Turmeric Rhizome (*Curcuma domestica Val.*) to Catfish (*Clarias sp.*) Bone Gelatin: An Antimicrobial Protein-Based Films for *Escherichia coli*

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Abstract

Edible film packaging is a relatively new food preservation technique. Many research has been carried out and have shown the extended shelf life and improved the quality of food products. One of the safest edible films for food was made from gelatin. In this study, we have made the gelatin from the wastes of catfish bone demineralization was carried out using 4% HCl for 5 hours, until it reached a pH of 5 extracted using 70C distilled water for 5 hours. The turmeric extract obtained from the maceration of 96% methanol for 2 days, the filtrate then evaporated and impregnated into gelatin as a specific antimicrobial for *Escherichia coli*, a gram-positive bacteria with the ratio of ossein catfish bone: distilled water: turmeric extract is (10:50:0); (10:50:5); and (10:50:10) % w/w. The films were applied to apples. The efficacy of the existing films against *Escherichia coli* bacteria for the coming 20 days was studied using the modified disc method. The acceptance test of film products was carried out using the organoleptic method

Keywords
Antimicrobial, catfish gelatin, Escherichia coli, edible film, turmeric rhizome

1. Introduction

The need for gelatin in Indonesia continues to increase, while there is no industry specifically producing gelatin so that imports of gelatin from several countries such as China, Australia, and several European countries continue to increase. According to Central Statistics data, imports of gelatin reached 2,715,782 kg with a value of 9,535,128 USD (BPS 2020). The use of gelatin is very wide in foodstuffs; as a gelling agent, thickener, emulsifier, foamer, and edible film, as well as in the pharmaceutical field (IMESON, 1992).

So far, the main raw material for gelatin comes from the skin and bones of cows or pigs. The use of fishbone gelatin is acceptable to Muslims as is the majority of the population in Indonesia, and there are few restrictions on Judaism and Hinduism (Mariod, A. A., & Adam, H. F, 2013). There are concerns about the use of raw materials from cows due to livestock diseases such as anthrax and mad cow disease (Gudmundsson, 2002). Gelatin is obtained from the conversion of collagen which was naturally found in animal bones or skin. Fish bones can be sources of gelatin because contain about 18.6% of collagen from 19.86% of the organic protein complex (Da Trindade Alfaro, Simões Da Costa, Graciano Fonseca, & Prentice, 2009). Several studies have been carried out on extracting gelatin from catfish bones with various types of immersion solutions, extraction temperature, extraction pH, extraction time, as for change variable (Liu, Han, & Guo, 2009), will affect the gelatin yield (Sanaei, Mahmoodani, See, Yusop, & Babji, 2013). In addition, the acid process is more profitable because it requires a shorter time and lowers costs.

Gelatin can protect food from the migration of water vapor, oxygen, carbon dioxide, aroma, and lipids as antimicrobials for packaging materials by paper or edible films. The mishandling of food can food deterioration by bacteria. *Escherichia coli* one type of gram-positive bacteria that grows in freshwater, seawater, soil contaminated with feces (Besser et al., 1993). The edible film will perform a good antimicrobial if spices such as turmeric (*Curcuma domestica Val*), temulawak (*Curcuma xanthoriza*), galangal (*Alpinia galangal L*), and ginger (*Zingiber officinale*) are added (Widiyono et al., 2021).

This research aims to study the efficacy of antimicrobial of catfish gelatin with turmeric on *Escherichia coli* to the shelflife of apples. Analyzed the property and the appearance of apples after 18 days with the support of an organoleptic.

2. Literature Review

Gelatin is the product hydrolysis of collagen found in animal tissues. The mechanism of the hydrolysis reaction can occur as follows:

$$C_{102}H_{149}N_{31}O_{38} + H_2O \rightarrow C_{102}H_{151}N_{31}O_{39} \quad (1)$$

Collagen water gelatin

Gelatin is a hydrocolloid that contains all essential amino acids excluding tryptophan. The usefulness of gelatin in food processing was due to its unique chemical and physical properties compared to its nutritional value as a protein source. Gelatin will expand and soften gradually absorbing water 5-10 times its weight if it is in water, easily soluble at a temperature of 71.1^0C, and forms a gel at a temperature of 48.8^0C, easily soluble in glycerol, mannitol, and sorbitol, but insoluble in alcohol, acetone, and other non-polar solvents. The molecular weight of gelatin is around 90,000 while the average molecular weight of commercial gelatin ranges from 20,000 – 70,000 (Wijaya & Junianto, 2021).

