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Antibacterial Edible Coating from Mandarin Orange Peel (*Citrus reticulata*) and Moringa Leaf (*Moringa oleifera*) Extract for Fish Preservation

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Abstract

Approximately 10% of total fish production is wasted due to decomposition. Excessive formalin use in fish preservation can be potentially lethal. One of the natural preservatives is an edible coating, which can be prepared from natural ingredients such as mandarin orange peel (*Citrus reticulata*) pectin, and Moringa leaves (*Moringa oleifera*) which contain antibacterial compounds. The purpose of this research was to examine the antibacterial influence of edible coatings made from pectin of mandarin orange peel with the addition of Moringa leaf extract and to determine its effect on the freshness of Nile tilapia (*Oreochromis niloticus*). Moringa leaf extraction was performed using the Soxhlet extraction method with 96% ethanol at 60–80 °C, while the mandarin orange peel was isolated by reflux using 1% HCl with a pH of 1.5 for 4 hours at 90e-r°C. The edible coating was synthesized from mandarin orange peel pectin and carboxymethyl cellulose (CMC) which was homogenized with distilled water, and glycerol was added as a plasticizer. Edible coatings were prepared with various concentrations of 0%, 50%, and 100% Moringa leaf extract. Nile tilapia with the addition of edible coatings experienced slower decay, especially in the variations of 100% Moringa leaf extract. This was evidenced by the quantitative test through the Total Plate Count (TPC) test which still did not exceed the limit of >5.6 log CFU/g.

Keywords: *antibacterial; edible coating; moringa leaf; pectin; preservative*

Introduction

Fish is one of the furthermost transacted commodities. In 2019, Indonesian fish consumption increased to 55.95 kg per capita per year. The supply of fish demand in Indonesia is projected to rise until 2030. Indonesian aquaculture is expected as the primary trader of fish in 2026[1]. However, the process of post-harvest distribution, handling, and storage of fish can reduce the quality of fish when consumed. In general, fish that is wasted due to decay reaches about 10%, which is up to 12 x 10^6 tons annually of the total production of capture fisheries and aquaculture[2]. Preservation of fish is very important to prevent the loss of nutrients in fish[3]. Fish can be preserved by using formalin. Formalin has the capability to prolong the shelf life of fish beyond that of ice. Unfortunately, prolonged

exposure to formalin in food can be potentially harmful[4]. Natural preservatives are generally preferred by consumers over synthetic preservatives because they are safer to consume. Natural preservatives can come from microorganisms, animals, and plants[5]. One example of a natural preservative is an edible coating.

The edible coating is a thin, biodegradable layer that functions as a preservative. These coatings are able to prevent mass, vapor, and gas transfer, as well as moisture barrier[6], carrier food additives such as antioxidants and antimicrobials[3], increase shelf life, and protect food from microbial contamination so that it can inhibit the spoilage process. A good edible coating usually has sufficient stabilizing activity and the ability to maintain good fish structure[7]. Edible coating and edible film are both comestible, but there are differences between the two; the edible coating is directly applied to the food surface, whereas edible film is used as a food wrapping material[8]. Edible coatings can be synthesized from pectin which will have the advantage of a smooth film. Pectin can be obtained through fruit peels, one of which is the peel of mandarin oranges (*Citrus reticulata*). Globally, the consumption rate of oranges is very high, and it is estimated that orange peel waste is produced at around 13.7 million tons[9].

Edible coatings with the addition of several functional compounds such as antimicrobials, plasticizers, and antioxidant agents will be able to minimize the threat of pathogenic microflora contagion and food quality reduction. Edible coatings show enormous prospects as a tool to improve the protection and quality of food products, specially fish products such as Nile tilapia, by minimalizing their weakness to spoilage and improving their shelf life[10],[11]. Various plants can be applied to edible coating formulations because they contain polyphenolic compounds that act as antimicrobial and antioxidant agents in edible coatings[12]. Other plants that contain an antibacterial is Moringa leaves (*Moringa oleifera*). The *M. oleifera* leaves consist of tannins, steroids, flavonoids, saponins, and alkaloids that can inhibit bacterial growth, which thus, the product has a longer shelf life. Various type of edible coating was prepared in this study by utilizing mandarin orange peel pectin and Moringa leaf extract. The effect of applying the edible coating on the quality of Nile tilapia was then evaluated at room temperature, and its efficacy was demonstrated by organoleptic tests, pH tests, and TPC tests.

