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Effect of basil (*Ocimum* sp.) essential oil addition in chitosan edible film on the quality of red snapper (*Lutjanus campechanus*) in cold storage

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Abstract. *Setyaningsih R, Pangastuti A, Farahdilla N. 2023. Effect of basil (*Ocimum *sp.) essential oil addition in chitosan edible film on the quality of red snapper (*Lutjanus campechanus*) in cold storage. Asian J Trop Biotechnol 21: 13-20*. One of the marine fish favored by the community is the red snapper (*Lutjanus campechanus* Poey, 1860). However, the quality of fresh fish will rapidly deteriorate during the auctioning, sorting, and marketing processes. Basil essential oil contains antimicrobial compounds that may improve the efficacy of edible film in preserving fish quality. This study aims to determine the effectiveness of using edible chitosan film with glycerol plasticizer and basil essential oil to increase the shelf life of red snapper and inhibit the growth of microorganisms. This study used two treatments consisting of basil essential oil (0%, 1.5%, 3%, and 4.5%), with sample testing on days 0, 3, and 6, and storing the samples at 4°C. The results showed that fish flesh's pH and water content revealed a statistically significant (P= 0.05) difference between treatments. However, Total Volatile Base (TVB) values for optimum treatment of 3% and 4.5% did not significantly differ. The Total Plate Count (TPC) showed that red snapper coated with an edible film treatment of 3% was the most optimal for inhibiting microbial growth. The panelists also preferred red snapper fillets with an edible film containing 3% basil essential oil because it increases the fillets' shelf life compared to other treatments.

Keywords: Basil essential oil, edible film, *Lutjanus campechanus,* red snapper, shelf life

INTRODUCTION

Red snapper (*Lutjanus campechanus* Poey, 1860) is a high-economic-value marine fish popular for public consumption and export commodities. Red snapper is highly nutritious and can improve health (Ihsan et al. 2019). Increasing market demand for fish, especially red snapper, needs to be followed by maintaining the quality of the fish. During the auctioning, sorting, and marketing process, the quality of fresh fish will quickly decline; one of the efforts to prevent the decline in fish quality is cold storage. Fish storage at cold temperatures can slow the growth of bacteria and affect the parameters of fish freshness. Unfortunately, preserving fresh fish in supermarkets and traditional markets is usually unhygienic, namely, only using ice placed in boxes or tables to attract consumers. This certainly allows contamination by microorganisms and accelerates fish spoilage (Syafitri et al. 2016).

Fish stored at cold temperatures have a longer shelf life than those stored at room temperature or without preservatives (Kresnasari 2021). However, to improve fish quality maintenance further, it is necessary to use effective packaging and preservative technologies. Therefore, as a substitute for plastic packaging, the edible film that can coat food products is better at maintaining humidity and oxygen levels and is antimicrobial. The edible film is also safe for consumption to reduce environmental pollution. In addition, natural preservatives can be added to increase the antimicrobials by edible packaging (Afrianti 2010; Qoeroti et al. 2021). One of the plants that can be used as an edible natural preservative is basil (*Ocimum* sp.).

Edible films are generally made of hydrophilic substances that have good mechanical and barrier properties to resist the transfer of gases, aroma compounds, and fats. Substances that are generally used as basic ingredients for edible films are proteins and polysaccharides. Polysaccharides have hydrocolloid properties which means they have low permeability to water vapor (Pavlath and Orts 2009). One of the natural ingredients that contain polysaccharides is chitosan. In making edible films, materials are needed to reduce fragility, increase flexibility and film resistance. These materials are commonly called plasticizers. One of the ingredients that can be used as a plasticizer is glycerol. The addition of glycerol greatly determines the quality of the film, especially if it is used for low temperature storage. Glycerol has a good correlation when combined with starch and can improve the physical and chemical properties of tensile strength, elongation, and transparency of edible films (Alfatahillah et al. 2021).

Basil leaves contain flavonoids, alkaloids, polyphenols, tannins, saponins, steroids, and essential oils, which can eradicate fungi and germs and inhibit the growth of food pathogens (Souhoka et al. 2019). Therefore, Basil leaves can be used as an active ingredient in edible films as a coating for the red snapper to extend its shelf life. In addition, edible films of basil essential oil are known to have antimicrobial activity. Therefore, the addition of basil essential oil is considered to have the potential as an antimicrobial agent and can maintain the quality of fish flesh (Singh et al. 2020). Moreover, optimum microbial growth in tilapia fillets can be inhibited by packaging using an edible film of basil with a concentration of 1.5%; this packaging can increase the shelf life of fish until the ninth day (Agustin et al. 2020).

