

Research Article

Modification of Pickle Goatskin with Silver Nanoparticles Using Brown Algae (*Padina sp.*) With Assisted Microwave

Eli Rohaeti^{1*}, Amalia Sultan Nanda Annisa², Rusita¹, Kun Sri Budiasih¹, and Nur Aeni Ariyanti³

¹Department of Chemistry Education, Faculty of Mathematics and Natural Science, Universitas Negeri Yogyakarta, 55281, Indonesia

²Department of Food Science and Technology, Faculty of Agricultural Technology, Institut Pertanian Bogor, Indonesia

³Department of Biology Education, Faculty of Mathematics and Natural Science, Universitas Negeri Yogyakarta, 55281, Indonesia

ORCID

Eli Rohaeti: <https://orcid.org/0000-0002-0930-732X>

Amalia Sultan Nanda Annisa: <https://orcid.org/0000-0002-5162-7757>

Rusita: <https://orcid.org/0000-0002-9738-4885>

Kun Sri Budiasih: <https://orcid.org/0000-0002-8116-5709>

Nur Aeni Ariyanti: <https://orcid.org/0000-0002-8995-8287>

Corresponding Author: Eli

Rohaeti; email:

eli_rohaeti@uny.ac.id

Published: 27 March 2024

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Selection and Peer-review under the responsibility of the ICMSCE Conference Committee.

Abstract.

The objectives of this study were to determine the effects of modification of goat skin with silver nanoparticles prepared using brown algae (*Padina sp.*) and assisted microwave on hydrophobic properties, antimicrobial activity, mechanical properties, and biodegradability of goat skin. The synthesis of silver nanoparticles was carried out using the microwave method with a bioreductor of brown algae extract (*Padina sp.*) and a stabilizer of a starch solution. Characterization of the resulting silver nanoparticles was conducted by determining the wavelength utilizing the UV-Vis instrument and the particle size with a particle size analyzer (PSA). Characterization of the modified goat skin was conducted by measuring hydrophobicity through the sessile drop method, the antimicrobial activity test on modified goat skin was done by determining the clear zone against *Escherichia coli* (gram-negative bacteria) and *Staphylococcus epidermidis* (gram-positive bacteria). The mechanical properties of modified goat skin were analyzed by measuring the tensile strength of the skin. The characterization with UV-Vis on the resulting silver nanoparticles revealed 426 nm in maximum absorption and the diameter of silver nanoparticles by using PSA was 58.2 nm. Based on atomic absorption spectroscopy (AAS), the study showed that as many as 99.25% of silver nanoparticle has coated the surface of pickle goat skin. The modification of goatskin by adding silver nanoparticle can increase antibacterial activity of goatskin. The contact angle and the tensile strength of goatskin after modification were 81.49° and 14.63 MPa. The clear zone of goatskin after modification against *Staphylococcus epidermidis* and *Escherichia coli* were 9.33 mm and 8.87 mm, respectively. Meanwhile, the best biodegradability was the unmodified goatskin. Thus, modification by nanoparticles can decrease the biodegradability of goatskin.

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Keywords: pickle goatskin, silver nanoparticles, brown algae

1. INTRODUCTION

Goatskin is one of hydrophilic material, could be attached by many microbes easily. In improving hydrophobicity and ant microorganism activity of goatskin could be modified by addition silver nanoparticles (AgNPs). The AgNPs could be prepared by *Padina sp.* The total tannin level of *Padina sp.* is higher than other brown algae, namely *Sargassum sp.* The Total tannin level in *Sargassum sp.* is 0.5152%, while in *Padina sp.* is 1.1321% [1]. This level can be used as a bioreductor in manufacturing nanoparticles. The reason is that tannin contains a hydroxyl group (OH) which will attack Ag^+ by releasing Hydrogen atom that Ag will bond to O. Then, through a hydrolysis reaction where H which is attached to O in the ortho position of O-Ag will be released thus, O will form double bonds and the reduction happens. Thus, the Ag bond is released from O to form Ag^0 or silver nanoparticle [2]. The ideal size for silver nanoparticles ranges from 1 - 100 nm. Nanoparticles have a larger surface area and volume compared to similar particles with the larger size. It leads to nanoparticles having more reactive properties [3]. The high toxicity to bacteria is in 1-10 nm size of the nanoparticles since it will be easier to bind to the cell membrane and enter the bacteria. Silver nanoparticles also cause increased permeability and cell death. It has a greater effect if it is generated on solid media rather than suspension in aqueous media [4, 5].

