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Manufacturing, Characterization, and Activity Testing of Edible Film from Sweet Orange Skin Pectin (*Citrus sinensis l.*) with the Addition of Tapioca Flour and Glycerine as Plastic

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Abstract. Preparation of edible films from the peel of sweet oranges (*Citrus sinensis L.*) has been done by the addition of tapioca starch and glycerol as plasticizers. Edible films are made by mixing pectin and starch with 0.6; 1.2; 1.8; 2.4; dan 3.0 g variations, by adding glycerin with a fixed composition of 1 mL. Once homogeneous, printed on an acrylic plate and dried at 35 to 45°C temperature in an oven for 2 days. Characterization of edible film physically by measuring thickness, tensile strength, and elongation. From the characterization obtained thickness 0.125 mm, tensile strength 0.32 kgF / mm^2 and elongation 10.2 %. Analysis of nutrient content of edible film produces 75.583% carbohydrate content, 1.707 % protein content, 0.725 % fat content, 21.2 % moisture content, and 0,785 % ash content. FTIR (Fourier Transform Infrared) spectrum analysis with absorption at 3603.03 cm^{-1} range showed hydroxyl group (OH), absorption at 2985.81 cm^{-1} and 2870,08 cm^{-1} ranges showed alkane group (CH), absorption at 1759.08 cm^{-1} range showed (C=O) group of carboxyl acid and absorption at 1168,86 cm^{-1}, 1126.43 cm^{-1} dan 1064.71 cm^{-1} ranges showed (C-O) groups of carboxyl acid. Morphology test of SEM (Scanning Electron Microscopy) produces edible film is a flat, tight, and compatible surface structure. Antioxidant activities tests with the DPPH method produce an IC_{50} value of 49.667 mg/L. Antibacterial activities tests with Kirby Bauer method produce positive tests in Staphylococcus aureus bacterial with antimicrobial index 0.374 and Escherichia coli bacterial 0.337 and Standart count Plate test in the edible film as layer cake wrapping is obtained the colony numbers less than layer cake without edible film wrapping.

Keywords: Edible Film, Pectin, Tapioca Starch, Glycerin, Activity test

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1. Introduction

Sweet orange (Citrus sinensis L.) is one type of citrus that is popular in the community (Winarno, F.G, 1992). Because too many citrus fruits are processed, there is also a lot of waste from citrus fruits in the form of the outer skin, inner peel, and seeds. Citrus fruit waste, after being researched and studied, turns out that it can produce results that have high economic value (AAK, 1994).

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Sweet oranges are oranges with about 30% of the fruit in the form of peels that have not been used, even though orange peels can be used as raw materials for the manufacture of pectin (Winarno, F.G, 1992). So far, research on pectin made from sweet orange peel has been carried out by treating pH extraction, extraction temperature, extraction time, and the ratio of the ingredients to the extracting solution (Lubis, 2003).

Several studies related to the manufacture of pectin have been carried out. Ahda and Berry (2008) stated that the pectin content in Kepok bananas ranged from 10.10% to 11.93%. The pectin content in lemon peel is 32.61% (Fitriani, 2003). In the study of extraction and characterization of pectin from Pontianak orange processing waste conducted by Hariyati (2006), the yield of pectin ranged from 4.87% to 6.95%.

One of the uses of pectin is as an ingredient for making edible films. The edible film is a thin layer made of edible material and is used for coating food (Bourtoom, 2006). Edible films formed from pectin are usually brittle, so the addition of a plasticizer is needed to change the physical properties of the film which can reduce intermolecular forces and increase the flexibility of the film by widening the vacant space of the molecule and weakening the hydrogen bonds of the polymer chains. The most common types of plasticizers used in the manufacture of edible films are glycerol, sorbitol, and polyethylene glycol (Suppakul, 2006).

Edible films have received a lot of attention in recent years because of their greater advantages over synthetic plastics. The main advantage is that edible films can be eaten together with packaged food products. Edible films are made of lipids as well as two-layer (bilayer) films or mixtures made of lipids and proteins or polysaccharides (Hui, Y.H. 2006).

