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CHARACTERISTICS OF BIODEGRADABLE PLASTIC FROM SEA CATFISH (ARIUS THALASSINUS) BY-PRODUCTS: GELATIN AND NANOCALCIUM

Fadia Haya Safitri1, Isdiana Fitriyani1, M. Dafa Rosa Putra Maulana2, Nuraeni Setya Ningrum1, Irfany Amelia Putri1, Dan Retno Ayu Kurniasih1*

[1]Department of Fishery Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University.

[2]Department of Capture Fisheries, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Soedarto, SH., Tembalang, Semarang.

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***Corresponding Author:** Retno Ayu Kurniasih
Department of Fishery Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University.
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ABSTRACT

Plastic is practical and inexpensive packaging material, but it does not decompose easily and can pollute the environment. Biodegradable plastic offer an alternative for eco-friendly containers and can be produced from sea catfish by-products, such as bones and swim-bladders. This study was conducted to examine the effect of different nanocalcium concentrations and gelatin on the characteristics of biodegradable plastic. The production method involved extracting gelatin from swim-bladder and extraction of calcium from bone using NaOH, followed by mixing the gelatin, nanocalcium, distilled water, CH3COOH, glycerol and alcohol. The results showed that different nanocalcium concentrations significantly affected (p < 0.05) the characteristics of biodegradable plastic. Higher nanocalcium concentration increased the thickness, solubility and elongation of the plastic. However, a 2% nanocalcium concentration was optimal for increasing tensile strength and producing a denser morphology. The addition of nanocalcium did not have a significant effect (p > 0.05) on the Water Vapor Permeability (WVP) and biodegradation values. The resulting biodegradable plastic exhibited a thickness of 0.14 to 0.37 mm, a solubility of 70 to 120 min, an elongation 32.22 to 115.56%, a tensile strength 3.60 to 12.42 MPa, and a WVP value of 1.49 to d. 1.72 g H2O/(m2.day). Based on these data, sea catfish swim bladder and bones can be used as an alternative raw material for biodegradable plastic production.

KEYWORDS: Sea catfish; Biodegradable plastic; Gelatin; Nanocalcium.

1. INTRODUCTION

Plastic are widely used in daily life due to their lightweight, elastic, durable, water-resistant, and cost-effective properties. However, plastic have the disadvantage of not being easily decomposed naturally by microbes in the soil. Therefore, they causes a buildup of plastic waste and contribute to pollution and environmental damage. According to data from the Ministry of Environment and Forestry of Indonesia (KLHK) (2019), the percentage of plastic waste in Indonesia was between 14% and 16% (10.43 million tons) of the total waste pile (65.2 million tons) in 2016 and the amount increased to 17% of the total waste of 67.8 million tons in 2019. However, it is undeniable that the use of plastic to package food products is irreplaceable. Therefore, an alternative is needed by using environmentally friendly plastic to package food products.

Biodegradable plastics can be an alternative to conventional plastic (synthetic plastics) which have the advantage of being able to degrade after being disposed of into the environment because they are decomposed by the activity of microbial enzymes in the soil (Handayani & Wijayanti, 2015). Kamsiati *et al*. (2017) added that biodegradable plastics can be made from natural polymer materials such as gelatin.

Gelatin is a type of protein derived from the natural hydrolysis of collagen in the skin and bones. Gelatin can be produced from fishery by-products, such as bones, swim-bladders, scales and fish skins (Nurilmala *et al*., 2017; Siburian *et al*., 2020). Meanwhile, the characteristics of the film layer formed from gelatin can be improved by the addition of nanocalcium. Petcharat dan Benjakul (2017) explained that proteins are able to bind to nanocalcium so that they form a strong, compact film layer that resists low temperature (<0ºC). Nanocalcium is calcium that has a super-small size of 500 x 10-9 nm, so it has a higher bioavailability than macro-sized calcium.

Research on making gelatin from various fish swim-bladders has been carried out, specifically from striped catfish (Sitepu *et al*., 2019), yellowfin tuna (Hadinoto *et al.*, 2019), and pike-conger (Kartika *et al.*, 2016). Furthermore, the manufacture of edible film using gelatin has been carried out by Sutono & Pranoto (2013), specifically from black tilapia skin gelatin. The results of nanocalcium research from various fish bones have also been reported, namely yellowfin tuna (Prinaldi *et al.*, 2018), striped catfish (Sumarto *et al*., 2021), and snakehead murrel (Tawali *et al.*, 2019). Furthermore, the application of nanocalcium from fish bones in food has also been carried out by Dewi *et al*. (2020) to increase the strength of the gel in kamaboko. However, research on the use of sea catfish swim-bladders for gelatin and sea catfish bones for nanocalcium and the manufacture of biodegradable plastics from a mixture of gelatin and nanocalcium has never been done before.

