality characters. The formulation of alginate and ginger oil combination is effective for reducing the pathogenic bacteria of peeled shrimp *Litopenaeus vannamei* shrimp until the sixth day of storage. Edible coating treatment formulation with 3.0 g alginate, 100 mL aquadest, 4 g $CaCl_2$, 15 mL glycerol, and 1.5 mL ginger oil was the most effective composition to inhibit TPC, *E. coli*, and *V. cholerae* bacteria with high-quality characters.

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