Concerning these problems, adding basil essential oil to chitosan edible film with glycerol plasticizer can potentially be used as packaging for red snapper, which is stored at cold temperatures before being sold. This study aims to determine the effect of the edible film added with basil essential oil on the quality of red snapper in cold storage. This treatment is expected to maintain the freshness of the fish in terms of its physical (appearance, texture of flesh, surface mucus, and odor), chemical (fish pH, water content, and total volatile base), microbiological, and sensory properties.

MATERIALS AND METHODS

Place and time of the study

The research was conducted from August to December 2021 at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, and the Integrated Laboratory of Universitas Sebelas Maret, Surakarta, Indonesia.

Materials and tools

This study used red snapper filets measuring 5 cm x 5 cm (purchased from Balekambang Fish Market, Surakarta), basil leaves (purchased from Hardjodaksino Market, Surakarta), food-grade chitosan (CV. Chimultiguna), water, distilled water, ice cubes, fish-balls (Teman Laut brand), perchloric acid 6%, phenolphthalein indicator, NaOH 20%, H_3BO_3 3%, hydrochloric acid (HCl), Butterfield's phosphate-buffered solution, Plate Count Agar (PCA), thioglycolate agar, organoleptic assessment blanks, glacial acetic acid 1%, glycerol, vacuum, and 2-methyl-3-furaanthiol.

The tools used in this study were an analytical balance, thermometer, pH meter, mortar and pestle, oven, desiccator, Conway dish, container, Erlenmeyer, petri dish, incubator, magnetic stirrer, hot plate, storage area, tape, stir bar, glass beaker, banana leaf, scissors, micrometer, ruler, and stationery.

Experiment design

This study used a Completely Randomized Design (CRD) with four treatments and three replications for each treatment. In addition, this study used two experimental factors, namely edible film packaging made from chitosan with glycerol plasticizer and basil essential oil at a concentration of 0%, 1.5%, 3%, and 4.5%, and the sampling time was on the 0^{th}, 3^{rd}, and 6^{th} day; data obtained from three replications. The fish was wrapped in edible film and stored at 4°C, then subjected to physical, chemical, and microbiological tests on the 0^{th}, 3^{rd}, and 6^{th} days. Furthermore, a completely randomized design was used based on the number of experimental factors.

Working method

*Manufacture of basil essential oil (*Ocimum *sp.)*

Basil leaves were dried using the dry wind method at room temperature for seven days. Next, steam distillation apparatus was set up. First, 10 liters of distilled water were put into the distillation tank. Next, dried basil leaves were put into the distillation tank and processed for 5 hours. Finally, the resulting essential oil is transferred to a sterile container and ready to use (Agustin et al. 2020).

Manufacture of the edible films

As much as 3 grams of chitosan was slowly dissolved with 100 mL of 1% glacial acetic acid. Next, glycerol was added to as much as 0.4 ml/gram of chitosan and basil essential oil with a concentration of 0%; 1.5%; 3%; and 4.5%. The homogeneous edible film solution lay on the banana leaf sheets measuring a 15 x 15 cm^2 with a 5-7 mL volume and let dry up at room temperature for 24 hours. (i) Thickness test, checking the thickness of the edible film was done using a tool, the Brookfield CT 3-4500, and the results were compared with Japanese Industrial Standards (JIS). (ii) Tensile strength, the Tensile Strength (TS) at the fracture point and the elongation percentage were measured using the Universal Testing Instrument (UTI). The water concentration in the edible film is first balanced with the moisture content of the environment, which has a relative humidity of 70% at 25°C. Sample weighing is done every 2 hours. When the weight of the edible film sheet is constant, the moisture content in the sample is in balance with the environment. Furthermore, the tensile strength of the edible film was analyzed using a Brookfield CT 3-4500. (iii) Water vapor permeability test, the edible film was cut into 2 cm by 2 cm squares. A container containing 15 mL of sterile aquadest was then prepared. The edible film was then dried at 180°C for 3 hours use oven. Finally, the dried edible film was added to the prepared container and stored at 25°C (Huri and Nisa 2014). (iv) Elongation, the elongation percentage was calculated by comparing the edible film length after breaking it with the edible film length before being pulled by the Brookfield CT 3-4500 tool (Huri and Nisa 2014).