The Ministry of Maritime Affairs and Fisheries of Indonesia has reported that the total production of sea catfish in Indonesia was 114,108.70 tons in 2018 and left waste around 50 to 60%. Sea catfish in Indonesia is consumed in the form of processed salted fish and smoked fish, which in the processing process leaves bones, skin, swim bladders, eggs, heads, and fins. Based on the description above, it is necessary to conduct study on the manufacture of biodegradable plastics from sea catfish swim-bladder gelatin and sea catfish bone nanocalcium. This study was conducted to examine the effect of the addition of nanocalcium with different concentrations on the characteristics of biodegradable plastics from gelatin. The results of this study are expected to provide solutions to environmental problems caused by plastics and fish processing by-products. The plastic is expected to have the same or better quality than conventional plastics, so it can be used to package food products and can be decomposed by microbes in the soil.

2. MATERIALS AND METHODS

2.1 Materials and equipment

The equipment used in this study included an oven (Sharp Corporation, Japan), a micrometer (Micrometer Gauge, China), a waterbath (WiseBath, Korea), a Scanning Electron Microscopy (SEM) (Jeol JSM 6510LA, Japan), a Universal Testing Machine Auto Strain (A PPT Group Company, UK), and a 200-mesh sieve (China Famous Manufacturer, China). The materials used are dried sea catfish swim-bladders and fresh sea catfish bones obtained from UD SI (Lamongan, Indonesia). The chemicals used included distilled water, 70% pure alcohol, 99% CH3COOH, glycerol, NaOH, and EM 4 bacteria (EM 4 Agriculture, Indonesia).

2.2 Gelatin Extraction

Dried sea catfish swim-bladders were cut into 1x1 cm pieces. The cut swim-bladders were then soaked in a 0.1 M NaOH solution in a ratio of 1:10 (b/v) for 8 h at a temperature of 4 oC with NaOH regulation every 2 h (Liu *et al*., 2015). Based on the modified research of Sompie *et al*. (2015), the swim-bladders were then washed with cold distilled water until a neutral pH was reached. Afterward, the swim-bladders were soaked in an acetic acid solution (0.5 M CH3COOH) with a ratio of 1:2 (w/v) for 52 h. Gelatin extraction was carried out by heating the swim-bladders in a ratio of 1:3 (w/v) at 80 ºC for 3 h. The gelatin solution was filtered using a cheesecloth and dried at 60 ºC for 3 d (until dry gelatin was obtained). The dried gelatin was crushed and sifted through a 200-mesh sieve to produce gelatin powder.

2.3 Preparation of Nanocalcium

Based on a modified by Benjakul *et al.* (2017), the preparation of nanocalcium began by washing the fish bones with water to remove adhering dirt and blood. The fish bones were then boiled, followed by the removal of any remaining attached meat. Next, the fish bones were boiled again for 2 h using distilled water. The bones were then dried at a temperature of 60−65 ºC. Calcium extraction was carried out using a NaOH solution with a ratio of a 1:3 (w/v) for 3 h at 100 ºC, whit the NaOH solution being replaced every hour. The extracted fish bones were neutralized, filtered, and dried at 50 ºC for 12 h. Finally, the bones were ground and sifted through a 200-mesh sieve..

Table 1. Formulation of Materials for Biodegradable Plastic Production.

Material Name	**Quantity (%)**			
	0%	**2%**	**4%**	**6%**
Gelatin	3	3	3	3
Nanocalcium	0	2	4	6
Alcohol	10	10	10	10
Glycerol	0.75	0.75	0.75	0.75
Aquatics	84.25	82.25	80.25	78.25
CH3COOH (v/v%)	2	2	2	2

2.4 Preparation of Biodegradable Plastic

Based on modified method by Fachry & Sartika (2012), the biodegradable plastics were carried out by dissolving 0%, 2%, 4% and 6% (w/v) nanocalcium in alcohol. Gelatin was dissolved in distilled water, and then added with CH3COOH was added at a temperature 80 oC. The dissolved gelatin and nanocalcium solutions were then mixed with glycerol in a container at 80 oC for 45 min until a homogeneous solutionwas formed. Next, the solution was cast and dried at 60 oC for 6 h. The formulation of the ingredients is presented in Table 1.