Nanoparticles can be synthesized using various methods, for example using microwave irradiation method [6], ultrasound irradiation method [7], photochemical or photosynthesis method [8], and extraction method [9]. In this study, silver nanoparticles were synthesized using a bioreductor from brown algae extract (*Padina sp.*). This synthesis is known as biosynthesis or green chemistry synthesis [10].

One of the nanoparticles applications is on goatskin pickle. Goatskin has the highest collagen level rages from 30-33%, in which the collagen will bind to silver nanoparticles [11, 12]. Nanoparticles will function as a breaker of bacteria's cell walls. There are two common types of bacteria, namely gram-positive bacteria and gram-negative bacteria having two different types of cell wall structure [13]. The lipid level in gram-positive bacteria is lower than in gram-negative bacteria. In gram-positive bacteria, the lipid content is only about 1-4%, while in gram-negative bacteria, the lipid content is around 11-22%. *Escherichia coli* is a gram-negative bacteria whose optimum growth is at 37°C [13, 14]. *Staphylococcus epidermidis* is gram-positive bacteria which is facultative anaerobic and grows optimally at 37°C [15].

As nanoparticles are physiochemical or antimicrobial, they are tested on fungi such as *Candida albicans*. This fungus has a cell wall consisting of several layers which are

proteins. The silver nanoparticles tested on the fungus *Candida albicans* will react and turn into ions when they undergo oxidation. The silver ion will bind to the thiol group (-SH) resulting in the inactivation of a certain protein in *Candida albicans* [16].

Goatskin pickle is hydrophilic that its modification with silver nanoparticles is also tested for its hydrophobicity property. The amino acids in the skin form collagen, a triple helix structure protein. These amino acids will affect the hydrophobicity of the skin [13, 17, 18]. The skin will have hydrophobic properties with a contact angle of 90° - 150° , while 150° - 180° indicates that the skin is super hydrophobic. If the contact angle is less than 90° , then the skin is hydrophilic and is super hydrophilic if the value is 0° - 5° . The method used in determining the hydrophobicity properties is the sessile drop method which measures the contact angle [18, 19].

The tensile strength test includes the tensile strength test, flexibility, and modulus young. The purpose of conducting the tensile test on the skin is to determine the maximum stress, yield stress, and strain. This test results in a change in properties from elastic to plastic [20]. Tensile strength is inversely proportional to elongation [21].

Biodegradation is a process of microbiological attack of polymers by microorganisms such as bacteria or fungi. The biodegradation method can be carried out using mixed cultures. The growth pattern of microorganisms in mixed culture was made to determine changes in the mixed culture of microorganisms that play an active role in fermentation by isolating the product microorganisms [22]. Characterization carried out in biodegradation is the characterization of mass loss. This mass loss is determined by weighing the polymer mass before and after the biodegradation process at a certain time. The actual mass loss can be calculated by including a mass correction factor for the mass of the sample before biodegradation, which was obtained from negative control [23, 24]. The objectives of this study were to prepare AgNPs by using *Padina sp* and to apply AgNPs in improving characteristics of goatskin, especially antibacterial, antifungal activities, mechanical properties, and also hydrophobicity property.

2. RESEARCH METHOD

2.1. Materials and Instrumentation

Materials were goatskin pickle, brown algae leaves (*Padina sp.*), solid AgNO₃, distilled water, isolated microorganism, PA starch powder, *Whatman* filter paper No.41, *nutrient broth* (NB), *nutrient agar* (NA), PDA, ethanol 70%, and activated sludge. The tools

which used in this study were Shimadzu UV-2400 UV-Vis Spectrophotometer, Particle Size Analyser (PSA) Microtac Nanotrac Wave II, Tensilon Tensile Strength Tester, Microwave, Sonication Tool, blender, Laminar Air Flow (LAF) Shimadzu SCB-1000A, Autoclave HICLAVE HVE-50, Colony counter SIBATA CL-560, analytical scale, Petri dish, callipers, ruler, micropipette, beaker, Erlenmeyer flask, measuring flask, glass stirrer, pipette, thermometer, measuring cup, ball pipette, crucible clamp, funnel, magnetic stirrer, hotplate, flacon bottle, oven, aluminium foil, plastic wrap, spatula, shaker, wire loop, dryglasky, and camera.

