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

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Abstract

Production an edible films packaging is a rather new food preservation approach. 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 produced the gelatin from catfish bone wastes, demineralization was carried out using 4% HCl for 5 hours until it reached a pH of 5, extracted by distilled water for 5 hours with the temperature of 70^0C where the ratio of catfish bone ossein: aquadest was (1: 2 w/w). The turmeric was extracted using the maceration method with 96% methanol for 2 days. The filtrate was evaporated, then impregnated into gelatin as specific for a gram-positive bacteria *Escherichia coli*. The formula for edible films was prepared with the ratio of catfish bone ossein: distilled water: turmeric extract (10:50) w/w with turmeric 0%; 5%; and 10 %. The edible films were then applied to apples. The efficacy of the existing films was improved by turmeric 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. Fishbones 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 accomplish 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 be deteriorated by bacteria. *Escherichia coli* is 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 aimed to produces edible films from catfish gelatin with the addition of turmeric and study the efficacy as antimicrobials to *Escherichia coli* to the shelflife of apples. Investigate the appearance of apples in 20 days with the support of paper disc methods and sensory attributes.

2. Literature Review

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

$$\underset{\text{Collagen}}{C_{102}H_{149}N_{31}O_{38}} + \underset{\text{water}}{H_2O} \rightarrow \underset{\text{gelatin}}{C_{102}H_{151}N_{31}O_{39}} \qquad (1)$$

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 semi gluten and hemi colin (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 extensively carried out and has been confirmed extent the shelf life and convalesce character 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 fundamental ingredients of protein can come from corn, proteins, and protein derivatives. Polysaccharides used in assemble edible films are cellulose and its derivatives etc (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 shaped by the content of active compounds in turmeric (Nguyen Van Long, Joly, & Dantigny, 2016), 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^{0}C where the optimum is 37^{0}C, 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.Producing 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 (Liu et al., 2009). 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.Producing 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 to remained durable.

3. Producing Antimicrobial Edible Film

The ossein of catfish bone in the form of gelatin powder, distilled water, was weigh in the ratio 10:50 w/w to turmeric 0 %; 5%; and 10%. 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 outer of polyethylene sheet, then leveled by emplying 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 diffusing 1 gram of sample in 100 ml of CO_2-

free water at 55^0C. An amount of 4 ml of the sample solution was compounded 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 Escherichia 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 (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, edible films 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 liquefying 1 gram of sample in 100 ml of CO_2-free water at 55^0C. An amount of 4 ml of the sample solution was blended with 0.1 ml of 125g/l $CuSO_4.5H_2O$ solution and 1 ml of 85g/L NaOH solution. The purple 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 summarized in Table 1.

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

Edible Films	Diameter of Inhibition Zone (mm)	Response of Inhibition 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 apple fruit conditions on day 5, day 10, and day 15 had the same trend. This was 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.