Physical properties test of red snapper fish

The external appearance of the fish was observed and assessed based on the assessment blank provided. The physical assessment consisted of four parameters: appearance, flesh texture, surface mucus, and odor. The texture of fresh fish flesh is elastic and compact. The texture of the fish is compared to fish balls and fresh fish. The smell of fresh fish is very distinctive according to its type and is similar to that of seaweed. The odor assessment parameters are based on ISO 8586:2012 with four odor descriptions, such as a comparison with vinegar to indicate a sour odor or comparison with expired nuggets; smelling rancid indicates a rancid odor; comparison with fresh fish indicates the smell of seaweed, while comparison with 2-methyl-3-furaanthiol indicates a mushroom odor (SNI 2346-2015).

Chemical properties test of red snapper fish

pH. Fish flesh samples weighed as much as 10 grams. Next, the sample was dissolved in 20 mL of sterile distilled water. The dissolved sample was then homogenized using a mortar for 1 minute. Finally, the pH of fish flesh is measured using a pH meter (Bawinto et al. 2015).

Water content. A porcelain cup was dry-sterilized using an oven at 105°-110°C for 1 hour. When finished, the cup was cooled in a desiccator for 30 minutes. The weight of the cup is weighed as weight (A). A total of 2 grams of sample was transferred to a cup and then weighed (B). Next, the sample cup was dried in an oven at 105°-110°C for 24 hours. When finished, the sample cup was cooled in a desiccator for 30 minutes and then re-weighed as weight (C). The water content formula calculates the sample's water content (Bawinto et al. 2015).

$$\text{Water content} = \frac{(B-C)}{(B-A)} \text{ X } 100\%$$

Total Volatile Base (TVB). The initial procedure was carried out by weighing 10 grams of sample, which had been added to 90 mL of 6% perchloric acid and homogenized for 2 minutes. The homogenization results were then filtered to obtain a clear, colored filtrate. Next, 50 mL of the filtrate was placed in a distillation tube and drip-dried with the phenolphthalein indicator. Then, as much as 10 mL of 20% NaOH was added to the solution until the color changed. Erlenmeyer was prepared and filled with 100 mL of 3% H_3BO_3 and 5 drops of Tashiro indicator. Then steam distillation was carried out for 7 minutes until 200 mL of distillate was obtained. Finally, the distillate was titrated using a 0.0197 N HCl solution.

Microbiological test with Total Plate Count (TPC) (SNI 2332-3: 2015)

Sample preparation. The fish flesh weighed 2 grams and was transferred to a sterile container. Next, 27 mL of Butterfield's Phosphate Buffered solution was added to the sterile container containing fish flesh and homogenized for 2 minutes. Next, as much as 1 mL of the homogenate was transferred to a test tube, and 9 mL of Butterfield's Phosphate Buffered solution was added (as a 10^{-2} dilution). The dilution step was repeated until the dilution was $10^{-5;}$ for each dilution, the sample was homogenized by rotating it 25 times with rotary evaporator.

Total Plate Count (TPC) test. A total of 1 mL of homogenate from each dilution was transferred to sterile petri dishes in duplicate. Next, as much as 12-15 mL of PCA agar was added to the petri dish, which already contained the homogenate. It was then homogenized and incubated at 37°C for 24 hours. If the sample contains bacteria, the bacteria will grow on PCA media after incubation, forming round, milky white colonies. All colonies formed were read and recorded. Microorganism incubation results were calculated using the Total Plate Count (TPC) method and compared to the quality standards listed in SNI 2729: 2013, with a maximum fish flesh of 5.0 x 10^5 colonies/g.

Sensory test (SNI 2346-2015)

Hedonic test. The hedonic test assessed several samples with a score of 1-9 according to the level of preference for the blank provided. The higher the score, the more the panelists like the assessed object. The assessment of very strong dislike have 1 point , and the assessment of very strong liking at 9 point.

Scoring. Samples were assessed for appearance, smell, and texture with a score of 1-9 on the blank provided. The higher the score, the higher the specifications of the flesh according to common fresh fish. Score 9 is given for its bright, intact appearance, strong aroma suitable for fresh fish, and very compact texture. Scores of 7 are given for intact but less bright appearance, strong odor, and compact texture. A value of 5 is given for a dull and incomplete appearance, starting to smell sour, and a less compact texture.

