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Functional Bioplastic Based on Chitosan/Sago Starch (*Metroxylon sago*) with the Addition of Black Fruit (*Haplolobus sp.*) Leaves Ethanolic Extract

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ABSTRACT. Bioplastic as an antioxidant and antibacterial packaging has been synthesized from chitosan and sago starch with Black fruit leaves ethanolic extract. The bioplastic synthesis was done by mixing a solution of chitosan, sago starch, glycerol, and ethanolic extract of Black fruit leaves, pouring it into a petri dish, and then drying it in an oven. The bioplastic produced was then characterized using Fourier transform infrared spectroscopy (FTIR). The results show that increasing the weight ratio of chitosan/sago starch increased tensile strength, decreased elongation, decreased thickness, and increased biodegradation time. The addition of ethanolic extract of black fruit leaves reduces tensile strength at a chitosan/starch weight ratio of 4:1, increases tensile strength at a chitosan/starch weight ratio of 1:1, tends to minimize elongation at both Chitosan/starch ratios, tends to increase thickness at a chitosan/starch weight ratio of 4:1, decreases thickness at a Chitosan/starch weight ratio of 1:1, and increases biodegradation time. The best bioplastic formulation in this study was found in CS11E1 (weight ratio of Chitosan/starch 1:1, extract concentration 1%), with a tensile strength of 4.71 MPa, elongation of 29.33%, thickness of 0.07 mm, and biodegradation time of 6 days.

INTRODUCTION

Environmental pollution by the accumulation of non-biodegradable plastic is increasingly worrying. Substituting packaging plastic with biodegradable bioplastic is a good solution to this problem (Ortiz, 2023). Bioplastics are biodegradable plastics produced from biological materials or renewable sources (Atiwesh *et al.*, 2021). Bioplastics can be synthesized from natural polymers, such as cellulose (Isroi *et al.*, 2017), pectin (Listyarini *et al.*, 2020), carrageenan (Fadhallah *et al.*, 2024), alginate (Dalal *et al.*, 2023), chitosan, and sago starch (Alves *et al.*, 2022; Duma *et al.*, 2023). The use of natural polymers in the synthesis of bioplastics has many advantages because of their biocompatible, good bioavailability, biodegradable, and low cost properties, both in acquisition and production (Paradowska-Stolarz *et al.*, 2021), which attracts many research groups interested in developing bioplastics from natural polymer materials.

Starch is a natural polymer abundant on Earth and a common bioplastic material. However, there is a disadvantage in the nature of starch, namely, it is hydrophilic, which makes it unsuitable for use as a single ingredient in bioplastics. To increase the hydrophobicity of starch-based bioplastics, starch can be mixed with chitosan (Tan *et al.*, 2022). Chitosan is a polysaccharide that has hydroxyl and amine groups. When viewed from these functional groups, chitosan is hydrophilic, but experimental results show a hydrophobic character in Chitosan films (Cunha *et al.*, 2008).

The addition of specific fillers and additives to bioplastic materials produces bioplastics with unique properties and characteristics for specific uses (Abang *et al.*, 2023). Bioplastics based on natural ingredients with

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antioxidant and antibacterial properties are useful in health, agriculture, and packaging industries (Kong *et al*., 2023). In developing bioplastics that have antioxidant and antibacterial properties, compounds or extracts that have antioxidant and antibacterial properties can be added in bioplastic synthesis, such as Black fruit leaf extract (*Haplolobus sp.*) (Aronggear *et al.*, 2022; Lestari *et al.*, 2024). The ethanolic extract of Black fruit leaves contains secondary metabolites that are antioxidant and antibacterial, namely alkaloids, tannins, saponins, polyphenols, steroids, and flavonoids (Lestari *et al.*, 2024). Tannins in bioplastics can act as stabilizers that improve the thermomechanical properties of bioplastics (Bonnenfant *et al*., 2022). The phenolic hydroxyl group of tannin can form hydrogen bonds with the hydroxyl group of glycerol and starch. In addition, the presence of benzene rings in tannins helps increase the mechanical strength of thermoplastic starch (Ma *et al.*, 2022).