Several factors that affect the formation of gelatin, among others, are the hydrolysis temperature. The higher the hydrolysis temperature, the faster the reaction and the darker the color of the gelatin because the protein in the collagen is damaged. If it is run above 95^0C, the gelatin will break down into semiglutin and hemicolin (Amertaning, Bachrudin, ., Chin, & Erwanto, 2019). Gelatin is distinguished based on the processing process, there is type A which in the process is soaked in an acid solution or an acid process. While type B is treated with an alkaline solution or an alkaline process. The advantages of catfish compared to other animal products are high levels of leucine and lysine. Leucine is an essential amino acid that is needed in growing children while maintaining nitrogen balance.

Currently, research on edible coatings has been widely carried out and has been proven to be able to extend the shelf life and improve the quality of food products. The antimicrobial ingredients tend to increase because of the potential dangers of synthetic preservatives.

In the manufacture of edible film composites, the basic ingredients of protein can come from corn, soybeans, casein, collagen, gelatin, milk protein, and fish protein. Polysaccharides used in the manufacture of edible films are cellulose and its derivatives, starch and its derivatives, pectin, marine algae extract (alginate, carrageenan, agar), gum (gum arabic and gum karaya), xanthan, chitosan, and others(Firdaus, Purnamasari, & Gunatama, 2018). Medicinal plants contain a lot of antibacterial compounds such as turmeric (*Curcuma domestica Val*). The response of the inhibition of microbial growth is influenced by the content of active compounds in turmeric such as essential oils, alkaloids, flavonoids, tannins, curcuminoids, and terpenoids (Nguyen Van Long, Joly, & Dantigny, 2016) and Flavonoid compounds can damage cell walls, causing cell death. E.coli bacteria are gram-negative pathogenic bacteria, is a facultative anaerobic bacterium in both aerobic and anaerobic conditions in the range temperature 10-40^0C where the optimum is 37^0C, optimum pH is 7.0-7.5, lives in humid places, It can survive in difficult environments, survive at high acidity levels in the human body spread through feces. The main source of E.coli contamination in fruit is due to E.coli contamination from animal feces (Besser et al., 1993).

3. Method

Material

Catfish bone, turmeric rhizome, HCl (p), Whatman filter paper no. 42, Aquadest, Methanol 96%, Plastic sheet, Gelatin extract sample, $CuSO_4.5H_2O$, NaOH, CO_2 free water

3.1. Preparing The Antimicrobial Films

1.Procedure of Gelatin Extract from Catfish Bone

The catfish bones are washed with warm water to remove the remaining meat and dirt, then washed with lime to remove the fat that sticks to the fish bones, then dried in the oven 60°C. After drying, the bone was cut ± 1 cm. The dried bones were weighed 20 grams and demineralized in 100 mL of 4 % HCl for 5 hours. The demineralized bone formed an ossein were washed using running water until reached pH 5. The extraction process was carried out using aquadest at 70°C with a mass ratio of ossein: aquadest volume 1: 2 w/w and for 5 hours. The extraction results were filtered by Whatman filter paper no. 42, and put in a plastic-coated cup, dried in an oven at a temperature of ± 60°C to form gelatin crystals. Then grind into powder.

2.Procedure of Turmeric Extraction

The method of extraction used was maceration using methanol then evaporated (Da Trindade Alfaro et al., 2009). Fresh turmeric was washed, drained, and dried for 3 days until completely dry, and ground into powder. The sifted turmeric powder was soaked with 96% methanol until homogeneous, for 2 x 24 hours. The maceration results were filtered using whatman filter paper No. 42. The filtrate obtained then evaporated at 40°C. The extract was a form of a paste. The extract was then stored in a sterile bottle at a temperature of 5°C so remained durable.