2.2. Extraction of Brown Algae and Preparation of Silver Nanoparticle

The brown algae were washed and dried for 24 hours. The dried brown algae were cut into small pieces and weighed 100 grams. These pieces then were immersed in 1000 mL of water and were heated for 15 minutes at 80 ° C with stirring once in a while to avoid the saturated solution. Then, the mixture was cooled and filtered with *Whatman* filter paper No.41 to obtain brown algae extract. The extracted filtrate was put into 1000 mL bottles and the period could be extended by storing it in the refrigerator.

Biosynthesis of silver nanoparticles using the microwave method was conducted twice (*duplo*) by mixing AgNO_3 1×10^{-1} M solution with brown algae extract in a 9: 1 ratio. Then, 100 mL starch solution was added to the mixture as a stabilizing agent [24]. Lastly, it was put in the microwave for four minutes at 300W. The characterization was performed using a UV-Vis spectrophotometer and Particle Size Analyser to determine the particle size of silver nanoparticle.

2.3. Modification of Goatskin by Deposition of Silver Nanoparticle

Goatskin Pickle was cut to 15 cm x 15 cm in size then washed using distilled water and dried at room temperature. The colloidal silver nanoparticles that have been made (microwave, ultrasound, and extraction methods) were then immersed in an Erlenmeyer flask containing 100 mL of colloidal silver nanoparticles and were shaken for 24 hours at 155 rpm speed [24]. The skin was dried for 24 hours to produce NP-skin.

2.3.1. Test of Antibacterial Activity, Antifungal Activity, Hydrophobicity, and Mechanical Properties

Testing on bacteria was done by planting them on NA and NB media in Petri dishes. Then, those were tested for antibacterial on the Petri dish according to the written label. The Petri dishes were sealed with plastic wrap and placed in a room at 37°C. Observation of the antibacterial clear zone was carried out every three hours for 48 hours.

Testing on fungi was conducted by planting it on PDA media in the Petri dish. Thereafter, the sample was tested for antifungal on a Petri dish according to the written label. The Petri dish was sealed with a plastic wrap and placed in a room with low temperature. Observation of the antifungal clear zone was carried out every six hours for 24 hours, then the observation done once in 24 hours for two days.

The hydrophobic test of goatskin pickle was carried out using the sessile drop method. The skin sample was placed on a flat surface and put the sample in the same position. Then, distilled water was dripped from 1 cm height using a pipette on the skin surface. After dripping the distilled water, then seized the distilled water droplets on the skin surface with the adjusted settings. After that, the photo results were processed utilizing Corel Draw X5 with the Angular Dimension Tool to determine the resulted contact angle.

Goatskin pickle samples before and after modification were cut as dog bone or dumbbell specimens with SNI 06-1795-1990 standards amounting to two pieces per sample, then those skin samples were tested for their tensile strength and elongation by pulling them until they broke. This tensile strength test used Tensilon tensile strength meter with 2000 kgf Lod cell and 50 mm gauge length.

2.4. Biodegradation of Goatskin

Biodegradation was done by making the media using 14 grams of NB and dissolving it in 500 mL of distilled water and boiling it. The tools and materials were then autoclaved for one hour and then stored in the oven for one night. The media was then poured on the Petri dish. The skin sample was then immersed in activated sludge and then implanted in the media. Measurement of skin degradation was carried out for 15 days with media replacement every five days.

3. RESULTS AND DISCUSSION

3.1. Characteristics of Silver Nanoparticles

Synthesis of silver nanoparticles was carried out by assisted microwave. A stabilizing agent was used to keep the formed silver nanoparticles from agglomeration [24, 25]. The solution colour before heating using microwave was light orange. It changed after the heating process into a slightly darker colour.