Black fruit is already commonly known to be eaten directly or can be added as a food additive. A study has reported that the toxicity level of Black fruit and its leaves is almost the same (Lestari *et al.*, 2024). Therefore, the leaves should also be applicable as a food additive. The antibacterial properties of the ethanolic extract of Black fruit leaves include a broad spectrum because it is able to kill gram-positive and gram-negative bacteria (Lestari *et al.*, 2024). This prompted this research to utilize the ethanolic extract of Black fruit leaves as an antioxidant and antibacterial additive in bioplastics based on chitosan, sago starch, and glycerol.

RESEARCH METHODS

The materials used are Sago flour from the Sanggeng Manokwari traditional market. Samples of Black fruit leaves (Haplolobus monticola) were from Manokwari, Papua Barat, Indonesia. Commercial chitosan from shrimp shells (Industrial Grade from CV. ChiMultiguna Cirebon), glycerol, NaOH, and glacial acetic acid were purchased from Merck (New Jersey, USA). Meanwhile, the equipment used in this research are a hot plate, oven, analytical balance, 8-cm-diameter petri dish, magnetic stirrer, blender, filter cloth, 200-mesh sieve, aluminum foil, glassware, vernier calipers, and FTIR spectrophotometer (Shimadzu IR Prestige-21).

Methods

Black Fruit Leaf Extraction

Black fruit leaves were washed clean, dried, ground with a blender, and extracted by maceration with 96% ethanol solvent for 3 × 24 hours. After every 24 hours, the liquid from the maceration process was filtered, and the residue was re-extracted with a new solvent. After 3 days, the filtered filtrate was collected and then concentrated using a rotary evaporator.

Bioplastic Synthesis

Bioplastic synthesis was carried out using the casting method. The chitosan solution (1%) was placed into an erlenmeyer flask, and a 1% sago starch solution, glycerol, and Black fruit leaf extract were added and stirred at 80 °C for 5 minutes, then cooled. In a petri dish with a diameter of 8 cm, 16 mL of the mixture was poured. Bioplastics were taken from petri dishes by immersion in a 1% NaOH solution. The resulting bioplastic was washed with distilled water and dried in an oven. The composition of the material mixture is presented in Table 1.

Table 1. Composition of bioplastic materials.

No	Sample Code	Weight Ratio Chitosan/ Starch	Chitosan 1% Volume (mL)	Starch 1% Volume (mL)	Extract Conc. (%)	Extract Volume (mL)	Aquadest Volume (mL)	Glycerol Volume (mL)
1	CS41	4:1	40	10	0	0	2.5	5
2	CS41E1	4:1	40	10	1	2.5	0	5
3	CS41E5	4:1	40	10	5	2.5	0	5
4	CS11	1:1	25	25	0	0	2.5	5
5	CS11E1	1:1	25	25	1	2.5	0	5
6	CS11E5	1:1	25	25	5	2.5	0	5

The resulting bioplastic was then characterized using FTIR, and its performance was tested, which included tensile strength, elongation, thickness, and biodegradation tests. Tensile strength and elongation testing were carried out using a universal testing machine, which applied a tensile force to the sample until it broke. The tensile strength was counted according to Equation 1, while elongation at break was determined using Equation 2.

$$Tensile\ Strength\ (MPa) = \frac{F}{T \times L} \quad (1)$$

Description:
F = maximum force (Newton)
T = thickness (mm)
L = length (mm).

$$\text{Elongation at Break (\%)} = l - lo \times 100 \quad (2)$$

Description:
l = length extended at the breaking point of the sample (mm)
lo = initial length of sample (mm)

The biodegradation test was carried out by planting samples in the soil at a depth of 30 cm, leaving them for 15 days, and observing every 3 days until the samples were degraded.