Experimental

Materials

The materials used in this study were mandarin orange peel, Moringa leaves, Nile tilapia, hydrochloric acid (HCl) (Merck, 38%), Mg powder (Merck), glycerol (Merck, 98%), ethanol (C_2H_5OH) (Merck, 96%), $FeCl_3$ (Merck), Wagner's reagent, PCA (plate count agar) (Merck), CMC (carboxymethyl cellulose) (Koepoe), filter paper, phosphate buffer pH 7.2 and aluminum foil.

Instruments

The instruments used in this research include an oven (Memmert), pH meter (ATC), digital thickness gauge (Qifeng), and Spectrophotometer FTIR (Shimadzu IR Prestige 21).

Methods

Moringa Leaf Extraction

Moringa leaves were cleaned, dried over sun exposure, and crushed into powder. Moringa leaf powder of as much as 30 g was used in a series of Soxhlet extractors. Ethanol was added in a ratio of 1:50 w/v. Extraction was done at a temperature of 60-80 ^oC until the last extract was colorless and then the extract was filtered. The filtrate was separated from ethanol using a rotary evaporator. The resulting extract was then subjected to the phytochemical test.

Pectin Isolation

The mandarin orange peel was dried in a 70°C oven for 48 hours and mashed using a blender until it formed a powder. A total of 50 g of orange peel powder was put in a 1000 mL

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Erlenmeyer to be followed by distilled water addition. The mixture was stirred until it forms a runny slurry. Then 1% HCl was added slowly until it reached a pH of 1.5, refluxed for 4 hours at 90°C, filtered, and cooled at room temperature. Ethanol (50 mL) was added and acidified with 2 mL of concentrated HCl. The mixture was allowed to stand for 1 h. to form a gel. The resulting gel precipitate was washed with ethanol, acidified using 2 mL concentrated HCl, and cooled for 17 h. The resulting precipitate was filtered with a vacuum pump and dried over an oven at 40°C for 8 h. The obtained pectin was ground and sieved over a 100-mesh sieve. Pectin was characterized by an FTIR spectrophotometer.

Edible Coating Preparation

The mandarin orange pectin powder (3 g) was put into a 100 mL volumetric flask and distilled water was added until the volume reached 100 mL. The solution was transferred to a beaker, magnetically stirred, and heated at 60°C for 30 minutes. CMC (1 g) and 10% glycerol plasticizer were added until homogeneous. The Moringa extract with concentrations of 0%, 50%, and 100% was added. The resulting edible coating was measured for its thickness using a digital gauge.

Edible Coating Application

Fresh Nile tilapia was dipped in an edible coating solution for 1 minute. The dipping was carried out twice and then the tilapia was transferred to a clean container and dried by aerating. The applied Nile tilapia with edible coating was stored at room temperature (28-30^{o}C).

Edible Coating Testing on Fish

Organoleptic Test

Samples of tilapia were scored test with criteria 1 for the lowest value and 9 for the highest value on the assessment of the eyes, slime, body surface, texture, odor, color, and appearance of the flesh.

pH Test

Tilapia with 4 treatments i.e., without edible coating, with 0%, 50%, and 100% Moringa leaf extract were tested for pH using a digital pH meter.