Testing the AgNO_3 solution show absorption at 210 nm (Figure 1), however the silver nanoparticles have absorption at 426 nm (Figure 1). The diameter size of silver nanoparticles (AgNPs) produced synthesis using microwave was 58.2 nm with a percentage of 79.5% (Figure 2). The particle size of biosynthesized silver nanoparticles using algae (*Padina sp.*) extract with microwave method showed good results. It can be seen from the theory explaining that the size of silver nanoparticles is considered good when it is between 1-100 nm [29-31]. Observing the diameter size of silver nanoparticles synthesized using brown algae extract (*Padina sp.*), they showed an agglomeration as that the particle size was more than 100 nm.

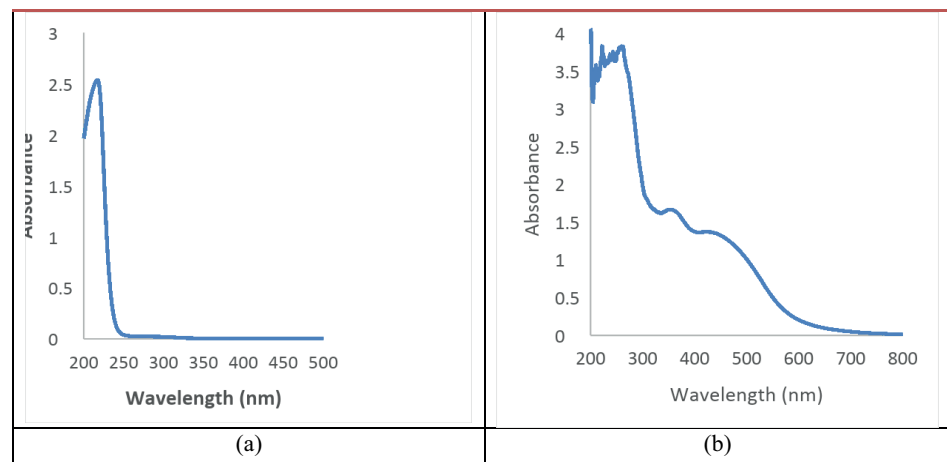


Figure 1: The UV-VIS spectrum of silver nitrate solution (a), and silver nanoparticle (b).

3.2. Modification of Goatskin Pickle

The modified goatskin pickle with silver nanoparticles showed different results prior to the modification. This can be seen from its colour becoming slightly more greyish. This colour change indicated that the silver nanoparticles were bound to the goatskin pickle. This change also occurred in colloid fluids before and after modification.

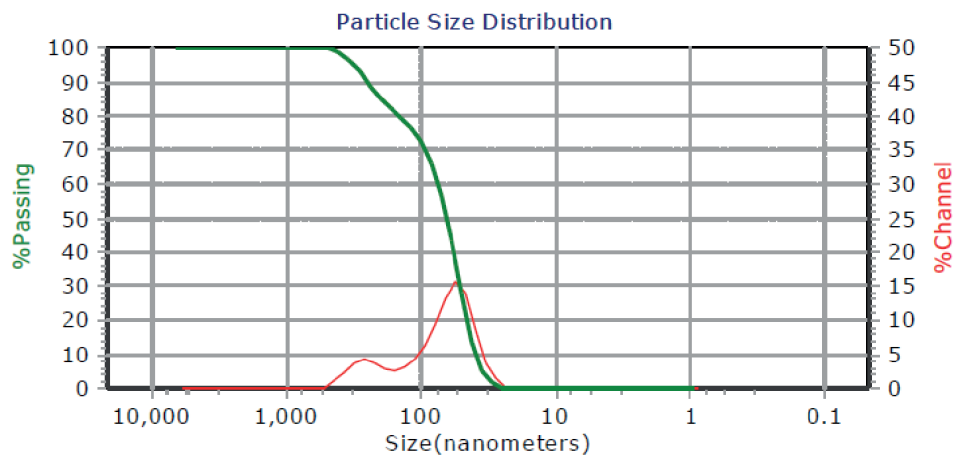


Figure 2: Distribution of silver nanoparticles.

