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DEVELOPMENT ANTIMICROBIAL PACKAGING MADE FROM CARRAGEENAN AND CHITOSAN TO EXTEND SAVE SHELFLIFE OF MEATBALLS MACKEREL

Adrian Zulfikar*, Iis Rostini, Evi Liviawaty, Rusky Intan Pratama

Faculty of Fisheries and Marine Science Students University of Padjadjaran
Jl. Raya Jatinangor KM.21 Sumedang, Indonesia

*E-mail address: adrianzulfikar1997@gmail.com

ABSTRACT

This research aimsed to determine the ratio of the combination of carrageenan and chitosan as an antimicrobial packaging to extend the shelf life of meatballs mackerel. This research was conducted in January and February 2019 in the Laboratory of Fisheries (PHP) FPIK Padjadjaran University in Indonesia, manufacture edible coating solution, Total Plate Count (TPC) testing, measuring pH and bacterial inhibition zone diameter. Research methods using experimental procedures with 4 treatment is treatment without edible coating, chitosan proportion of 25%: 75% carrageenan, chitosan proportion of 50%: 50% carrageenan and chitosan proportion of 75%: 25% carrageenan. Observations included TPC test to see microbial activity, pH and inhibition zone against *Pseudomonas aeruginosa*. The research results showed that the best edible coating is a treatment D (chitosan proportion of 75%: 25% carrageenan) with TPC value of 1.02 x 10^5 cfu / g with a shelf life of 11 days with a final pH value of 6.90 and a diameter of inhibition zone against *Pseudomonas aeruginosa* of 12.86 mm. The addition of antimicrobial edible coating of chitosan on carrageenan can extend the shelf life of meatballs mackerel in cold temperatures.

Keywords : **Edible Coating, Chitosan, Carrageenan, TPC, Zone of Inhibition**

1. INTRODUCTION

Active packaging is packaging that is designed to extend the shelf life, maintain or improve the condition of packaged food. The concept of this technology is based on, namely by adding certain components into a packaging system that can release or absorb certain substances from or into the packaged food or the surrounding environment (Widiastuti 2016)[1]. Active packaging is also known as interactive packaging, it is due to their active interaction between the packaging materials packaged food ingredients (Rooney 1995; Brody et al. 1997; Vermeiren et al. 1999). Antimicrobials and antimicrobial coating films are part of active packaging (Appendini & Hotchkiss 2002; Warsiki et al. 2010). According to Kerry et al. (2006), Pavlah and Orts (2009), the edible film is a thin layer made of edible ingredients, formed by wrapping the product or by placing the product among the components that function as a barrier to mass transfer (water vapor, gas and light) as well as to improve the handling of a food cover, while edible coating is a thin layer of coating material that is used as food. Is applied by wrapping, soaking or spraying in order to provide protection against mechanical damage (Baldwin et al., 2012).

Antimicrobial packaging is one form of active packaging (active packaging) which can increase the shelf life of food products and prevent, reduce or even kill pathogens in food packaging materials (Sarikus and Seydim 2006 in Yogeswara et al., 2015). Antimicrobial packaging can be classified into two types, that is packaging containing antimicrobial materials that can migrate to the surface of the food (contact with food) and an effective antimicrobial surface that inhibits the growth of microbes in food without any displacement (Widiastuti 2016). Some types of antimicrobial agents such as organic acids (propionic acid, benzoic acid, etc.), enzymes (lysozyme), protein (conalbumin) can be used.

Food products selected in the application of active packaging is the meatball mackerel, because the food product has a high water content and high protein content that causes fish balls have a short shelf life that is of around 12 hours to 1 day in the storage room temperature (Syamadi 2002), in addition to the fish balls product is susceptible to damage (Kurniawati 2008). Fish balls have a nutrient content and moisture content that is high thus affecting the storability of meatballs and fish balls can only survive one day at room temperature (Cahyono 2013). Nowadays, a lot of use of formaldehyde as a preservative for fish balls to prolong its shelf life. Preservatives are very dangerous for human health. Therefore, there is need for alternative to replace chemical preservatives by using natural ingredients that consist of carrageenan and agar plus chitosan as an antimicrobial compound. Use of Antimicrobials of natural ingredients can maintain product quality and extend the shelf life of processed food products.