2.5 Visual and Morphological Observation of Biodegradable Plastics

The resulting biodegradable plastic were visually observed for their color, texture, presence of air bubbles, and cracks. The surface morphology of the biodegradable plastic was examined using a Scanning Electron Microscope (SEM) to determine the surface structure, cracks, and surface smoothness of the biodegradable plastic products (Eich *et al*., 2015). Morphological observations were conducted using the SEM at an accelerating voltage of 10 kV and a magnification of 1,000x. The biodegradable plastic samples were first coated with platinum before being visualized with the SEM.

2.6 Water Solubility Test

Based on a modified method by Negim *et al*. (2014), the water solubility test of the biodegradable plastic was conducted by cutting the plastic into small specimens of 3x3 cm2. The specimens were then placed into a measuring cup containing 30 mL of water at room temperature. Next, the time required for the plastics to completely dissolve in the water was recorded.

2.7 Thickness Test

The thickness of the biodegradable plastic test was measured using a micrometer (Rhim, 2011). The ability of a biodegradable plastic to package and protect a product is significantly influenced by an important parameter, namely thicknes. Elongation and tensile strength are mechanical properties that can be related to the thickness of a plastic, which also affects gas permeability. A lower gas permeability value, often resulting from increased plastic thickness, ensures that the packaged product is well protected.

2.8 Elongation and Tensile Strength Test

Elongation and tensile strength tests were carried out with plastic samples tested using the Universal Testing Machine at a speed of 10 mm/min. Plastic samples were stored at 30 °C for 24 h before taking measurements. This tensile strength was determined based on the maximum load, while the elongation was calculated based on the biodegradable plastic break (Apriyani & Setyadi, 2015).

2.9 Water Vapor Permeability (WVP) Test

The WVP test was conducted by cutting a bioplastic sample measuring 6x6 cm with a known thickness. The plastic was then sealed using double tape onto the top of a glass cup containing 20 g of silica gel (previously dried to a constant weight). The sealed cup was then stored in an dessicator containing distilled water for 6 d and subsequently weighed. The increase in the weight of the silica gel was recorded to calculate the WVP of plastic (Dwimayasanti, 2016).

2.10 Biodegradation Test

The biodegradation test was carried out using the soil burial method. Biodegradable plastic samples measuring 2.5x4 cm were weighed to determine their initial weight (day 0). Next, the samples were buried in a container filled with 1 kg of soil and 10% EM4 bacteria (v/w). The plastic were then removed, cleaned, and weighed on days 2, 4, 6, 8 and 10.

2.11 Data Analysis

Data were presented as the mean of three replicates ± standard deviation. The obtained data were analyzed based on a Completely Randomized Design (CRD) using Analysis of Variance (ANOVA). A Tukey's Honestly Significant Difference (HSD) test was then conducted to determine significant differences between treatments ($p < 0.05$). SPSS version 16 (International Business Machines Corporation, USA) was used for the data analysis.

3. RESULTS AND DISCUSSION

3.1 Biodegradable Plastic Film Observation

Table 2 and Figure 1 show that the addition of nanocalcium in the preparation of biodegradable plastics from gelatin affected the visual characteristics of the resulting films, including color, texture, air bubbles, and cracks. Based on the visual observations of biodegradable plastics, higher concentration of nanocalcium resulted in a yellowish-white color of the plastics, which was attributed to the natural white color of the nanocalcium. Similarly, the texture became slightly more rigid after the addition of nanocalcium, along with the absence of air bubbles in the plastic. However, minor crack were observed on the plastic films with 4% and 6% nanocalcium concentrations. According to Udjiana *et al*. (2021), the optimal bioplastic from durian seeds with added calcium exhibited a transparent color, almost no bubbles, and a smooth surface. The presence of air bubbles is typically caused by trapped air during the plastic casting process.

Table 2. Observation of Biodegradable Plastics with Different Concentrations of Nanocalcium.