Based on AAS data showed that initial concentration of AgNO_3 as many as 170 ppm and final concentration of AgNO_3 as many as 1.2730 ppm. The modified skin of silver nanoparticles as the preparation's result of microwave method absorbed the percentage of nanoparticles amounting to 99.25%. The binding of Ag to the skin was carried out by binding the secondary metabolites resulting from a reduction reaction with skin collagen. The reason was that the metabolites had polyphenol groups and carboxylic acids [26].

3.3. Contact Angle of Goatskin

Goatskin without silver nanoparticles modification produced the average value of contact angle by 47.077° . In contrast, goatskin with silver nanoparticles modification had the higher contact angle value, it was 81.4933° . This can occur because silver nanoparticles fill the fibres first causing the surface to be more evenly and homogeneous so that that the roughness of surface and chemical composition can increase the hydrophobicity of material. The silver nanoparticle produced a rough surface and a dense layer that supported a hydrophobic surface [27].

Goat leather contains protein, especially collagen which is composed of amino acids. The basic structure of collagen is three chains joined to form a triple helix conformation containing peptide groups (-NH) and (-C = O) [28]. Based on the results of this study indicate that the addition of silver nanoparticles to pickle goat leather gives an increase in the value of the contact angle, but the value of the contact angle is still in the hydrophilic category because it is less than 90° .

3.4. Antibacterial Activity of Goatskin Pickle against *Escherichia coli* and

3.4.1. *Staphylococcus epidermidis*

Based on the observations, a clear zone has resulted in some periods from each sample (Table 1). Based on this data, a graph shows the relation between the diameter of the clear zone and time of incubation (Figure 3). The order of inhibition of sample against *Escherichia coli* from the largest to the smallest was positive control (chloramphenicol), goatskin + AgNPs, goatskin, and negative control.

TABLE 1: The diameter of clear zone of goatskin before and after modification against *E. coli*.

Time (hours)	Diameter of Clear Zone (mm)		
	Skin	Skin + AgNP	Positive Control (Chloramphenicol)
0	0	0	0
3	0	0	0
6	7.33	7.90	11.97
9	7.41	7.07	13.17
12	5.13	5.30	10.03
15	5.90	6.90	11.30
18	5.20	5.97	9.20
21	4.63	6.30	10.00
24	4.93	5.94	9.73
27	6.73	6.23	13.00
30	6.73	7.97	11.73
33	4.80	6.00	10.73
36	5.23	4.53	10.40
39	6.87	5.40	11.07
42	6.20	7.03	12.37
45	6.70	7.07	10.93
48	6.23	8.87	11.80

Based on Table 1 shows that the incubation time is up to 3 hours, all samples have not shown antibacterial activity. Starting at 6 hours, all samples showed antibacterial activity. Thus, these samples need time to adapt to inhibit the growth of *E.coli*. Figure 3 shows that the goatskin + AgNPs had lower antibacterial activity than the positive control, but higher than the skin without the addition of silver nanoparticles. Metal nanoparticles have antimicrobial activity because of their ability to bind to protein molecules in microbial cells so that they interfere with microbial metabolic activity and subsequently are able to kill microbes. Silver metal is a metal that is often used because

of its non-toxic nature to human skin. The diameter of the clear zone formed around the sample shows the ability to inhibit bacterial growth. The greater the diameter of the clear zone formed, the stronger the inhibitory power of the compound against bacterial growth.

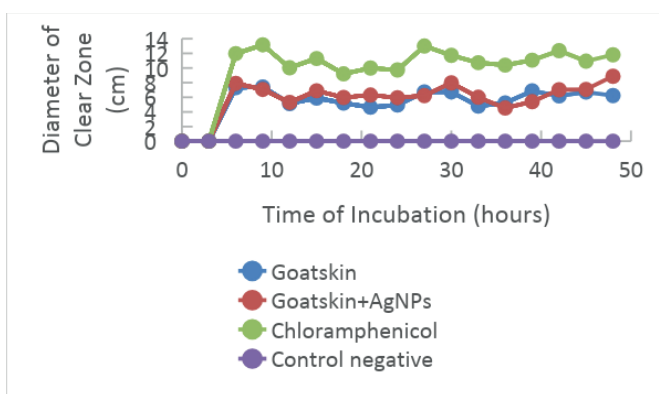


Figure 3: The diameter of clear zone versus time in antibacterial testing of skin against *Escherichia coli*.