Carrageenan is widely used in food products, because it serves as an emulsifier, the manufacture of the gel, stabilizers as well as materials that can increase the food viscosity. Saparinto (2011), proposed that seaweed extract, together with stabilizers, such as carrageenan can be used. Carrageenan will work to improve / stabilize the structure of the chitosan. In addition carrageenan can also increase the water-binding power (Keeton 2001), so that decay can be inhibited.

Chitosan was selected as an antimicrobial ingredient because it contains enzymes aminopolysacharida lysosim and groups that will inhibit bacterial growth and inhibition efficiency of chitosan against bacteria (Wardaniati and Setyaningsih 2009). According to Fernandez and Kim (2008), chitosan provide antibacterial effect against the bacteria E. coli, *Pseudomonas aeruginosa*, *S. aureus* and *Salmonella paratyphi*. The use of chitosan carrageenan 0.5% and 0.1% in the fish balls can inhibit the activity of bacteria or provide preservation for 3 weeks in cold storage that is equal to 2.1×10^4 colonies/plate and 8 weeks in freezing temperatures in the amount of 2.65×10^3 colonies/plate. Based on the description above then research was done that applied the edible coating on mackerel meatballs to extending the shelf life of the food.

2. MATERIALS AND METHODS

2.1 Time and Place

This research was carried out from January to February 2019 in the Laboratory of Fisheries Technology Faculty of Fisheries and Marine Sciences, University of Padjadjaran.

2.2 Data Collection Methods

The method used in this research is an experimental design to determine the shelf life of meatballs mackerel by using antimicrobial packaging. Antimicrobial packaging that was used to coat the meatballs mackerel were carrageenan and chitosan. This research used carrageenan concentration of 0.8% and 1.5% chitosan concentration. The proportion of the volume of chitosan and carrageenan are used in meatballs mackerel consisted of three replicates, including the following:

A: Control (without edible coating)
B: 25% chitosan: 75% carrageenan
C: 50% chitosan: 50% carrageenan
D: 75% chitosan: 25% carrageenan

This research procedure of two stages, where the first stage is making edible coating in accordance with the desired treatment, then the edible coating applied to fish balls. The second stage is to determine the inhibitory zone of edible coating to the tested bacteria, to determine the pH and TPC threshold of microbial growth after a predetermined storage period.

2.3 Research procedure

The procedure carried out refers to Ariandoko's (2015) research which includes several stages, namely the prepation of carrageenan edible coatings with the addition of chitosan, the manufacture of mackerel meatballs, the application of edible coatings on mackerel meatball products, determination of inhibition zones, pH measurement and Total Plate Count (TPC) testing.

1. **Manufacture of Edible coating from Carrageenan**

Aquades is heated with a hot plate to a temperature of ± 80ºC and controlled by using a thermometer. Carrageenan flour was added as much as 0.8% (b / v) and stirred for ± 3 minutes at ± 80ºC, then added 1% (b / v) glycerol was added until homogeneous ± 6 minutes at ± 80ºC.

2. **Manufacture of Edible coating from Chitosan**

1.5% (b / v) chitosan powder into a 100 ml measuring cup then added an acetic acid solution of 1% (v / v), glycerol 1% (v / v) to a volume of 100 ml, then stirred with a magnetic stirrer and heated with a hot plate stirer to a temperature of 60 ° C, stirred and heated to 80°C which is maintained for 5 minutes.

3. **Edible coating application on meatballs**

Edible coating solution from carrageenan and chitosan mixed with the following proportions namely 75% carrageenan: 25% chitosan, 50% carrageenan: 50% chitosan, 25% carrageenan: 75% chitosan and control (without edible coating) were used. The next process is coating the meatballs with edible coating according to the treatment and then stored at a temperature of 5ºC - 10ºC. The method used is the dyeing method for 1 minute.

2.4 Making of Fish Meatballs

The meatballs was made from mackerel meatballs. Procedure for making fish meatballs was based on SNI (2014). The formulation of ingredients for making mackerel meatballs is shown in Table 1.

Table 1. Fish Meatballs Formulation

Materials	Proportion
Mackerel Meat	700 g
Tapioca	15%
Onion	3%
Garlic	3%
Pepper	0,3%
Salt	3%
Sugar	2%
Ice Cubes	25%

2.5 Data Analysis

Data analysis was carried out in a descriptive comparative manner supported by the presented tables and images. In addition, the data that was obtained was compared with finding of previous research.