Several studies on the manufacture of antimicrobial edible films have been carried out. Antimicrobial-alginate film incorporating 0.4%v/v garlic oil showed its antibacterial activity against Staphylococcus aureus and B.cereus tested bacteria using the agar diffusion method. The incorporation of garlic oil at concentrations of 0.3% and 0.4% v/v gave a significant change ($p<0.05$) in the tensile strength and elongation of the alginate film (Pranoto, 2004).

2. Materials and Methods

2.1 Equipments

The tools used in this study were: acrylic plate, caliper, cutter, blender, dropper, spatula, Erlenmeyer, reagent bottle, distilled water bottle, magnetic stirrer, hotplate, oven, analytical balance, beaker, measuring flask, measuring cup, thermometer, torsion apparatus, SEM (Scanning Electron Microscopy), FT-IR Spectrophotometer, funnel, filter cloth, ordinary filter paper, universal indicator, stative and clamps, furnace, sieve, petri dish, Bunsen, test tube, ose

needle, serology pipette, incubator, UV-Visible Spectrophotometer, autoclave, volume pipette, hockey stick, and vortex.

2.2 Materials

The material used includes sweet orange peel, 96% ethanol, 90% ethanol, tapioca flour, glycerin, distilled water, 1N HCl, Nutrient Agar (NA), Mueller Hinton Agar (MHA), McFarland standard solution, blank disc, Plate Count Agar (PCA) and 2,2-diphenyl-1-picryl-hydrazil.

2.3 Sample Preparation

The sample in the form of sweet orange peel was obtained from the Sweet Jeruk Garden in Berastagi, North Sumatra. Citrus fruit has the Latin name Citrus sinensis L. The sample preparation process was carried out by extracting sweet orange peels that had been dried at room temperature and mashed by a solid-liquid extraction method using acidified water as a solvent and then precipitation of pectin compounds with alcohol.

2.4 Extraction of Pectin from Sweet Orange Peel Powder

Weighed 200 grams of sweet orange peel powder, and put it into a 2 L glass beaker. Add 1000 mL of distilled water which has been acidified with 1 N HCl until the aquadest has a pH = 3. It was heated on a hotplate at 60ºC for 75 minutes while the magnetic stirrer was running. Then filtered with 3 layers of filter cloth and the filtrate was taken which was poured into a 1000 mL glass beaker. The filtrate was evaporated to half the initial volume and then cooled. After cooling, 96% ethanol was added and precipitated for 1 night. After 1 night, filtered with filter paper. The precipitate in the form of pectin must be purified by dissolution using hot distilled water, then re-precipitated with 90% ethanol. Filtered using a filter cloth. The pectin gel was obtained and dried in an oven at a temperature of 80 to 100ºC. Then it was smoothed and analyzed by the FT-IR test.

2.5 Making Edible Film

The preparation of two types of solutions was initially prepared in advance, namely, the first solution was a solution of 6 grams of sweet orange peel pectin dissolved in 150 mL of distilled water. The second solution is a solution containing tapioca flour with variations in the addition of 0.6 g; 1.2 g; 1.8 g; 2.4 g and 3.0 g were dissolved in 150 mL of distilled water, heated on a hot plate (until the color changed to clear) and continued with stirring using a magnetic stirrer. Then the tapioca solution is poured into a glass beaker that already contains a solution of sweet orange peel pectin. Next, add 1 mL of glycerol, then stir and heat continuously to 75ºC (for 5 minutes). Heating was continued while stirring until the temperature was 80-85ºC (for 10 minutes). The solution was poured into molds and dried in an oven at 35-45ºC for 2 days.

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2.6 Testing of the Mechanical Properties of Edible Film

Thickness Test

For each film sample to be tested, the thickness was measured at five different points using a caliper five times, namely the upper left corner, the upper right corner, the lower left corner, the lower right corner, and the middle corner. Then, find the average thickness.