Nanocalcium Concentration Treatment	Color	Texture	Air Bubbles	Cracks
0%	Clear Yellow	Flexible	Slight	None
2%	Clear Yellow	Slightly stiff	None	None
4%	Yellow White	Slightly stiff	None	Slight
6%	Yellow White	Slightly stiff	None	Slight

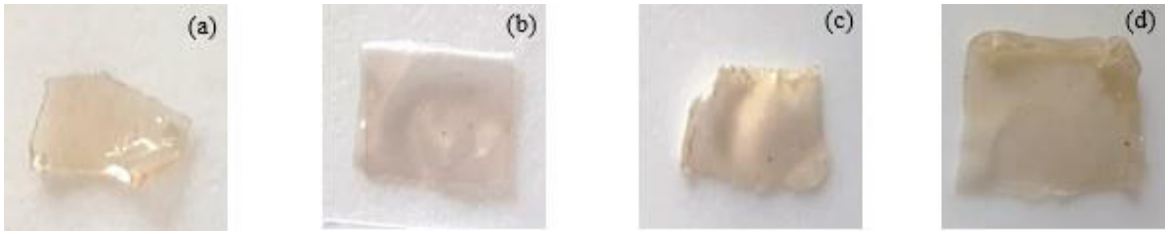

Figure 1. Biodegradable Plastic Film with Different Concentrations of Nanocalcium. (a). 0%; (b). 2%; (c). 4%; dan (d). 6%.

3.2 Plastic Morphology Observations

The SEM observations revealed that different nanocalcium concentrations affected the surface morphology of the biodegradable plastics. The biodegradable plastic with 2% nanocalcium exhibited a more compact film structure with no visible pores (Fig. 2). Furthermore, no nanocalcium agglomeration was observed, unlike in the 4% and 6% nanocalcium treatments. This agglomeration was likely caused by the excessive amount of nanocalcium used, which prevented it from interacting optimally with the gelatin matrix. According to Udjiana *et al*. (2021), cioplastics can exhibit different morphological appearances, where a well-integrated mixture of filler and polymer matrix produces a uniform texture that contributes to good tensile strength. Matondang et al. (2013) added that the presence of small grainules in bioplastics indicates filler particles that failed to interact properly with the matrix. The properties of the nanocalcium might also have been affected by a decrease in purity during the production and packaging processes, leading to poor homogenization.

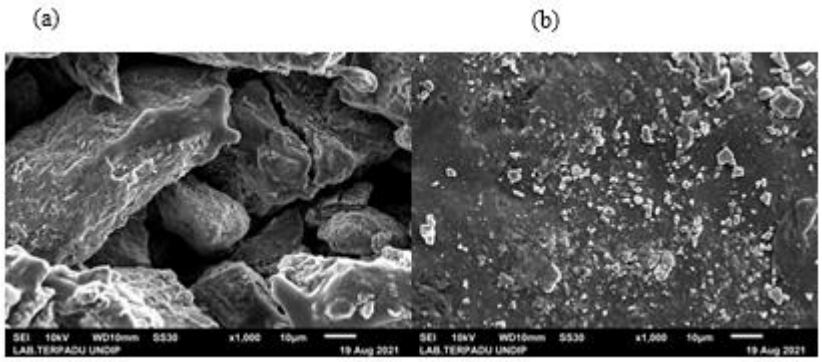

Figure 2. Morphological Observations of Biodegradable Plastics with Different concentrations of Nanocalcium. (a). 0%; (b). 2%; (c). 4%; and (d). 6%.

3.3 Plastic Thickness

The different concentrations of nanocalcium had a significant effect on the thickness of the plastic ($p < 0.05$), indicating that higher amounts of nanocalcium increased the thickness of the resulting biodegradable plastic (Table 3). Biodegradable plastics with 6% nanocalcium exhibited the greatest thickness among the samples in this study. Similar results were reported by Rusli *et al*. (2017), who stated that the thickness of the produced plastic can increase due to the addition of dissolved solids during manufacturing process. The increase in thickness could also be attributed to the unique association of colloidal compounds acting as thickeners and suspending agents,facilitating stronger interactions among the constituent components. The thickness of the plastic in this study ranged from 0.14 to 0.37 mm. These values are higher than those of the biodegradable plastics produced by Pramananta *et al.* (2019) using fresh seaweed *Ulva lactuca* which ranged from 0.10 to 0.22 mm.

Table 3. Physical Characteristics of Plastics with Different Nanocalcium Concentration.