RESULTS AND DISCUSSION

Sago Starch Content

The results of the sago starch content test can be seen in Table 1. Table 1 shows that the composition of sago used in this study is dominated by carbohydrates (90.33 ± 0.01%wb) with low fat, protein, and crude fiber content. The starch content in sago is 89.32 ± 0.06%wb, with amylose content of 38.14 ± 0.06% wb and amylopectin 51.17 ± 0.00%wb. In other studies, this level is almost the same: amylose 37.0 ± 1.46% and starch 87.6 ± 2.50% (Adawiyah *et al*., 2013). The starch content of sago starch can vary according to environmental conditions, such as the type of soil where the sago tree grows (Ayulia *et al*., 2021).

Physical Appearance of Bioplastics

The bioplastic produced in this research is in the form of a thin, translucent film. The color of the bioplastic is influenced by the ethanolic extract content of Black fruit leaves, where bioplastic without added extract ((e) CS41 and (f) CS11) is colorless. In contrast, bioplastic with added extract ((a) CS41E1, (b) CS41E5, (c) CS11E1, (d) CS11E5, (e) CS41) are greenish yellow (Figure 1). The more concentrated the ethanolic extract content of Black fruit leaves, the higher the intensity of the greenish-yellow color.

Figure 1. Bioplastics (a) CS41E1, (b) CS41E5, (c) CS11E1, (d) CS11E5, (e) CS41, and (f) CS11.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR measurements are intended to determine the interaction of functional groups contained in the materials that make up bioplastics, namely chitosan, starch, glycerol, and ethanolic extract of Black fruit leaves at a wavenumber of 4000 – 400 cm^{-1} with a resolution of 1 cm^{-1}. The FTIR spectrum of bioplastics is shown in Figure 2. The stretching vibration of the –OH (Karua and Sahoo, 2020) in standard bioplastics (CS11 and CS41) and in bioplastic variants 1 and 2 (CS41E1 and CS41E5) appears at a wavenumber of 3425 cm^{-1}. However, the –OH vibration in variants 3 and 4 (CS11E1 and CS11E5) occurs at lower wavenumbers at 3417 and 3410 cm^{-1}, respectively. In the other study, the starch biopolymer films' FTIR spectra indicate that glycerol and starch join together to form many hydrogen bonds. Shifts to lower wavenumbers (redshift) indicate stronger and more stable hydrogen bonds, representing increased interactions and structural integrity in the films (Pooja *et al*., 2024). In this study, the shift to lower wavenumber of peaks at 3417 and 3410 cm^{-1} of the 1:1 Chitosan/starch sample (sample codes CS11E1 and CS11E5) shows that the –OH groups from the ethanolic extract of Black fruit leaves (tannin) and glycerol tend to have better molecular interactions (hydrogen bonding) with bioplastics with higher starch content in the range of this study (ratio of Chitosan/starch 1:1) (Lestari *et al.*, 2018). The hydrogen bonds formed cause the weakening of the X–H bond (X = O or N), so the stretching vibration frequency decreases (Sen and Patwari, 2018). These strong hydrogen bonds directly affect the mechanical properties and degradation of bioplastics. Tensile strength increases because strong intermolecular interactions strengthen the cohesion between polymer chains (Figure 3). However, hydrogen bonds also limit the mobility of the polymer chain, so that elongation tends to decrease (Figure 4), making the film stiffer and more brittle (Tafa *et al*., 2023). Meanwhile, the film thickness is reduced (Figure 5) because dense intermolecular bonds can produce thinner films. In addition, the polymer network structure reinforced by hydrogen bonds makes the material more resistant to microorganism attack and water penetration, thereby extending the biodegradation time (Figure 6). This finding is in accordance with other research (Kusumaningrum *et al.*, 2023).