Data analysis

Analysis of the data results using one-way ANOVA (One-Way ANOVA) with a 95% confidence level and continued with the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Edible film properties

Thickness is one of the parameters for assessing the quality of edible films. In this study, basil essential oil was added at 0%, 1.5%, 3%, and 4.5% concentrations. The thickness of the edible film obtained will affect other parameters such as tensile strength, water vapor transmission rate, and Elongation, whose quality will be compared with the Japan Industrial Standard (JIS). The thickness of all films ranged from 0.05 to 0.21 mm. Based on JIS, the films produced meet the standards because they have a thickness of no more than 0.25 mm (Nurindra et al. 2015).

The thickness of a film is affected by the area of the print and the volume of solution used. For example, a print area of 15 cm x 15 cm with a volume of 5-7 mL of edible film solution produces a thickness that meets JIS standards. Appropriate film thickness can reduce the risk of breaking when used as packaging. Film thickness is an important parameter because the thicker the film, the stiffer and physically harder the film will be, and the more quickly the film will break (Alfatahillah et al. 2021).

Tensile Strength (TS) is one of the feasibility parameters of edible films for food packaging. The results of the tensile strength test on edible films with the addition of basil essential oil at different concentrations are directly proportional to the thickness of the edible film. The thicker the edible film, the higher the tensile strength value produced. The higher tensile strength value becomes one of the quality parameters of edible films used in food packaging (Nguyen et al. 2020). The tensile strength value of the resulting edible film ranges from 3.65-8.52, and there are significant differences in each treatment of adding basil essential oil to make edible films (Table 1). This value meets the minimum tensile strength standard based on JIS of 0.392266 MPa. Therefore, the edible film with the addition of basil essential oil meets the standards and has good quality by observing the results of the film tensile strength test (Nurindra et al. 2015).

The third parameter of edible film quality is water vapor permeability, also known as water vapor transmission or WVP. The film's resistance to inhibiting the transmission of food moisture in and out is one of the reasons this film can inhibit bacterial decay and prevent bacterial contamination. The results of the WVP edible film test that will be used as packaging for fresh red snapper fillets are directly proportional to the film's increase in thickness and tensile strength (Table 1). The Water Vapor Transmission rate (WVP) in the edible film produced in this study follows previous studies results. The thicker the edible film, the more WVP there will be in proportion to the amount of chitosan and other additives (Pramono et al. 2018). The denser the film, the easier it is for WVP to be inhibited so that the interaction between food and moisture from outside the package can be inhibited to maintain food quality (Agustin et al. 2020).

Determination of the best edible film used as packaging can be observed from the lowest WVP value (Katili et al. 2013). The range of WVP edible film values is 0.2579-0.37535 g/hour m2, which complies with JIS standards. According to the JIS standard, the maximum WVP value of the film is 0.4167 g/hour m2 (Setijawati 2017). The edible film that meets the JIS standard is good for packaging, especially food packaging, because it can optimally inhibit water vapor transmission.

The elongation value of the edible film, or the percent elongation of the film, is one of the parameters of film quality in food packaging. Film flexibility is important when a film is used as food packaging. Films that break easily are unsuitable for packaging food because they cannot completely cover the surface of the food and allow bacterial contamination. Edible film elongation in this study was inversely proportional to the film's thickness, WVP, and tensile strength. The higher the concentration of essential oil added, the more the elongation value of the film should decrease. The elongation value decreases due to decreased bond distance between the molecules. However, the test using the Brookfield CT 3-4500 showed an increase in Elongation directly proportional to adding essential oils to manufacture edible films. Nevertheless, the film elongation in this study ranges from 15.43% to 17.57%. The elongation value is low because it is less than 50% (Suryaningrum et al. 2005).

Physical properties of red snapper fillets

Physical assessment of red snapper fillets was measured from the external appearance of the fish body, the brightness of the eyes, the elasticity of the fish flesh, and the condition of the fish flesh. Fish that are still fresh have different bodies, flesh, and other organ colors from fish that have started to decompose due to biochemical reactions during the capture and marketing process, causing physical changes in the fish. Fish start to rot, has a faded flesh color, and secrete mucus due to biochemical reactions of enzymes and microbial decomposition. The fresh fish eyes are bright, while those fish starting to rot are cloudy and coated with mucus. The flesh of fresh fish is chewy and still watery because its protein is good enough to bind to water. Damaged fish flesh is characterized by a loss of elasticity (Waluyo and Kusuma 2017). Fresh fish has chewy and wet flesh because it has not lost much fluid from the flesh, and there is no dull body surface mucus, which makes fish unattractive (Nurilmala et al. 2018).