3. Procedure and Formula of Antimicrobial Edible Film

The ossein of catfish bone in the form of gelatin powder, distilled warer, and the extracted turmeric were weigh in the ratio 10:50:0 (%, w/w) Formula I; 10:50:5 (%, w/w) Formula II, and 10:50:10 (%, w/w) Formula. Heat and stir the mixture using a hotplate at 70°C until the gelatin dissolves and forms bubbles. The mixture was then poured manually on the surface of a smooth polyethylene sheet, then leveled using a plate for 2 days at room temperature. Then remove the edible film from the plastic from the polyethylene sheet. Edible films ready to be applied to apples. The weight test on apples was carried out by packing the apples with Edible Film and then weighing the apples periodically, stored at room temperature, and weighing the apples every 5 days for 20 days.

3.2 Testing of Antimicrobial Edible Film

1. The qualitative test of Gelatin

The Principle of Gelatin Qualitative Test Principle. The proteins are made up of amino acid molecules. Amino acid molecules are linked together by bonds called peptide bonds. The presence of peptide bonds in proteins can be tested using the biuret test, which gives a purple precipitate. Identification of gelatin was done by dissolving 1 gram of sample in 100 ml of CO_2-free water at 55^0C. A total of 4 ml of the sample solution was mixed with 0.1 ml of 125g/l $CuSO_4.5H_2O$ solution and 1 ml of 85g/l NaOH solution. Stir and observed the results.

2. The inhibition test of Antimicrobial to Eschericial Coli using paper disc Method

Nutrient Agar Solid Media, Edible Film Sample, Antibiotic Amoxilin as control, Aquadest, Edible film paper as disc paper. The bacteria were diluted by mixing 1 ose of *E. coli* bacteria suspension into a test tube that already contained a NaCl solution. It was homogenized using a vortex and the turbidity was standardized with a concentration of 0.5 Mc Farland so that the number of bacteria met the sensitivity test standard of 105-108 / mL. Then the standardized solution was smeared on Nutrient Agar or NA media. The edible film whose diameter is adjusted like a disc. Then it was incubated in an incubator at 37^0C for 24 hours. The next day, the clear zone formed was measured using a ruler.

3. Consumer Acceptance Using Organoleptic Test

Three samples were provided, namely edible film without turmeric, the edible film with 5% turmeric, and edible film with 10% turmeric. There were 10 non-professional panelists to test samples referred to color, taste, and aroma according to their respective preferences.

4. Result and Discussion

1.Gelatin Identification Test Results

The identification test of gelatin in catfish bones was carried out qualitatively. The aim was to ensure and know that the sample in the form of solid crystals produced from the extraction of catfish bones is a gelatin compound. Identification of gelatin was done by dissolving 1 gram of sample in 100 ml of CO_2-free water at 55^0C. A total of 4 ml of the sample solution was mixed with 0.1 mL of 125g/L $CuSO_4.5H_2O$ solution and 1 mL of 85g/L NaOH solution. The violet color of the gelatin identification test on the samples produced from the extraction of catfish bones showed positive results.

2. The inhibition of antimicrobial films to *Escherichia coli* strain

The test was conducted using a modified disc method applied to the design formula of the antimicrobials. The result was compared to amoxicillin as the positive control, where the formula of 10% addition of turmeric was the best amongst others. The inhibition zone for 10% turmeric was 17.5 mm, it was higher than 5% and 0% was 11.5 mm and 0 mm respectively as can be seen in Table 1.

Table 1.The Inhibition of The Antimicrobials to *Escherichia Coli*

Edible Films	Diameter of Inhibition Zone (mm)	Response of Inhibiton Growth
OCB+Extracted Turmeric 0 %	0	non
OCB+Extracted Turmeric 5%	11.5	weak
OCB+ExtractedTurmeric 10%	17.5	medium
Control positive: Amoxicillin	40	strong

3.Apples Weight Test Results

The weight test on apples was carried out quantitatively. Through periodic weighing of apples packaged with edible film, it can be seen the effect of edible film packaging on the spoilage of apples. Apples were stored at room temperature and apples were weighed every 5 days for 20 days. It was known that during 20 days, unpackaged apples experienced a weight loss of 4.38%, apples packaged with edible film gelatin + 0% turmeric extract experienced a weight loss of 2.11%, apples packaged with edible film gelatin + 0% turmeric extract experienced a weight loss of 2.11%, apples Packaged with a mixture of edible film gelatin + 5% turmeric extract experienced a weight loss of 1.44%, and apples packaged with a mixture of edible film gelatin + 10% turmeric extract experienced a weight loss of 1.18%.