TPC (Total Plate Count) Test

The TPC Test began with the media preparation from PCA. PCA was poured into sterile petri dishes as much as 12-15 mL, then cooled. A total of 5 g of fish samples were weighed aseptically, put into an Erlenmeyer, and added 45 mL of butterfield's phosphate buffered solution. The mixture was then stirred for 2 min. until homogeneous. This homogenate is a 10^{-1} dilution solution. A total of 1 mL of 10^{-1} homogenate was taken by a sterile measuring pipette and put in a test tube containing 9 mL of butterfield's phosphate buffered solution to obtain a 10^{-2} dilution. Furthermore, for the 10-3 dilution obtained from taking 1 mL of the 10^{-2} dilution. Each dilution was shaken and 1 mL of fish sample for each dilution (10^{-1}, 10^{-2}, and 10^{-3}) was pipetted into sterile petri dishes containing PCA media. The sample was allowed to soak for 1 hour and it was incubated in an inverted petri dish for 48 hours at 37^{o}C.

Results and Disscusion

Moringa leaf extraction was done by the Soxhlet extraction method. This method is often used because it is easy, does not require high costs, the solvent used can be recovered, and the extraction time is relatively short[13]. In this Moringa leaf extraction, ethanol solvent is used. Moringa leaf extract will tend to dissolve in polar solvents[14]. Ethanol will be able to extract active compounds such as tannins, flavonoids, alkaloids, polyphenols, polyacetylene, terpenoids, and sterols[15]. The extract obtained was dark green which has a distinctive leaf aroma in 9.24% yield.

Moringa leaf extract contains secondary metabolites as antibacterial compounds such as flavonoids, alkaloids, saponins, and tannins[16]. The mechanism of flavonoid compounds in inhibiting bacteria is by binding to bacterial extracellular proteins, inhibiting bacterial cell metabolism by protein deactivation, and

engaging through the cell walls of bacteria. The flavonoid isolation has been carried out by utilizing the polarity dissimilarity among the lipid components of the microbial cell and the hydroxy group in flavonoids. Via hydrogen bonding, it forms a complex with microbial lipids. Thus, the cell wall arrangement and cytoplasmic membranes will be unstable triggering the microbial cell loss in biological potency. Moreover, the permeability of microbial cells might be disturbed and encounter lysis or breaking down, triggering microbial cell death[17].

Saponins are natural surfactants from plant extracts. The molecular structure of saponins consists of hydrophilic sugars attached to aglycones (steroids and triterpenoids)[18]. Saponins exhibit biological roles and medicinal properties such as anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, and anticancer[19]. The mechanism of action of saponins in killing bacteria is to reduce the surface tension of the bacterial cell wall which results in cell escape and the discharge of intracellular constituents[20].

Once the microbial wall is lysed or ruptured owing to saponin and flavonoid, tannin could possibly pass into microbial cells and bind to polypeptides cell wall inhibiting bacteria in synthesizing new cell walls. Tannin might prevent microbial growth through deactivating substantial enzymes or genetic components and complex formation with proteins via hydrophobic interaction. This process would create protein denaturation causing microbial cell death. Meanwhile, alkaloids work by disrupting the peptidoglycan of microbial cells. Thus, the formation of the cell wall layer would not complete causing the cell slowly dies[21]. The proof of the presence of antibacterial compounds of flavonoids, alkaloids, saponins, and tannins can be done by phytochemical tests (Table 1).

Pectin Isolation

Pectin isolation was carried out using the reflux method at a temperature of 90° because it will form bonds between protopectin molecules that are easily separated and dissolve in water so that more pectin is produced. Isolation of pectin from orange peel was carried out at pH 1.5 because the low pH value can increase the release of pectin since the pectin bonds with hemicellulose are disconnected[22]. The addition of 96% ethanol was carried out to bind HCl from the pectin gel so that the pectin obtained did not contain acid. The yield of pectin produced was 9.715%. The results obtained are close to the reported research that the pectin yield of mandarin orange peel is 9.45%[22].

Pectin was characterized by using an FTIR spectrophotometer (Figure 1). At a wavenumber of 3399 cm^{-1}, it indicates that the pectin spectrum contains O-H group absorption caused by inter- and intramolecular hydrogen. Absorption at 2926 cm^{-1} and 2859 cm^{-1} exhibit the existence of a CH_3 group. The absorption band at 1744 cm^{-1} shows the C=O vibration of the polysaccharide, namely pectin, the absorption band at 1646 cm^{-1} reveals a carboxylate group and the absorption at 1431 cm^{-1} indicates the presence of –C-H bonds. These groups are functional groups owned by pectin compounds[23].