Ten panelists assessed red snapper fillets, resulting in a different assessment each day of testing (Table 2). Overall, the parameters for assessing mucus, flesh, smell, and texture of red snapper fillets packaged using edible film show that the value of fish packaged with the edible film but without the addition of basil essential oil (0% concentration) is the lowest compared to the addition of basil essential oil. Furthermore, adding basil essential oil at a 4.5% concentration on day 6 obtained the highest score for the mucus parameter, with a score of 7.63. Adding basil essential oil at 3% concentration obtained the highest rating from panelists until day six compared to 0%, 1.5%, and 4.5% concentrations.

On the sixth day, it was observed that the panelists preferred the physical appearance of the fish based on the parameters of mucus, flesh, smell, and texture of the fillets packaged using edible film with the addition of basil essential oil at a 3% concentration. Edible coating as packaging influences the appearance of fish flesh. Packaging fish fillets using edible coatings with high concentrations of essential oils makes the appearance of the flesh duller compared to low concentrations, which are more stable in maintaining the brightness of the flesh (Azzahra et al. 2013).

The normal pH value of fresh fish is 7, or to be precise, 6.9-7.2, with a decrease ranging from pH 2-6.5 that occurs during the rigor mortis phase. Rotten fish has an alkaline or high pH between 8 and 10 due to protein breakdown into alkaline compounds in the fish's body. The decrease in pH occurs due to the formation of lactic acid from anaerobic glycolysis results after the fish dies or does not receive oxygen (Waluyo and Kusuma 2017; Yuniarti et al. 2021). The higher the concentration of essential oils added to the edible coating, the more stable the pH of the fish fillets. At the highest concentration, the pH of fish fillets experienced a lower increase compared to fish packed with edible coatings with lower concentrations of essential oils. This is because essential oils contain antimicrobial compounds, which cause protein decomposition in fish fillets to occur more slowly (Azzahra et al. 2013).

The pH of red snapper fillets that experienced the most significant decrease was found in fillets treated without edible film packaging and with edible films but without the addition of basil essential oil. Red snapper fillets can be consumed until the 6th day in 1.5%-4.5% edible film packaging. Compared to concentrations of 1.5%, 3%, and 4.5%, treatment at 0% inhibits pH changes, causing acid accumulation in red snapper fillets to increase significantly and the pH value to decrease (Figure 1).

Table 1. Physical properties of chitosan edible film with the addition of basil essential oil

Component	Edible film's physical properties				Japanese Industrial Standard (JIS)
	Basil ess. oil 0%	Basil ess. oil 1.5%	Basil ess. oil 3%	Basil ess. oil 4.5%	
Tensile strength (MPa)	3.65a ± 0.12	5.04b ± 0.06	7.09c ± 0.04	8.52d ± 0.12	TS > 0.392266
WVP (g/hour m2)	0.27a ± 0.02	0.28a ± 0.06	0.32a,b ± 0.04	0.37b ± 0.24	WVP < 0.4167 g/hour m2
Elongation (%)	15.43a ± 0.13	15.78a,b ± 0.15	16.07b ± 0.09	17.57c ± 0.15	10% < E < 50%

Note: ess: Essential; a,b,c: Similar letter notation in one line indicates no significant difference for each test parameter at Duncan's test level of 5%

Table 3. Total Plate Count (TPC) value of red snapper fillets packaged using chitosan edible film with the addition of basil essential oil

Storage time	Total Plate Count value (x105)				
	Control	Basil ess. oil 0%	Basil ess. oil 1.5%	Basil ess. oil 3%	Basil ess. oil 4.5%
0th Day	$2.00^{ay} \pm 0.00$	$1.93^{ax,y} \pm 0.06$	$1.93^{ax} \pm 0.06$	$1.93^{ax} \pm 0.06$	$1.9^{ax} \pm 0.10$
3rd Day	$3.20^{by} \pm 0.00$	$2.80^{bx,y} \pm 0.00$	$2.63^{bx} \pm 0.06$	$2.57^{bx} \pm 0.06$	$2.27^{bx} \pm 0.12$
6th Day	$5.63^{cy} \pm 3.78$	$3.30^{cx,y} \pm 0.00$	$3.03^{cx} \pm 0.06$	$2.80^{cx} \pm 0.10$	$2.53^{cx} \pm 0.06$

Note: ess: Essential; a,b,c: Similar letter notation in one column shows no significant difference for storage time at 5% Duncan test level; x,y: Notation of similar letters in one row indicates no significant difference for the treatment of different concentrations at Duncan's test level of 5%

Table 2. Physical value of red snapper fillet packaged in chitosan edible film with basil essential oil