Absorption of C–H alkanes appears at wavenumbers between 2924 and 2931 cm$^{-1,}$ which is the C–H stretching area, which is the basic structure of starch and Chitosan polymers (Karua and Sahoo, 2020). The C=O carbonyl bond appears at 1651 cm^{-1} and 1635 cm^{-1}. This bond can come from chitin amide bonds in chitosan and tannin ester bonds in the extract. A new absorption appeared in the sample with the addition of extract at wave numbers 1589 – 1604 cm^{-1}, which is associated with aromatic ring stretching vibration of tannin (Koochakzaei and Sabaghian, 2023). The –OH absorption band also widens at 3402 cm^{-1} and 3425 cm^{-1}, indicating intramolecular hydrogen bond vibrations of the –OH group attached to the aromatic group. These peaks strengthen the presence of tannin compounds (Karua and Sahoo, 2020).

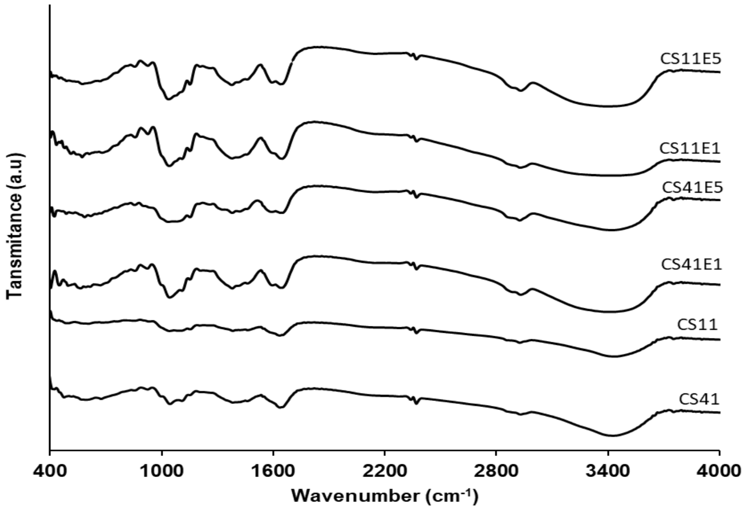

Figure 2. FTIR spectra of bioplastics.

The C–O group on the polymer backbone appears at a wavelength of 1033 – 1041 cm^{-1}. At a Chitosan/starch ratio of 1:1, the addition of extract caused a shift in CO absorption to shorter wavenumbers from 1057 cm^{-1} (CS11) to 1042 cm^{-1} (CS11E1) and 1034 cm^{-1} (CS11E5), while at a chitosan/starch ratio of 4:1 there was no shift in absorption from 1049 cm^{-1} (CS41) to 1049 cm^{-1} (CS41E1). However, there is a shift in absorption to longer wavenumbers from 1049 cm^{-1} CS41 to 1057 cm^{-1} (CS41E5), showing that at a chitosan:starch ratio of 1:1, the addition of the extract strengthens the bonds between polymers. This is possibly due to phenolic OH groups, including tannin from the extract, which form hydrogen bonds between chitosan and starch. The interplay between tannin and chitosan primarily arises from intricate electrostatic interactions; these components may establish a substantial network of hydrogen bonds, functioning as crosslinkers for Chitosan (Aliabadi *et al.*, 2021).

Tensile strength

Mechanical tests on bioplastics were carried out through tensile strength and elongation tests. The tensile strength value is the force required for the bioplastic to break. A low tensile strength value for a bioplastic indicates that the nature of the bioplastic is easily damaged. Meanwhile, a high tensile strength value suggests that the bioplastic produced is able to protect the product from mechanical disturbances such as friction or impact on the product. Figure 3 shows that the lowest tensile strength in this study was 1.97 MPa for CS41E1 bioplastic, and the highest was 18.13 MPa for CS41 standard bioplastic. The tensile strength of CS41, CS41E5, and CS11E1 bioplastics meets the JIS standard, namely 3.92 MPa (Japanese Industrial Standard, 2019; Brilianti *et al.*, 2023). In standard bioplastics (without ethanolic extract of Black fruit leaves), increasing the amount of chitosan increases the tensile strength. This was also reported in another study by Tan *et al.* (2022). Adding the ethanolic extract of Black fruit leaves gave different effects according to the chitosan/starch weight ratio. Adding the extract reduces the tensile strength at a chitosan/starch weight ratio of 4:1. In contrast, at a chitosan/starch weight ratio of 1:1, the addition of the extract increases the tensile strength. This is possibly due to the tendency of the hydroxyl groups of polyphenols or tannins in the extract to form hydrogen bonds with the –OH groups of starch rather than the NH groups of Chitosan (Ma *et al.*, 2022). Tannins have –OH groups, which can act as plasticizers in bioplastics (Ma *et al.*, 2022); however, too high a plasticizer concentration will reduce the tensile strength of the plastic (Budiman *et al.*, 2022).