Tensile Strength Test

The tensile strength at break is the tensile strength that can be achieved until the film can remain intact before film breaks or tears. Measurement of tensile strength at break is useful for knowing the magnitude of the force achieved to achieve maximum tension in each unit area of the film to stretch or elongate.

Elongation Test

The percent elongation of the edible film was obtained from the results of the tensile strength test of the product so that 2 data were obtained, namely the initial length (before the tensile strength test) and the final length (after the tensile strength test) of the edible film.

2.7 SEM Analysis

An electron beam with a diameter of 5-10 nm is directed at the specimen. The interaction of the electron beam with the specimen produces several phenomena, namely backscattering of the electron beam, X-rays, secondary electrons, and electron absorbance. In this case, the surface of the mixing of tapioca flour with sweet orange peel pectin and glycerin was observed based on the optimal mechanical properties of the edible film.

2.8 FT-IR Analysis

Analysis of the interaction of the compounds contained in the edible film in the form of stretching or indentation of functional groups displayed in the form of a wave spectrum. In this case, the functional group interaction spectrum of the edible film from a mixture of sweet orange peel pectin with variations of tapioca flour and glycerin was observed based on the optimal mechanical properties of the edible film.

2.9 Determination of Nutrient Contents

Determination of Water Content

2 g of the edible film was weighed in a weighing dish with a known weight. Dry in the oven at a temperature of 105^{o}-110^{o}C for 3 hours. Cool in a desiccator. Then weighed until a constant weight is obtained.

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Determination of Ash Content

Weighed the edible film as much as 2 g in a porcelain dish whose weight was known. It was dried in the oven. It was pulverized in an ashing furnace at a maximum temperature of 600^{o}C for 3 hours. Cool in a desiccator and then weighed until a constant weight is obtained.

Determination of Fat Content

Weighed as much as 2 g of edible film, and put it in a paper sleeve. Dry in the oven at a temperature of not more than 80^{o} C for about 1 hour. Then put into a Soxhlet tool that has been connected to a bottom flask that already contains boiling stones. Extracted with hexane for approximately 6 hours. Hexane was distilled and the fat extract was dried in an oven at 105^{o} C, cooled, and weighed to a constant weight.

2.10 Determination of Protein Content

Weighed 1 g of edible film and put it into a 100 mL Kjeldahl flask. Added 2 g of selenium and 25 mL of $H_2SO_{4(p)}$. Heated over an electric heater or burner until it boils and the solution becomes clear greenish (about 2 hours). Allowed to cool, then put into a 100 mL volumetric flask and diluted with distilled water to the marked line. Pipette 5 mL of 40% NaOH(aq) and 1-2 drops of mixed indicator. Distill for about 10 minutes. NH_3(g) was accommodated in an Erlenmeyer glass containing 10 mL of a 2% borate solution that had been mixed with the Tashiro indicator. Rinse the tip of the cooler with distilled water. Titrate with 0.1 N HCl solution.

2.11 Determination of Carbohydrate Content

Determination of carbohydrates (including fiber content) by difference is calculated as 100% minus the content of water, ash, protein, and fat.

2.12 Edible Film Antioxidant Test with DPPH Method

Preparation of 0.3 mM DPPH Solution

As much as 0.3 mM DPPH solution was prepared by dissolving 11.83 mg of DPPH powder in ethanol p.a in a 100 mL volumetric flask, then homogenized.

Preparation of Variations of Edible Film Solutions

The edible film was made from 1000 ppm mother liquor: by dissolving 0.025 edible films with ethanol as a solvent in a 25 mL volumetric flask. Then from the mother liquor a solution of 100 ppm was made, and from a solution of 100 ppm made again variations in the concentration of the solution 10, 20 and 40 ppm to be tested for antioxidant activity.

2.12 Antioxidant Test

A total of 1 mL of 0.3 mM DPPH solution was added with 2.5 mL of ethanol p.a, homogenized in a test tube, and left for 30 minutes in a dark room. After that, the absorbance was measured with a maximum wavelength of 515 nm.