Parameter	Treatment	0th day	3rd day	6th day
Outer mucus	Control	7.47	7.53	7.03
	0%	8.30	7.57	7.03
	1.5%	8.37	7.60	7.30
	3%	8.37	7.80	7.40
	4.5%	7.47	7.77	7.63
Flesh	Control	7.03	6.70	6.40
	0%	7.60	6.80	5.80
	1.5%	7.57	6.57	6.07
	3%	7.50	7.40	6.57
	4.5%	6.73	7.10	6.40
Odor	Control	7.53	6.73	5.17
	0%	7.60	6.67	3.77
	1.5%	7.43	7.07	5.10
	3%	7.70	7.43	5.53
	4.5%	7.47	7.30	5.47
Texture	Control	6.87	7.57	5.73
	0%	7.17	7.70	4.77
	1.5%	6.83	7.20	4.23
	3%	7.37	7.67	6.17
	4.5%	7.30	7.03	5.60

Note: Score 8.1-9: Clear mucus layer, transparent, shiny, bright, brilliant cutlet, the flesh tissue is very strong, the smell is fresh, and the texture is dense, compact, very elastic; Score 7.1-8: Clear mucus layer, transparent, quite bright, the cutlet is brilliant, strong flesh tissue, the smell of fresh fish, solid texture, compact, very elastic; Score 6.1-7: The mucus layer is starting to get a little cloudy, the cutlet is a little less bright, strong flesh tissue, the less specific smell of fresh fish, dense texture, compact, very elastic; Score 5.1-6: The mucus layer is getting cloudy, the cutlet is less bright, slightly less strong flesh tissue, neutral odor, slightly soft texture, a little less elastic; Score 3.1-5: The mucus has thickened and begun to change color, and the cutlet has faded, there is less strong flesh tissue, a slightly sour odor, a soft texture, and less elasticity

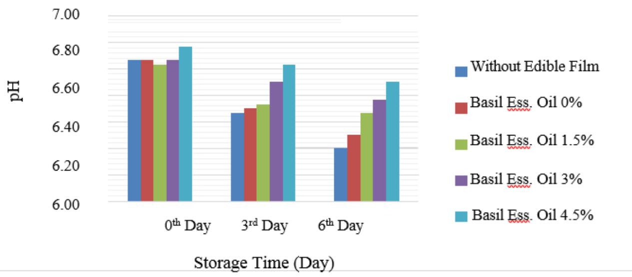

Figure 1. pH value of red snapper fillets packaged using edible chitosan film with the addition of basil essential oil

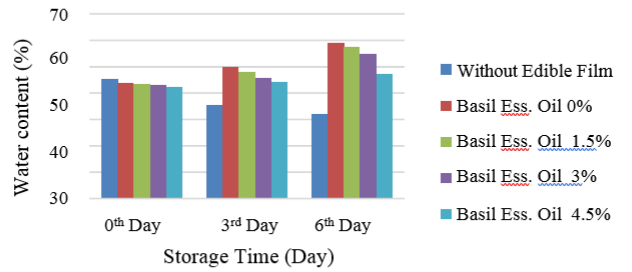

Figure 2. The water content of red snapper fillets packaged using edible chitosan film with the addition of basil essential oil

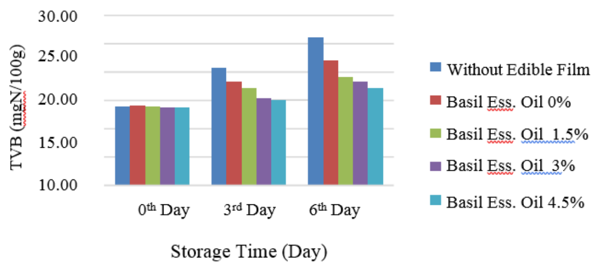

Figure 3. Total Volatile Base (TVB) of red snapper fillets coated with edible chitosan film with basil essential oil added

The water content is the second chemical parameter that determines the quality of fish fillets. The longer the fish fillets are stored in edible film packaging, the water content value will increase. That can happen because of chitosan's ability to inhibit water's evaporation in fish flesh. These results follow previous studies: fresh anchovies' water content with edible film increased within three days of storage (Mardyaningsih et al. 2014). However, fillets with edible film increased significantly in water content without adding basil essential oil, and at a concentration of 4.5%, the increase in water content occurred more slowly (Figure 2). The slow increase in water content in fillets with high concentrations of basil essential oil edible film indicates that the higher the concentration of essential oil added, the better the ability of the edible film to inhibit the rate of transmission of water vapor, which is one of the causes of rapid fish spoilage.