The percentage of weight loss on the four apple fruit conditions on day 5, day 10, and day 15 had the same trend. This is proven that the edible film packaging affects the length of rotting apples. Turmeric extract added in the composition of the edible film was also capable of acting as an antimicrobial. The higher the concentration of turmeric extract in the composition of the edible film, the higher the effectiveness as an antimicrobial packaging on the edible film as can be seen in Figure 1.

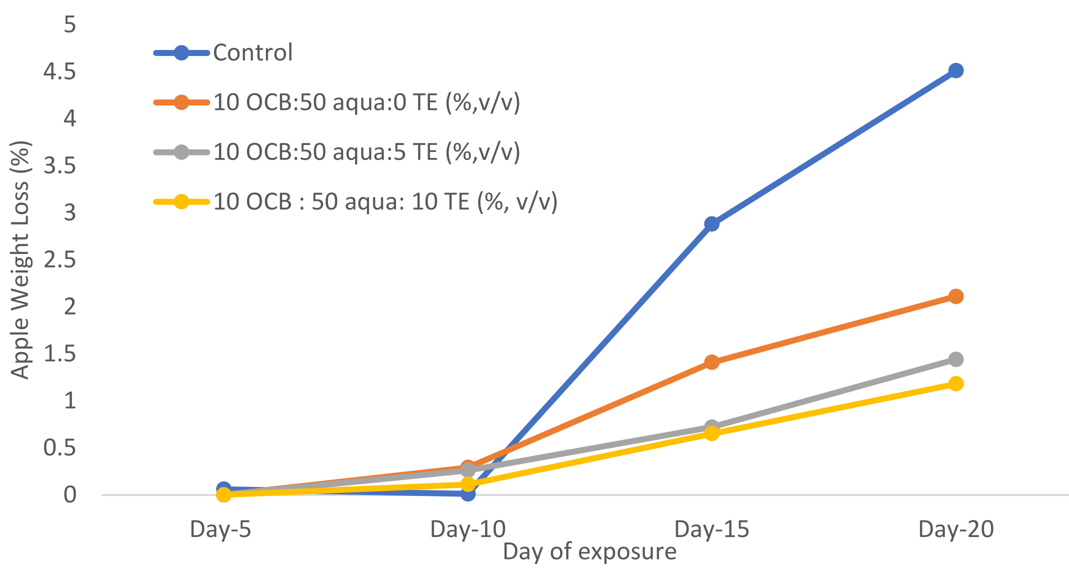

Figure 1. The weight loss of Apples (%)
*OCB: ossein catfish bone; TU: turmeric extract

4 Edible Film Organoleptic

The sensory attributes and consumer acceptance of the antimicrobial using 3 formulas, which differentiate the turmeric concentrations. The purpose of the organoleptic test was directly related to consumer tastes and acceptance of a product. The organoleptic test of the edible film was carried out by 10 non-professional panelists with test parameters including color, taste, and aroma on 3 edible films sample products. The level of liking was divided into 5 groups, namely very poor, poor, average, good, and excellent. The results of the edible film organoleptic test, is summarizes in Figure 2 and the average of the all sample feature can be seen in table 1.