A total of 1 mL of 0.3 mM DPPH solution was added to 2.5 mL of 10 ppm edible film solution, homogenized in a test tube, and left for 30 minutes in a dark room. After that, the absorbance was measured with a maximum wavelength of 515 nm. It was carried out in the same way with 20 ppm and 40 ppm edible film solutions.

2.13 Antibacterial Activity

The antibacterial activity test was carried out aseptically using the Kirby Bauer method. Each bacterial suspension was inoculated on the surface of Mueller Hinton Agar (MHA) media. Then the edible film that has been cut into discs with a diameter of 6 mm is placed on the surface of the media that has been inoculated with bacterial suspension. Bacterial cultures were incubated in an incubator upside down at 32 to 34^{o}C for 24 hours. The antimicrobial zone formed around the disc was measured using a caliper.

3 RESULTS AND DISCUSSION

3.1 Mechanical Properties of Edible Film

The results analysis of edible film characteristics of sweet orange peel pectin (C*itrus sinensis l.*) with the addition of tapioca flour and glycerin as plasticizers.

Table 1. Mechanical Properties of Edible Film

No.	Parameters	Adding Tapioca Flour	
		2.4 g	**3.0 g**
1.	Tensile strength	0,3091 kgf/mm^2	0.32 kgf/mm^2
2.	Thickness	0.124 mm	0.125 mm
3.	Elongation	9.6 %	10.2 %

3.2 Nutritional Contents of Edible Film

The results analysis of nutritional content of edible film from pectin peel of sweet orange (*Citrus sinensis l.*) with the addition of tapioca flour and glycerin as plasticizer

Table 2. Nutritional Contents of Edible Film

No.	Parameters	Adding Tapioca Flour	
		2.4 g	**3.0 g**
1.	Water	22.2 %	21.2 %

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2.	Ash	0.65%	0.785 %
3.	Fat	0.285 %	0.725%
4.	Protein	1.269 %	1.707 %
5.	Carbohydrate	75.596%	75.583 %

3.3 SEM (Scanning Electron Microscopy) Analysis

The results of the SEM examination showed the surface shape of the edible film of sweet orange peel pectin (Citrus sinensis L.) with the addition of tapioca flour and glycerin. From the characterization of the nutrient content of the edible film with the addition of 3.0 grams of tapioca flour and 1 mL of glycerin as a plasticizer, the best results were shown, so a physical SEM (Scanning Electron Microscopy) test was carried out at 500 times magnification which showed a flat surface and compatible with the type of shape. regular morphology. In the SEM analysis, it can be seen that the mixing process is almost uniform so that the edible film morphology is regular and has quite dense pores, which are between 120-720 nm.

3.4 FT-IR (Fourier Transform Infra Red) Analysis

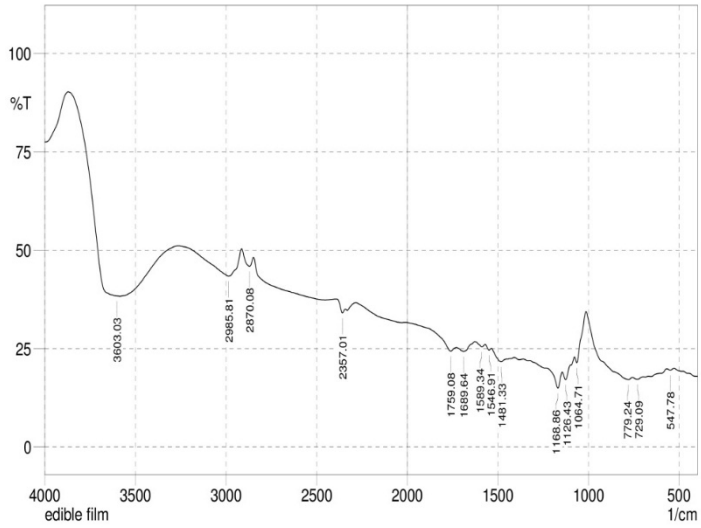

Figure 1. The FTIR spectrum of edible film

Table 3. Comparison of the frequency of functional groups of research results with theory