Nanocalcium Concentration Treatment	Thickness (mm)	Solubility (min)	Elongation (%)	Tensile Strength (MPa)	WVP (g H2O/m2.day)
0%	0.14±0.01a	70±1.24a	76.35±9.47b	7.26±0.65c	1.71±0.11a
2%	0.24±0.04ab	78±3.39a	32.22±0.54a	12.42±1.02d	1.49±0.06a
4%	0.28±0.05bc	105±7.36b	103.53±2.62c	3.60±0.40b	1.60±0.05a
6%	0.37±0.02c	120±4.98b	115.56±4.08c	1.26±0.42a	1.72±0.11a

Note: Different superscript letters within the same column indicate significant differences ($p < 0.05$).

3.4 Plastic Solubility

Table 3 shows that the different concentrations of nanocalcium had a significant significant effect on the water solubility of the plastic ($p < 0.05$). The longest dissolution time was observed at the 6% concentration with an average of 120 min, while the shortest time was recorded at the 0% concentration with an average of 70 min. This aligns with the findings of Ningsih *et al.* (2019), who reported that a higher addition of calcium powder particles makes it more difficult for the plastic to absorb water. The incorporated calcium particles can increase the water resistance of the plastic. The biodegradable plastics produced in this study dissolved slower than biodegradable plastics reported by Hidayati *et al.* (2019), which exhibited dissolution times ranging from 54 to 98 min for bioplastics made from seaweed waste.

3.5 Elongation

Elongation, or elongation at break, is a crucial parameter for determining the elasticity and flexibility of biodegradable plastic films. The addition of nanocalcium in the preparation of the biodegradable plastics had a significant effect on the elongation percentage ($p < 0.05$). Specifically, the addition of 2% nanocalcium resulted a lower elongation value compared to the control plastic (without nanocalcium). However, the elongation of the plastics increased at the 4% and 6% nanocalcium concentrations. The nanocalcium incorporated into the biodegradable plastics can enhance the protein (gelatin) bonds within the matrix. However, higher concentration of nanocalcium resulted in agglomeration (lumps) within the plastics. This is in line with the findings of Wariyah *et al*. (2014), who reported that analog rice treated with high calcium supplementation exhibited a lower degree of gelatin binding.

According to Coniwanti *et al.* (2014), the elongation percentage is inversely proportional to the tensile strength. A decrease in plastics elasticity is caused by a reduction in the intermolecular bond distance. Once the saturation point is exceeded, the excess plasticizer molecules separate from the polymer phase, which lowers the intermolecular forces between the polymer chains. This allows the chains to move more freely, thereby increasing flexibility. According to international plastic standards (ASTM 5336), the elongation percentage for Poly Lactic Acid (PLA) plastic from Japan is 9%, while Polycaprolactone (PCL) plastic from the UK reaches 500% (Utomo *et al*., 2013). The elongation values obtained in this study met the standards for PLA plastics.

3.6 Tensile Strength

Table 3 shows that the addition of 2% nanocalcium resulted in the highest tensile strength of the plastics ($p < 0.05$). However, at concentrations of 4 and 6%, the tensile strength decreased compared to control biodegradable plastic (gelatin without nanocalcium). This observation aligns with Setiani *et al.* (2013), who stated that nanocalcium particles can disrupt the compactness of the bioplastics matrix. Consequently, an increases in nanocalcium concentration can lead to a decrease in tensile strength. According to Rahmadani (2019), a higher tensile strength indicates a more compact and homogeneous molecular structure, resulting in a stronger and more fracture-resistant plastic.

The tensile strength of the biodegradable plastics from sea catfish swim-bladder gelatin and bone nanocalcium ranges from 1.26 to 12.42 MPa. The values are higher than the tensile strength of biodegradable plastic made from shrimp shells and cassava pulp, which ranged from 1.01 to 1.97 MPa (Astuti *et al.* 2019). However, they are lower than the tensile strength of conventional plastics such as PP and PE (LDPE, HDPE), which ranges from 24.7 to 302 MPa according to the Indonesian National Standard (SNI) No. 7188.7:2016 of 2016 on Ecolabel Criteria - Part 7: Product categories of biodegradable plastic and bioplastic shopping bags (BSN, 2016).

3.7 Water Vapor Permeability (WVP)

The WVP test results indicated that the nanocalcium concentration did not significantly affect the WVP of the biodegradable plastics ($p > 0.05$) (Table 3). However, contrasting results were reported by Wonoputri *et al*. (2020), who stated that the addition of calcium components in amounts that are either too or too high could affect the water vapor permeability. An increasing concentration of nanocalcium in bioplastics tends to form random structures within the film matrix, thereby hindering the transmission of water vapor. Furthermore, the presence of nanocalcium can lengthen the diffusion path (tortuosity) that water vapor must travel to penetrate the plastic.