3. RESULTS

3.1 Total Plate Count (TPC)

Based on the analysis performed on TPC for 15 days, can be seen in Table 2, which is a total change in the microbial colonies in meatballs mackerel during cold temperature storage (5-10ºC).

Table 2. Microbes in The Mackerel Total Meatballs Based Treatment

Storage day	Treatment			
	A (Control)	B (25% Ch: 75% K)	C (50% Ch: 50% K)	D (75% Ch: 25% K)
1	3.44 x 10^3	1.45 x 10^3	1.28 x 10^3	1.17 x 10^3
3	1.71 x 10^4	1,97 x 10^3	1.68 x 10^3	1.39 x 10^3
5	1.24 x 10^5	1.65 x 10^4	1.71 x 10^4	1.36 x 10^4
7	4.45 x 10^5	8.64 x 10^4	7,86 x 10^4	7.73 x 10^4
9	**1.09 x 10^6**	9.91 x 10^4	9.05 x 10^4	8.95 x 10^4
10	1.45 x 10^7	1.25 x 10^5	9.95 x 10^4	9.82 x 10^4
11	-	3,18 x 10^5	1.24 x 10^5	1.02 x 10^5
12	-	**1.36 x 10^6**	4.36 x 10^5	1.06 x 10^5
13	-	1.81 x 10^7	1.40 x 10^6	1.16 x 10^5
14	-	-	**2,86 x 10^6**	**2,09 x 10^6**
15	-	-	2,22 x 10^7	2.18 x 10^7

Description : The numbers printed ditebal is the maximum number of bacteria that are still safe for food to be consumed by Connell (1990), namely 10^6 cfu / g.
- : is not done because previous observations have exceeded 10^6 colonies / g.
Ch : chitosan and K: Carrageenan

In general, the number of microbes for each treatment continued to increase during the storage period can be seen on the first day of observation that, the number of bacteria for each treatment ranged from 1.17 x 10^3 cfu / g to 3.44 x 10^3 cfu / g. A meatball treatment mackerel have the highest number of bacteriathat is 3.44 x 10^3 cfu / g. It shows that the edible coating layer carrageenan and chitosan can inhibit the growth of microbial spoilage. Edible coating has the benefit of optimizing the quality of the food since it is a product that protects the product from the influence of microorganisms, prevent water, oxygen and prevent the product from fast decay (Handoko et al., 2005). Meatballs mackerel that are not coated with the edible coating made from carrageenan and chitosan has a threshold number of microbes by Connell (1990) is on the 9th day with a total of microbes of 1.09 x 10^6 cfu / g. Edible coatings with the proportion of 25% chitosan and 75% carrageenan (treatment B) was able to extend the shelf life of meatballs mackerel until the $12t^h$ day with the number of microbes of 1.36 x 10^6 cfu / g, while the edible coating with a proportion of 50% and 50% chitosan carrageenan (treatment C) was able to extend the save meatballs mackerel until the 14th day with a total of microbes by 2.86 x 10^6 cfu / g. Mackerel coated meatballs by edible coating with a proportion of 25% and 75% chitosan carrageenan are at the limit of microbial reception on day 14 with a bacterial count of 2.09 x 10^6 cfu / g. Treatment D has a long shelf life compared to other treatments, because the proportion of chitosan 75% and 25% carrageenan can inhibit bacterial pertmbuhan. According to research by Warsiki et al., (2013) edible coating with the addition of carrageenan on the meatballs can extend the shelf life for up to 12 days at a storage temperature of 5° C, because this carrageenan has a selective nature of the exchange of carbon dioxide and oxygen. Chitosan discount antimicrobial properties as an enzyme called lysosim and aminopolysacharida group capable of inhibiting the growth of bacteria and proved that chitosan is able to protect the meatballs (Wulandari et al., 2015).

Types of antimicrobial agents are incorporated into the matrix of edible coating is chitosan. The advantage of active ingredient that is added to the edible coating in addition to extending the shelf life, barrier properties derived from layers of film that is supported by an active component of antimicrobials that can inhibit spoilage bacteria (Winarti et al., 2012). Overall, the value of total microbes on mackerel meatballs to increase every day observations. This, due to the microbes always reproduce itself in accordance with the phase of bacterial growth is the lag phase, exponential phase, stationary phase and death phase.