Table 1. Phytochemical test results on Moringa leaf extract

Phytochemical Test	Reagent	Change Identification	Results
Flavonoid	Wilstater's Reagent	The brown color changed to yellow.	Positive
Alkaloid	Wagner's Reagent	A brown precipitate was formed.	Positive
Saponin	Distilled water	The stable foam was formed.	Positive
Tannin	$FeCl_3$	The color changed to greenish brown.	Positive

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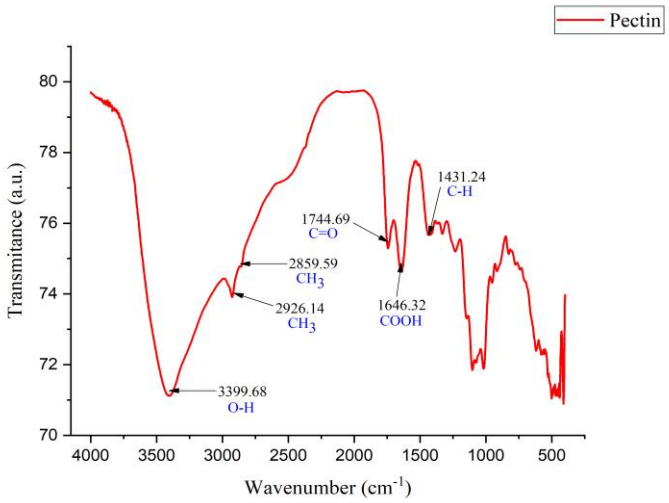

Figure 1. FTIR Spectra of Mandarin Orange Peel Pectin.

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Edible Coating Synthesis and Its Application

The synthesis of the edible coating was carried out with mandarin orange peel pectin because it contains high methoxy pectin group which could form jelly more quickly[24]. In this synthesis, CMC was added which aims to speed up the thickening process. The addition of glycerol in the synthesis of edible coatings aims as a plasticizer to improve the quality of edible coatings. The synthesized edible coating was applied to Nile tilapia by the double dipping technique. Four treatments were carried out, namely without applying the edible coating, applying edible coating with variations of 0%, 50%, and 100% Moringa leaf extract. Edible coating with the addition of 1 gram of CMC and 10% glycerol has a thickness for edible coating with 0%, 50%, and 100% Moringa leaf extract of 0.02 mm, 0.03 mm, and 0.04 mm. For all types of film were tested with a digital thickness gauge (Qifeng). According to these data, the variations in Moringa leaf extract affect the thickness of the edible coating.

Edible Coating Testing on Nile Tilapia Fish

The organoleptic test is a test performed to determine the level of freshness of fish by direct observation (Figure 2). Organoleptic tests were carried out by 10 panelists. Verify was done by performing an observation and a questionnaire to the panelists through several parameters such as odor, appearance, eyes, mouth, anus, and flesh. The graph shows that a value of 9 is given to fish with a fresh condition which is characterized by a bright, shiny, and not slimy appearance; has a fresh odor; have bright eyes that protrude outward and are not slimy; the anus is bright pink; mouth closed; as well as supple and chewy flesh. A score of 6 is given to fish with an almost fresh condition which is characterized by a dull, gloomy, and not slimy appearance; sour smell; slightly sunken eyes; closed mouth; colored anus; and soft and not chewy meat. A score of 3 is given to fish with almost rotten conditions by means of the physical appearance of a dull, gloomy, and slimy appearance; slightly pungent smell; the eyes are slightly sunken into the eye sockets;

mouth slightly open; the anus is slightly protruding and discolored; and soft but not chewy flesh. While the score of 1 is given to the condition of rotten fish with the characteristics of a dull, gloomy, and very slimy appearance; foul smell and very pungent; eyes sunken into the eye sockets; open mouth; the anus protrudes and is colored, and the flesh is very soft. Based on Figure 2, it can be perceived that the application of the edible coating on fish is confirmed to be more effective in reducing the deterioration of fish quality compared to fish without the application of edible coating. Moreover, the addition of Moringa leaf extract also affects the deterioration of fish quality. Edible coating with 100% Moringa leaf extract is more effective in slowing fish spoilage.