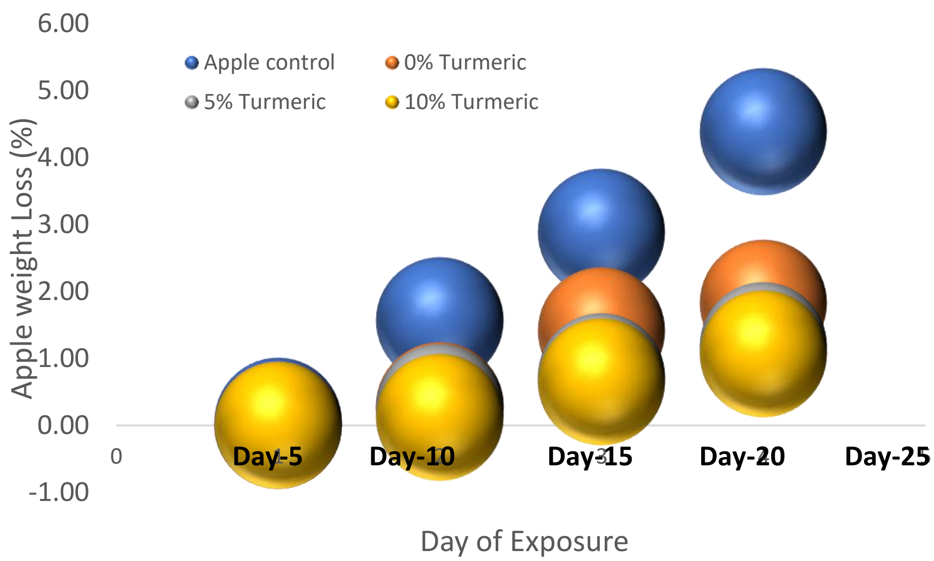

Figure 1. The weight loss of Apples (%) by blending of catfish based edible films to turmeric

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 following are the results of the edible film organoleptic test. The result was depicted in Figure 2, Figure 3, and Figure 4.