Functional Groups	Frequency Result (cm^{-1})	Frequency Theory (cm^{-1})
C-H	2985.81	2960 to 2850, 1470 to 1350
	2870.08	
O-H	3603.03	3650 to 3200

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C=O	1759.08	1690 to 1760
C-O	1168.86 1126.43 1064.71	1080 to 1300

3.5 Antioxidant Activity

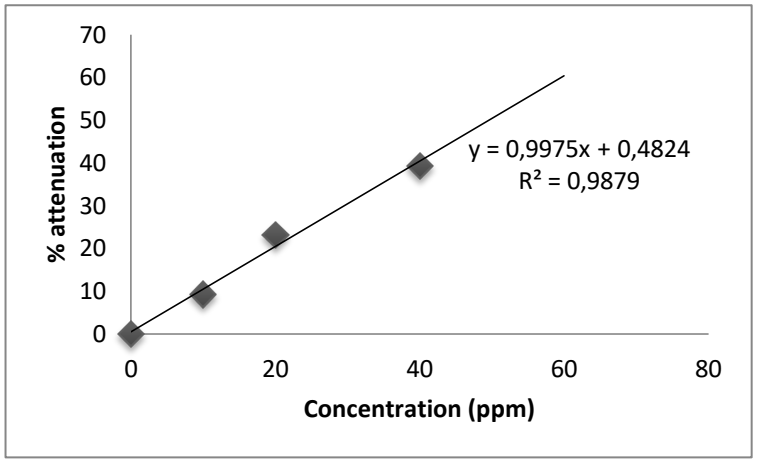

Figure 2. Edible Film Absorbance Measurement Results

From the linear regression equation, the IC_{50} value = 49.667 mg/L (49.667 ppm)

Table 4. Edible Film Absorbance Measurement Results

Sample	Absorbance	% Attenuation
Blank	0.995	0
10 ppm	0.903	9.246
20 ppm	0.764	23.216
40 ppm	0.604	39.296

3.6 Antibacterial Activity

Table 5. Antibacterial activity of edible film

No.	Bacterial Species	Inhibitory Zone Diameter (mm)	Antimicrobial Index
1	*Staphylococcus aureus*	I. 10.3 II. 9	I. 0.082 II. 0.374
2	*Escherchia coli*	I. 10.8 II. 12.3	I. 0.256 II. 0.337

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3.7 Counting the Number of Bacterial Colonies

The results of observations of colony growth on layer cakes wrapped in edible film (I) and those without wrapping (II).

Table 6. Comparison of the number of bacterial colonies

Days	Number of Bacterial Colonies	
	I	II
1	320	536
2	1,040	4,200
3	1,320	4,788

4 Conclusion

The characteristics of the best edible film of sweet orange peel pectin (*Citrus sinensis L.*) have a film thickness of 0.125 mm with a tensile strength value of 0.32 kgf/mm^2 and an elongation value of 10.2%. The nutritional content was produced from the best edible film with a carbohydrate content of 75.583%, protein content of 1.707%, fat content of 0.725%, ash content of 0.785%, and water content of 21.2%. The results of the SEM (Scanning Electron Microscopy) test have a smoother surface, small pores between 120 to 720 nm, and are dense and compatible. The results of the FT-IR (Fourier Transform Infrared) test give the edible film spectrum which shows the presence of a hydroxyl group (O-H), an alkane group (C-H), a carboxylic acid group (C=O), and a carboxylic acid group (C-O). The antioxidant test results yielded an IC_{50} value of 49.667 mg/L. The results of the antibacterial test of the edible film resulted in a positive test for *Staphylococcus aureus* bacteria with an antimicrobial index of 0.374 and *Escherichia coli* of 0.337 and the standard count plate as a wrapper for layer cakes resulted in fewer colonies than layer cakes without wrapping.

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