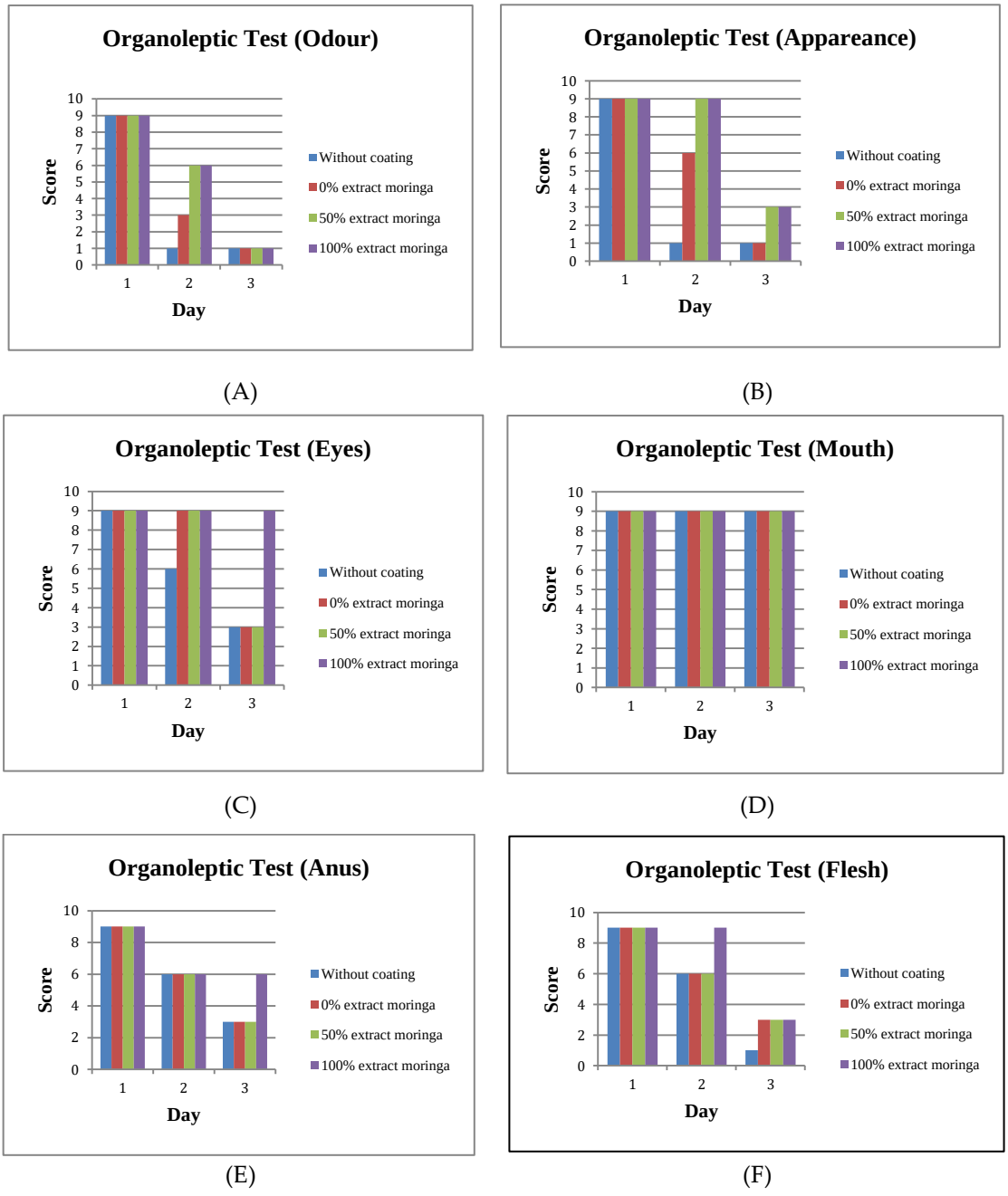

Figure 2. Organoleptic test results on odor (A), appearance (B), eyes (C), mouth (D), anus (E) and flesh (F).