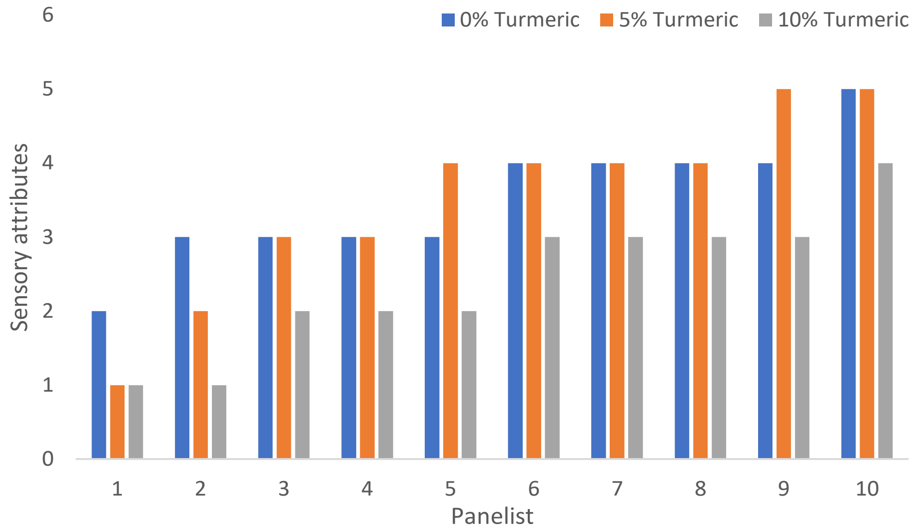

Figure 2. Data analysis on rating Color of Samples in this research

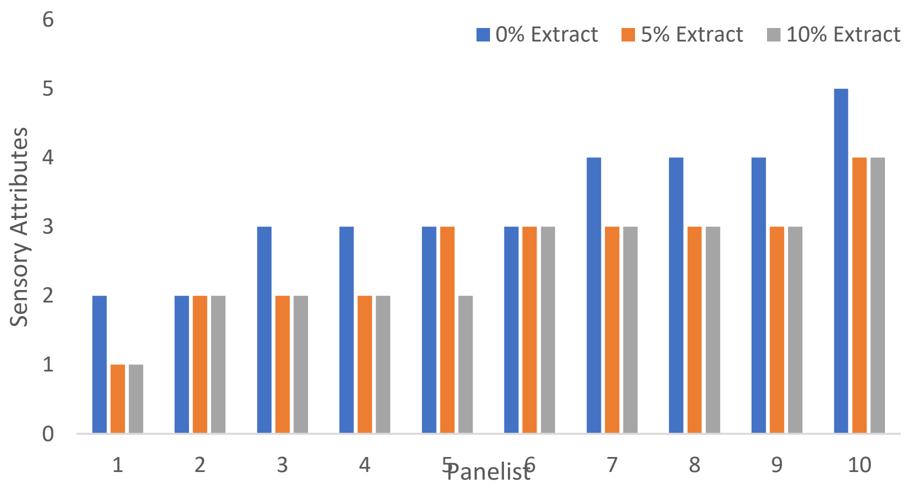

Figure 3. Data analysis on the rating of Taste of samples in this research

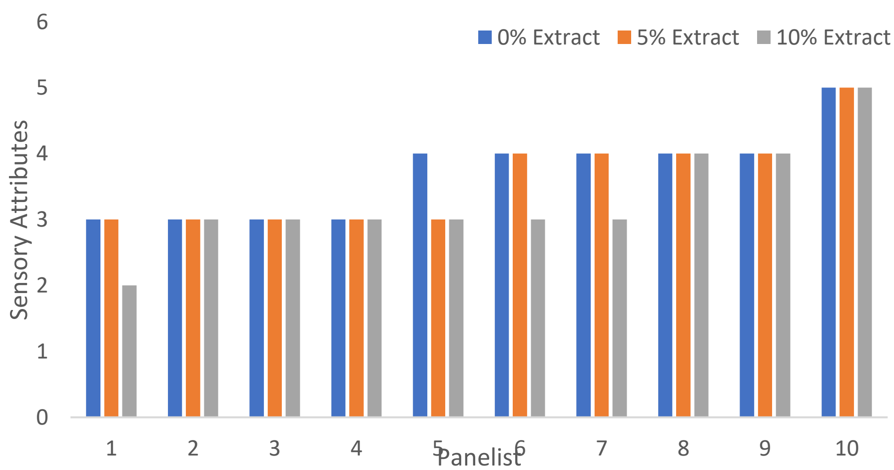

Figure 4. Data analysis on the rating of Aroma of samples in this research

As we analyzed the data obtained from the sensory attributes and related to consumer acceptance to color, taste, and aroma of edible film to the addition of 0%; 5%; and 10% of turmeric as summarized in table 2. There was H_0 rejection for color, it indicated the color differs significantly with higher turmeric concentration. For the taste the H_0 was accepted, it indicated that the difference formula does not significantly different to the taste, as the same thing happened to the Aroma where the H_0 was also accepted which indicates edible films with the addition of turmeric 5% and 10% do not differ significantly to the aroma.

Table 2. The average rating and Consumer Acceptance based on Sensory Attributes

	EF+ 0% Turmeric	EF + 5% Turmeric	EF + 10% Turmeric	Mean Acceptance ± SD	F	F Crit	P value	Result	
Color	3.5	3.5	2.4	3.5 ± 0.0	3.704	3.3541	0.0378	F > F Crit	H0 Rejected
Taste	3.3	2.6	2.5	3.2 ± 2.3	2.442	3.3541	0.1058	F < F Crit	H0 Accepted
Aroma	3.7	3.6	3.3	2.7 ± 0.33	0.8013	3.3541	0.4591	F < F Crit	H0 Accepted

*Hedonic scale:1= very poor 2= poor 3= average 4= good 5= excellent

A 5-point hedonic scale assessment test for approval was organized by 10 non-professional panelists. Data collected from the assessment forms were processed using descriptive analysis and ANOVA. The films involved in this research were also determined for antimicrobial inhibition to *Escherichia coli*, weight loss reduction (%) of applied covering films to apples, due to their direct obstruction to the films. The mean acceptance of sensory attributes of the five samples ranges from 2.7 to 3.5.

a b

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

a b

Figure 6. The extraction of catfish bone with Aquadest 70^{0}C for 5 hours
b) The maceration extraction of turmeric with methanol 96%

The best accomplished range of sensory attributes was sample 1 (ossein catfish bone/aquadest (10:50 w/w) with 0% turmeric with the mean value of acceptance 3.55±0.00. The less approved 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 the edible films of catfish bone-based gelatin were illustrated in Figure 5 and Figure 6.

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 for antimicrobial films. The testing of weight loss of apples within 20 days according to the data obtained, the uncovered apples (control) was 4.38% weight reduction which was the highest, the apples covered with edible films of catfish gelatin + turmeric extract 0% was 2.11% of weight reduction, apples packed with a mixture of edible films of catfish gelatin + turmeric extract 5% was1.44% d, and the weight reduction of apples packed with a mixture of edible film gelatin + turmeric extract 10% was 1.18%, it was the lowest among others.

One thing to be noticed is that the addition of 10% turmeric causes more thick films, 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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