Based on Table 2 shows that the incubation time is up to 3 hours, all samples have not shown antibacterial activity against *S. epidermidis*. Starting at 6 hours, all samples showed antibacterial activity. Thus, these samples need time to adapt to inhibit the growth of *S. epidermidis* bacteria. Figure 4 shows that the goatskin sample had lower antibacterial activity against *S. epidermidis* than the positive control, but higher than the skin with the addition of silver nanoparticles.

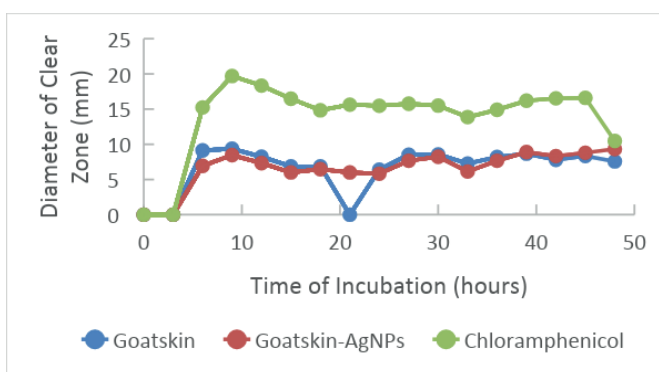


Figure 4: The diameter of clear zone versus time in antibacterial testing of skin against *Staphylococcus epidermidis*.

However, all sample has antibacterial activity against *Staphylococcus epidermidis* higher than against *Escherichia coli*. This provides information that the best inhibition of bacteria for modified goatskin pickle with silver nanoparticles was *Staphylococcus*

TABLE 2: The diameter of clear zone of goatskin before and after modification against *S. epidermidis*.

Time (hours)	Diameter of Clear Zone (mm)		
	Goatskin	Goatskin-AgNPs	Chloramphenicol
0	0	0	0
3	0	0	0
6	9.13	6.93	15.23
9	9.37	8.47	19.70
12	8.23	7.33	18.33
15	6.87	6.00	16.47
18	6.87	6.47	14.83
21	6.67	6.00	15.63
24	6.40	5.83	15.47
27	8.53	7.67	15.77
30	8.57	8.23	15.50
33	7.27	6.13	13.87
36	8.20	7.67	14.90
39	8.67	8.90	16.20
42	7.80	8.33	16.53
45	8.33	8.80	16.60
48	7.57	9.33	10.47

epidermidis bacteria. This is in accordance with the theory that *Staphylococcus epidermidis* or gram-positive bacteria have a thick peptidoglycan layer and their structure is dense. Moreover, it also contains teichoic acid which is a –OH group (eg ribityl and alcohol) and a phosphate group. These groups play a significant role in interactions with silver nanoparticles. Meanwhile, *Escherichia coli* only has a thin peptidoglycan layer [27–29].

3.5. Tensile Strength of Pickle Goatskin

The mechanical properties of modified goatskin pickle with silver nanoparticles can be seen by performing a tensile strength test (Figure 6). This graph can be used to determine the mechanical properties of goatskin pickle, such as hard and brittle, hard and strong, hard and tough, and also soft and tough. Based on the graph, the modified goatskin pickle with silver nanoparticles considered as hard and tough. The reason was that the curves were in the hard and tough areas. The tensile strength of the modified goatskin pickle was better than the unmodified version. The modified goatskin pickle with silver nanoparticles gave the more tough.

Meanwhile from the elongation data, the modified goatskin pickle with silver nanoparticles gave the longer than goatskin without modification. The adding of silver nanoparticles can increase elongation of goatskin.