Based on the TPC test that was carried out, showing that the entire meatball mackerel given treatment carrageenan and chitosan edible coating to inhibit the growth of microbial spoilage, meaning that chitosan can work effectively as an antimicrobial. Edible coating is best by treatment D (the

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proportion of 75% chitosan and 25% carrageenan) compared with treatment C (the proportion of 50% chitosan and 50% carrageenan), as seen from Table 2 the number of microbial treatment D on the 11th day fewer than treatment C that is equal to 2.09 x 106 cfu / g.

Mackerel coated meatballs by edible coating treatment D, the optimum for growth inhibiting activity and microbial spoilage in meatballs mackerel stored at cool temperatures (5-10^oC) as well as more efficient in applications carrageenan are used.

3.2 pH Test

Observation pH value aims to determine the level of acidity or changes in acidity in meatballs mackerel by means of a pH meter. These observations were made for 15 days. The pH value is one of the intrinsic factors that affect microbial growth.The pH value of meatballs mackerel during cold temperature storage (5-10^oC) are presented in Table 3.

Table 3. The pH value of Meatballs mackerel During Storage

Storage day	Treatment			
	A (Control)	B(25%Ch: 75%K)	C(50%Ch: 50%K)	D(75%Ch: 25%K)
1	6,80	6.70	6.70	6.75
3	6.70	6,80	6.75	6,90
5	6,80	6.85	6,90	6.95
7	6.85	6,80	6.85	6.85
9	7.00	6.70	6,80	6.75
10	-	6,80	6.75	6.70
11	-	6.95	6.85	6,90
12	-	7.00	7.00	6.95
13	-	-	7.15	7.10
14	-	-	7.15	7,20
15	-	-	7,20	7,20

Based on observations, it can be seen that the pH value of mackerel meatballs for all treatments on the first day ranged from 6.70 to 6.80. The pH value increases and decreases from the beginning of the observation to the end of the observation. Observation on the first day of the pH value of mackerel meatballs without treatment had a greater value than other treatments, namely 6.80. These results indicate that the more the addition of chitosan proportions the more acidic the pH value.

The presence of an acid solution in chitosan solution can affect the pH of mackerel meatballs, the more addition of the solution usually the pH tends to fall. According to (Ibrahim et al., 2009), this is due to the increasing proportion of chitosan solution,the lower pH in the solution is due to the use of acetic acid as much as 1%.

The pH value decreased during observation on day 9 for treatment B, C and D with a pH value of 6.70, 6.80 and 6.75 respectively. Decreasing is pH caused by the presence of microbes in meatballs during the storage period, in addition to the presence of natural fermentative microbes from fish meat that convert glycogen reserves to lactic acid (Sutherland 1995). The increase in pH on observation of the 10th day for treatment B is equal to 6.80, while treatment C and D have increased pH on observations on day 11 with values of 6.85 and 6.90 respectively. The increase in pH is caused by the metabolism of proteolytic bacteria in fish meat. According to (Liviawaty and Afrianto 2010), proteolytic bacteria produce proteolytic enzymes that break down complex compounds into simple ones such as amino acids, ammonia (causing foul odors) and hydrogen sulfide.

Overall the pH values of mackerel meatballs decreased and increased. Decreasing the pH value of mackerel meatballs is due to the process of reforming carbohydrates to lactic acid (Afrianto et al. 2015). When the pH value decreases, it becomes difficult for microbes to grow and develop. An increase in pH is caused by microbial metabolic activity that produces ammonia. Microbial growth continues until it reaches the ideal pH for microbes to grow, ie at neutral pH because most bacteria will grow at neutral pH (Forrest et al., 1975). According to Rahayu (1992), decomposition of proteins into amino acids will form ammonia compounds so that the pH value will increase, because ammonia provides an alkaline reaction. The increased pH value indicates decay in mackerel meatballs, which is indicated by mucus on the surface of meatballs.

Edible coatings have an important role to protect products from microbes originating from the outside environment (Winarti et al. 2012). Addition of chitosan solution to edible coating can inhibit microbial growth or activity, due to the nature of chitosan as an antimicrobial. Blocked microbial activity affects changes in pH values in mackerel meatballs. The proportion of chitosan 75% and carrageenan 25% is an effective treatment, because it can inhibit the microbial activity of decay on mackerel meatballs until the 14^{th} day.