The WVP value of biodegradable plastic from sea catfish swim-bladder gelatin and bone nanocalcium ranged from 1.49 to 1.72 g H2O/(m2.day). These values are higher than the WVP of

seaweed-based biodegradable plastics, which ranged from 1.25 to 1.58 g H2O/(m2.day) (Dewi *et al.*, 2017). Furthermor, the WVP of the bioplastics in this study is higher than that of conventional PP and LDPE plastics. Based on the research of Hamdayani *et al*. (2014), the WVP value of conventional plastics is 0.009077261 x 10-6 g/m.s.Pa, while the WVP of film without filler is 0.009635862 x 10-10 g/m.s.Pa. A lower WVP value (closer to zero) indicates a lower rate of WVP and absorption. Once the plastic absorbs moisture from the environment up to its maximum capacity, it acts as a better barrier against further water vapour transmission (Apriyanti *et al.,* 2013).

3.8 Plastic Biodegradation

Biodegradation is the breakdown of part or all of a compound's molecular structure through physiological reactions or microbial action. On day 0, the plastics exhibited a clean and intact appearance. By day 2, all plastic samples showed an increase in weight and has soil particles adhering to their surfaces. From days 4 to 10, continuous weight loss was observed in all samples.

Table 4. Biodegradation of Biodegradable Plastics.

Storage Length	Plastic Mass (%)			
	0%	2%	4%	6%
Day 0	0.55±0.00a	0.52±0.15a	0.79±0.13a	1.68±0.42b
Day 2	1.13±0.44a	0.87±0.22a	1.17±0.34a	2.27±0.34b
Day 4	0.82±0.13a	0.72±0.18a	0.95±0.28a	1.57±0.33a
Day 6	0.58±0.03a	0.68±0.17a	1.01±0.40a	1.05±0.15a
Day 8	0.50±0.00a	0.57±0.12a	0.80±0.21ab	1.00±0.08b
Day 10	0.46±0.02a	0.53±0.10a	0.68±0.16a	0.88±0.24a

Note: Different superscript letters within the same column indicate significant differences ($p < 0.05$).

Based on Table 4, the interaction between the nanocalcium concentration and the storage duration did not have a significat effect on the weight of the biodegrable plastics ($p < 0.05$). This lack of significant interaction can be attributed to the uniform composition of the test specimens, variations in initial sample weights, and the ambient humidity during the storage process at room temperature. Similarly, Dynanty & Alimin (2018) reported that the biodegradation of epoxy resin with added taro starch showed no significant differences among treatments due to uniform sample formulations. Variations in the initial mass of the specimens also affected the non-linear degradation calculations. Furthermore, soil moisture and the abundance of decomposing microbes are crucial factors influencing the degradation rate.

From day 0 to day 2, the biodegradable plastics experienced an increase in weight and a blackish-brown discoloration across all treatment. However, from day 4 to day 10, all treatments exhibited a gradual weight loss. Therefore, it can be concluded that the weight initially increased up to day 2, it subsequently decreased over time. According to Nurfauzi *et al*. (2018), such initial mass increases vary due to non-uniform water absorption caused by differences in plastic thickness. Additionally, uneven evaporation of soil moisture, resulting from the indoor placement of the soil medium, can also contribute to these variations.

These findings align with Purnavita & Wahyu (2018), who stated that bioplastics made from natural materials become more easily degraded the longer they are buried in the soil. The natural ingredients used in this study do not contain synthetic chemicals that would inhibit the biodegradation process.

4. CONCLUSIONS

Different concentrations of nanocalcium derived from sea catfish bones had a significant effect ($p < 0.05$) on the characteristics of the biodegradable plastic made from sea catfish swim-bladder gelatin. Higher concentration of nanocalcium can increased the thickness, solubility, and elongation of the plastic. However, a nanocalcium concentration of 2% was identified as the optimal concentration for maximizing the tensile strength. Biodegradable plastics with 2% nanocalcium exhibited a more compact morphology without any agglomeration. The addition of nanocalcium did not significantly affect ($p > 0.05$) the WVP of the plastics. Similarly, the interaction between nanocalcium concentration and storage time during the biodegradation test had no significant effect ($p > 0.05$) on the weight of the plastic.

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