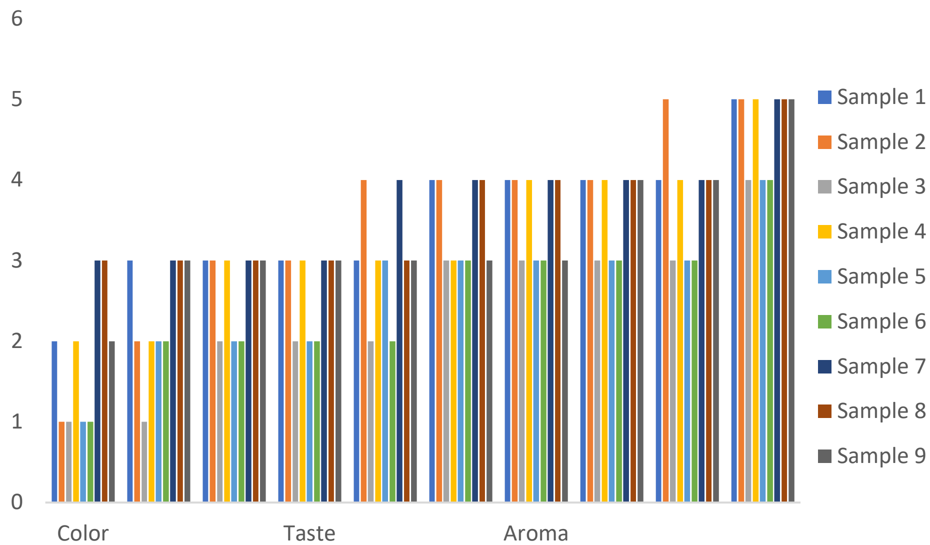

Figure 2. Data analysis on rating all features of all samples involved in this research

Table 1. The average rating of all features of all samples

Sample code	Color	Taste	Aroma	MeanAcceptance ±SD
Sample 1	3.5	3.3	3.7	3.5± 0.0
Sample 2	3.5	2.6	3.6	3.2±0.23
Sample 3	2.4	2.5	3.3	2.7± 0.33
Sample 4	3.5	3.3	3.7	3.49± 0.01
Sample 5	3.5	2.6	3.6	3.2±0.33

A 5-point hedonic scale evaluation test for acceptance was conducted with 10 non-professional panelists. Data collected from the evaluation forms were processed using descriptive analysis and ANOVA. The films involved in this research were also assessed for antimicrobial inhibition to *Escherichia coli*, weight loss reduction (%) of applied covering films to apples, due to their direct interference in sensory attributes of edible films. The mean acceptance of sensory attributes of the 5 samples varies from 2.7 to 3.5. The best-obtained range of sensory attributes was sample 1 (ossein catfish bone/aquadest/turmeric extract) (10:50:0) (%, w/w) with the mean value of acceptance 3.55±0.00. The less accepted with a mean value of acceptance 2.7±0.33 was samples 3 and 5. The addition of 10% (w/w) turmeric caused a thicker film even though the inhibition of the zone against *Escherichia coli* was the highest. The images of antimicrobial edible films based Catfish gelatin can be seen in Figure 3 and the images extraction of catfish and and turmeric in Figure 4.

Figure 3 The images of antimicrobial edible films based Catfish gelatin with a) 5% extracted turmeric b) 10% extracted turmeric

Figure 4. The images extraction of catfish bone with Aquadest 70^{0}C for 5 hours b) The maceration extraction of turmeric with methanol 96%

CONCLUSION

Based on the results of the study, it can be concluded that the higher the concentration of turmeric extract in the composition of the edible film, the higher the effectiveness of the edible film as an antimicrobial packaging. From the proven by the testing of weight loss of apples within 20 days according to the data obtained, the uncovered apples (control) was 4.38% weight reduction, apples covered with edible films of catfish gelatin + turmeric extract 0% was 2.11% of reduction, apples packed with a mixture of edible films of catfish gelatin + turmeric extract 5% was1.44% d, and apples packed with a mixture of edible film gelatin + turmeric extract 10% was 1.18%.

There was one thing that needs to be noticed that the addition of 10% turmeric causes more thick films so if applied commercially it would not be effective because the sheet was not flexible, even though it was able to withstand the *Escherichia coli* bacteria better than others. The darker yellow color appearance of the films with the 10 % turmeric seems to need to be modified to be appeared brighter while still in good inhibition against bacteria. The thick and stiff of existing films were necessary to be added a food-safe plasticizer.

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