3.3 Antimicrobial Activity Test

Testing of microbial activity was carried out for the best treatment, namely treatment D (proportion of 75% chitosan and 25% carrageenan) and treatment A (without edible coating). The method is was by measuring the inhibitory zone of bacterial growth which aims to determine the effectiveness of the best concentration of edible coating in inhibiting decay bacteria in fishery products namely mackerel meatballs. The bacteria tested to determine the inhibitory zone was *Pseudomonas aeruginusa*, this is supported by the opinions of Liviawaty and Afrianto (2010) that the decomposing bacteria contained in fish meat are the genus Pseudomonas, Flavobakterium, and Achromobacter. The results of testing the inhibition zone are presented in Table 4.

Table 4. Inhibition Zone Diameter Carrageenan and Chitosan Edible Coating

Bakteri	Inhibition Zone Diameter (mm)	
	A (Control)	D (25% carrageenan: 75% chitosan)
Pseudomonas aeruginosa	6,46	13,29
	7,30	12,43
Average	6,88	12,86

Based on the results it can be seen that treatment D has the ability to inhibit the bacterium Pseudomonas aeruginosa with an average of 12.86 mm, while treatment A in inhibiting bacteria only achieved an average of 6.88 mm. *Pseudomonas aeruginosa* is a gram negative bacterium that has thin cell walls compared to gram-positive bacteria, so that the cell walls of gram-negative bacteria are easily damaged (Pelczar and Chan 1986). This facilitates chitosan solution to kill gram negative bacteria, by damaging the cell wall. According to Pelczar and Chan (1988), the factors that can affect the work of antimicrobial substances, namely the higher the concentration of antimicrobial substances, the higher the inhibitory power. The results of observing the inhibitory zone for treatment A and D can also be seen in figure 1.

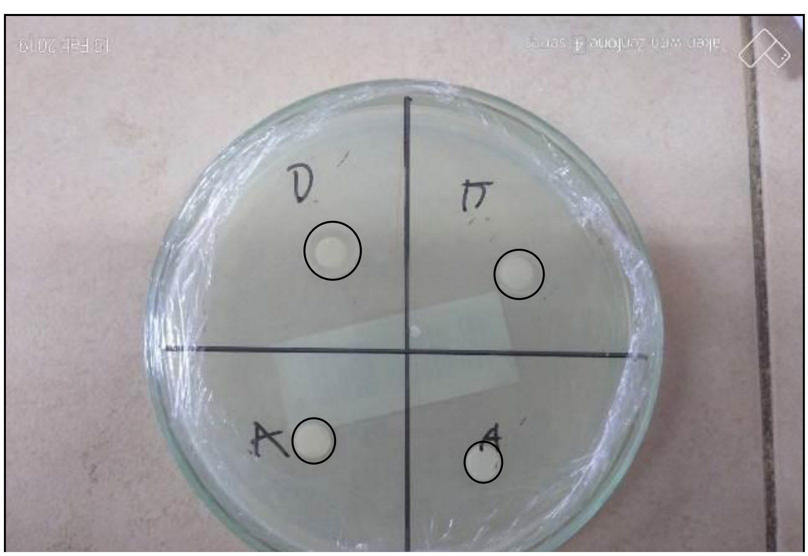

Figure 1. Zone of Inhibition of carrageenan and chitosan edible coating

The formation of inhibition zones, proves that the compound content in chitosan solution can inhibit bacterial growth. This is supported by the opinion of Sarjono *et al.,* (2008), that chitosan contains a free amino group that is positively charged so that it can bind to compounds that have a negative charge. The positive charge of $NH3^+$ on chitosan can interact with the negative charge on the surface of bacterial cells, namely teat acid in gram-positive and lipopolysaccharide bacteria in gram-negative bacteria. This interaction will interfere with the formation of peptidoglycan so that bacterial cells do not have a sheath that is strong and thus easy to experience lysis so that metabolic activity will be inhibited and eventually the bacteria experience death.

4. CONCLUSION

Based on research results that treatment D (chitosan proportion of 75% and 25% carrageenan) is the best edible coating, because it can extend the shelf life of meatballs mackerel up to 14 days in cold temperatures (5-10^{o}C). Treatment D managed to maintain the quality of meatballs mackerel and obtained the good values of TPC, pH and inhibition zone. TPC value obtained was equal to 2.09 x 10^6 cfu / g, a pH value of 7.20 and successfully inhibited bacteria *Pseudomonas aeruginosa* in diameter zone of inhibition produced by 12.86 mm.

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