Another chemical parameter used as a reference for fish quality is the Total Volatile Base (TVB) level. Storage of fish immediately after being caught is known to optimally inhibit chemical reactions in dead fish flesh (Nurilmala et al. 2018). Red snapper fillets without edible film packaging experienced a higher TVB increase than fish fillets with edible film with added basil essential oil. The higher the concentration of essential oils, the more the increase in TVB of fish flesh was inhibited (Figure 3). The addition of basil essential oil has a significant effect proportional to the concentration. In this study essential oils with the highest concentration of 4.5% were more effective in maintaining the TVB value of fish flesh than at 0%, 1.5%, and 3% concentrations.

The parameter TVB values for fresh values are as follows: TVB values for very fresh fish are 10 mg N/100 g; Fresh fish has a TVB value of 10-20 mg/100 g. Fish with a TVB value of 20-30 mg N/100 g can be consumed, but the higher than those cannot be consumed. Fish without coatings and with the addition of low concentrations of essential oils can be consumed until the 6th day, while fish with higher coatings can be consumed until the 9th day (Tambunan and Chamidah 2021).

Microbiological properties of red snapper fillets

The third determination of fish freshness after physical and chemical tests of red snapper fillets are microbiological testing. The preservative used in this study was edible film packaging with basil essential oil, known to have antimicrobial properties. That formulation is expected to optimize the function of the edible film as a component to prevent more microbial contamination and inhibit bacterial growth. However, it is known that the control treatment is not maximally effective in inhibiting microbial growth, even with edible film packaging added with basil essential oil, which is only considered capable of inhibiting microbial growth. Moreover, the ANOVA statistical test showed a $P = 0.292$ or $P > 0.05$ value. Controlled treatment and packaging of red snapper fillets with edible film significantly impacted the 6-day shelf life. At the same time, the difference in concentration did not make a significant difference in the inhibition of bacterial growth (Table 3).

After the fish dies, it will undergo autolysis because enzymes in the body break down non-protein nitrogen (NPN) so that nitrogen levels in the form of amino acids, amines, and glucose in the fish's body increase and provide an optimum environment for bacterial growth. As a result, bacteria can produce fish decay marker compounds in trimethylamine, ammonia, amines, fatty acids, aldehydes, indol, sulfides, and mercaptans (Nurilmala et al. 2018).

According to Ababutain (2019), basil essential oil has antimicrobial activity against *Candida albicans, Escherichia coli,* and *Staphylococcus aureus* molds. *Ocimum basillicum* L. can be an inhibitor against *E. coli* and *S. aureus* bacteria which are resistant to erythromycin antibiotics. On the other hand, Agustin et al. (2020) stated that the higher the concentration of basil essential oil added to manufacture edible films, the more directly proportional it is to the inhibition of bacteria, which continues to increase. At a concentration of 1.5%, the antimicrobial activity of basil essential oil was in the moderate category. In addition, basil essential oil contains several terpene or antimicrobial compounds that can inhibit microbial growth in tilapia fillets.

Essential oils contain antibacterial compounds. Therefore, bacterial growth will be more inhibited if fish fillets coated with the edible film are stored at refrigerator or freezer temperatures. The results of the total bacteria test showed that the treatment of coating fish fillets with edible film with the addition of essential oils at chiller temperatures was close to the quality standards of SNI 7388:2009 (Mulyadi et al. 2016). Control treatment fish fillets stored for 6 days showed microbial growth of 5.63 colonies which exceeded the SNI quality standard with a maximum of 5 colonies at 10^5 dilution.

Sensory properties of red snapper fillets

Moreover, determining fishery product quality uses the human senses (organoleptic) as an assessment parameter. The fish flesh used as the test sample is in the form of fillets. One way to find out the freshness of fish flesh is by observing by slicing the fish from the tail. While the fish flesh from the tail is difficult to slice, indicating the fish belongs to the fresh fish category. Then, if the fish's flesh is bent and can immediately return to its original shape, it indicates that the fish is still fresh (Nurilmala et al. 2018). A decline in fish flesh quality occurs due to autolysis by the cathepsin enzyme, which decomposes protein into simpler compounds and is followed by a decrease in the fish flesh pH (Nurilmala et al. 2018).