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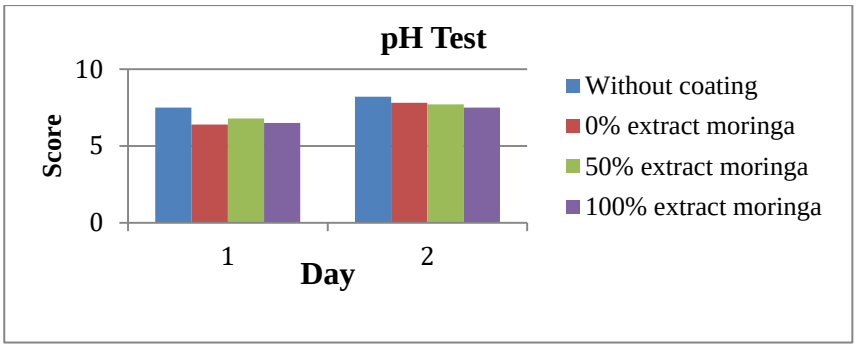

Figure 3. Results of pH Test

Table 2. TPC Test Results

Edible Coating Treatment	log CFU/gram	
	Day-0	Day-2
Without edible coating	4.25058	4.73156
0% moringa leaf extract		4.53300
50% moringa leaf extract		4.49112
100% moringa leaf extract		4.42975

The pH test on fish aims to measure the acidity level of fish in each treatment variation (Figure 3). It can be seen that the pH of each fish has increased. On the first day, the fish treated without edible coating had reached a pH of 7.5. While the treatment with edible coating had a pH <7 at 0%, 50%, and 100% extract variations. This means that the fish treated without edible coating had rotted on day 1, while the fish treated with the edible coating application of 0%, 50%, and 100% Moringa leaf extract variations showed that the pH was still acidic, which means the freshness of the fish was still quite good. On the second day, the fish in the four treatments had reached pH > 7. Fish with an alkaline pH showed spoilage because, at an alkaline pH, there was activity from bacteria that degraded amino acids into basic components i.e., ammonia, trimethylamine, and other volatile compounds[25]. Judging from the data produced, it is proven that the addition of edible coating on fish is able to inhibit the deterioration of fish quality.

The TPC test is a quantitative bacterial test that counts the number of colonies. This TPC test demonstrates that microbial contamination in fish is one of the factors influencing the decline in fish freshness. Bacterial growth can be influenced by several factors including time, temperature, and humidity of the media. Incubation was carried out for 48 hours at 37°C. TPC test results are presented in Table 2. The acceptable total microbial limit was 5.6 log CFU/g[26]. The results showed that the number of bacteria in each treatment was still within acceptable limits. According to the research of Rasulu et al. (2020) which stated that the application of an edible coating extended the shelf life of fresh tuna at room temperature for 24 hours of observation with organoleptic, pH, and TPC tests[27].

Conclusions

Edible coating prepared from mandarin orange peel pectin with the addition of Moringa leaf extract was able to inhibit the deterioration of fish quality. The organoleptic results showed that fish with the addition of edible coating deteriorated more slowly, especially fish with

the application of edible coating with a variation of 100% Moringa leaf extract. The pH test results also proved that fish with edible coating application was a more acidic pH than fish without a more alkaline application. Fish with a more acidic pH has a higher degree of freshness. The effectiveness of edible coatings was also proven by the TPC test where the results showed that the TPC value was not exceeded the limit >5.6 log CFU/g.

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