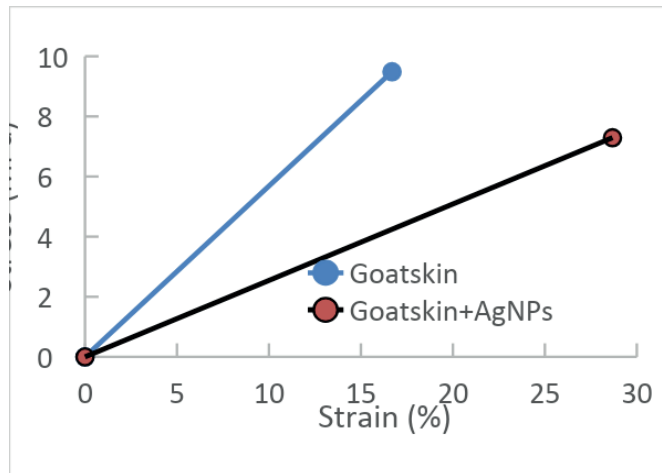


Figure 5: Curve of stress-strain of goatskin and goatskin+AgNPs.

3.6. Biodegradation of Silver nanoparticles Modified Goatskin Pickle

Based on Figure 7 and 8, mass loss tends to increase along with the incubation time. Meanwhile, the degradability or mass loss rate tends to decrease as the incubation time increased. Based on the graph, it can be seen that the skin without modification with silver nanoparticles gives the best degradability.

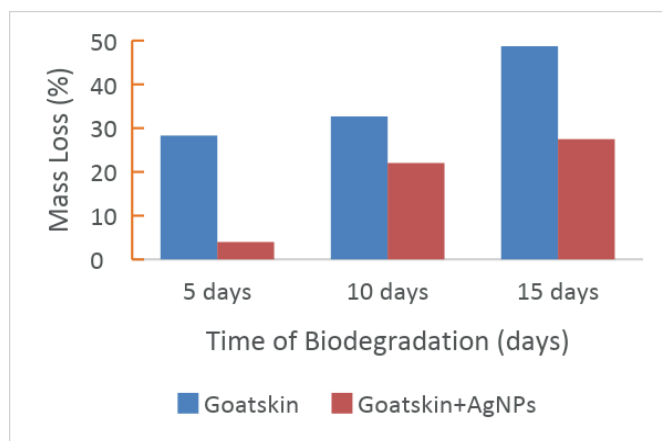


Figure 6: Mass loss versus time of biodegradation of goatskin before and after modification.

Based on Figure ?? shows that the degradability of goatskin before modification with silver nanoparticles is higher than degradability of goatskin after modification.

Thus, addition nanoparticle can decrease ease of degradation of goatskin. However, nanoparticle in goatskin can inhibit microbes in hydrolyzing of functional groups in goatskin [26].

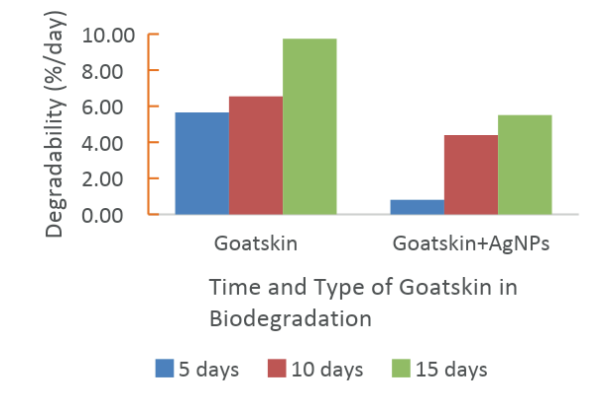


Figure 7: Degradability versus time of biodegradation of goatskin before and after modification.

4. CONCLUSION

Based on the UV-Vis test, there was an absorption for the microwave method at a wavelength of 426 nm and an absorbance of 0.024. Meanwhile in the PSA test, the nanoparticle size was 58.2 nm. Modification of goatskin pickle with silver nanoparticles by microwave method revealed significant results on the antibacterial and the antifungal properties. Modification of goatskin pickle with silver nanoparticles gave the best inhibition results in gram-positive bacteria (*Staphylococcus epidermidis*). The skin modification giving the best increase in contact angle was the skin modified with silver nanoparticles by the microwave method. The increase in time of degradation has an effect on the increase of mass loss, but the increasing incubation time has the effect on decreasing the rate of degradation (decreased degradability).

Acknowledgments

This research was funded by the Ministry of Education and Culture, Research and Technology, Republic of Indonesia by Applied Research, year 2022.

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