Hedonic test

The affective test includes the hedonic test, which aims to measure the panelist's level of preference for the product. For example, the results of the assessment by ten panelists on red snapper fillets stored at cold temperatures using edible film with the addition of basil essential oil showed that on the 6th day, they had started to show signs of decay; thus, they received a score of 2-5 (Table 4).

Table 4. Hedonic values of red snapper fillet packaged with chitosan edible film and basil essential oil

Parameter	Treatment	0th Day	3rd Day	6th day
Control		6	6	4
	0%	6	6	5
Appearance	1.5%	7	6	5
	3%	5	5	5
	4.5%	6	6	5
Control		5	5	4
	0%	6	6	2
Aroma / odor	1.5%	6	6	4
	3%	6	6	5
	4.5%	5	5	4
Control		6	6	4
	0%	6	6	4
Texture	1.5%	6	6	5
	3%	6	6	5
	4.5%	6	6	5

Note: Value 7.1-9: Bright intact appearance, very strong aroma (typical of fresh fish), and very compact texture; 7-5.1: Less bright intact appearance, strong aroma (typical of fresh fish), and compact texture; 1-5: Appearance is dull and incomplete, starting to smell sour, and the texture is less compact

Table 5. Scoring values of red snapper fillet packaged with chitosan edible film and basil essential oil

Parameter	Treatment	0th Day	3rd Day	6th day
Control		7	7	6
	0%	7	7	7
Appearance	1.5%	8	7	7
	3%	7	7	6
	4.5%	6	7	5
Control		7	6	5
	0%	6	7	6
Aroma / odor	1.5%	7	7	7
	3%	7	6	6
	4.5%	7	6	5
Control		7	7	6
	0%	7	7	6
Texture	1.5%	8	7	7
	3%	7	7	7
	4.5%	7	7	6

Note: Score: 8.1-9: Most favored, 7.1-8: Like it a lot, 6.1-7: Just like, 5.1-6: Quite like it, 4.1-5: Neutral

Scoring test

The scoring test is carried out to determine the level of quality on a value scale of 1-9. The results of the panelists' assessment of red snapper fillets stored at cold temperatures using edible film with basil essential oil tended to favor still the fish's appearance, smell, and texture (Table 5).

Based on the panelists' organoleptic tests, both the hedonic tests and the scoring showed that the panelists preferred the appearance of fish with edible film packaging of basil essential oil at 1.5% and 3% concentrations. In addition, panelists liked the smell of fish fillets with an edible film of basil essential oil at 3% and the texture at 1.5% to 4.5% concentrations. On the sixth day, 0% gave a poor rating for the odor and texture parameters compared to 3% and 4.5% concentrations. This is possible because the basil essential oil covered the distinctive fresh smell of basil. In comparison, the texture changes are difficult to inhibit due to the metabolism's rapidity.

Moreover, organoleptic changes in fish can be caused by microbial activity and chemical reactions in the fish's flesh. Protein and fish fat degradation reactions occur due to proteolytic enzymes that degrade protein and lipase enzymes for degrade fat. The quality of fish flesh changes occurs during the pre-rigor phase, marked by the release of mucus from the fish skin surface. During the next phase, rigor mortis, the fish body will become stiff due to contraction and relaxation between actin and myosin to produce actomyosin (Nurilmala et al. 2018).

Changes in fish appearance, apart from being caused by chemical reactions in the flesh, are also caused by additives during the storage period, or in this case, by the edible film used. The higher the concentration of basil essential oil added to the edible film, the duller the resulting edible film will be, so while it is used to package fish, it will affect its appearance. The higher the concentration of essential oils added, the stronger the smell of basil will be to prevent the odor of rotting fish flesh. This is comparable to the results of the organoleptic test (Tables 4 and 5), in which panelists prefer the smell of fish fillets with edible film packaging with the addition of a high concentration of basil essential oil (3%). The basil essential oil odor can neutralize the bad smell of fish flesh compared to the 1.5%, 3%, and 4.5% concentrations.

This study concludes that edible film produced from chitosan with basil essential oil and used as a packaging material meets Japanese Industrial Standards (JIS) standards for thickness, tensile strength, water vapor transmission rate, and Elongation so that it can be used as packaging for red snapper fillets. Meanwhile, edible films with basil essential oil at a concentration of 3% were more acceptable to panelists and inhibited more changes in fillet quality (TVB, moisture content, and growth of microorganisms). Furthermore, adding basil essential oil makes the film brownish-yellow in color. It provides a distinctive fresh smell of basil, which can reduce the rotten or fishy smell of red snapper fillets that have been stored for several days.

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