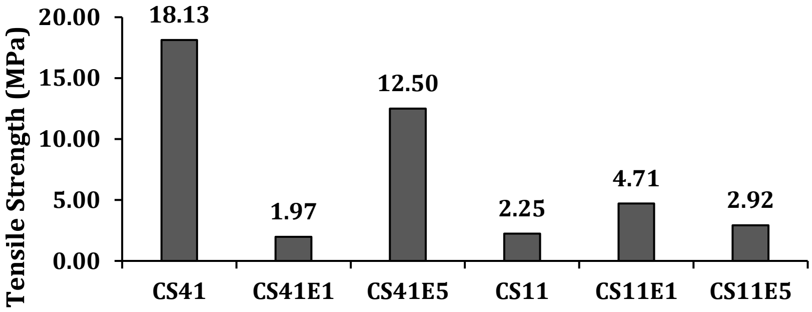

Figure 3. Results of bioplastic tensile strength tests.

Elongation at Break

Elongation at break is the ratio of the initial and final lengths of the plastic material before it breaks (Budiman *et al.*, 2022). Figure 4 shows the percent elongation at break, which is inversely proportional to the composition of Chitosan in bioplastics. This is in accordance with the results of research in (Gabriel *et al.*, 2021), which stated that adding chitosan filler reduced elongation. This is because the large amount of Chitosan filler causes a reduction in the bond distance between molecules; therefore, the plasticizer molecules reside in a separate region outside the polymer phase. The decrease in bond distance is caused by the increase in the number of hydrogen bonds formed between Chitosan molecules with amylose and amylopectin. Bioplastics become stiffer and less elastic. The addition of extracts tends to reduce elongation. This can also be caused by reduced interaction between the glycerol plasticizer and starch. Even though the extract contains secondary metabolite compounds of plasticizers, its plasticizing ability is lower than that of glycerol. Based on the elongation test, all bioplastics do not meet the elongation in JIS, namely 50%, but bioplastics CS11, CS11E1, and CS11E5 meet SNI, namely 20% (Brilianti *et al.*, 2023).

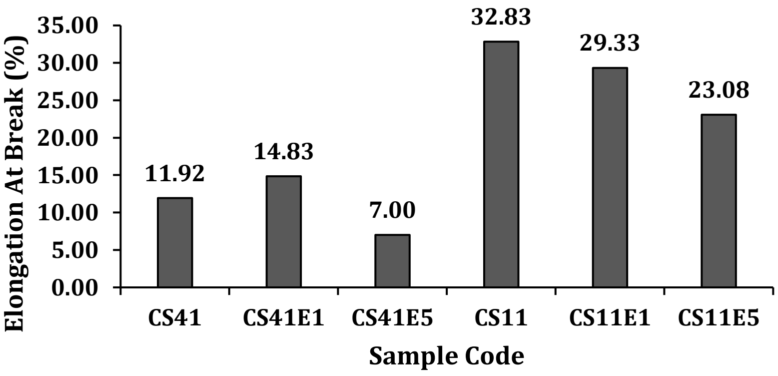

Figure 4. Bioplastic elongation test results.

Thickness

The thickness of bioplastic is influenced by the total amount of solid material that comprises the bioplastic (Budiman *et al.*, 2022). Figure 5 shows the effect of bioplastic composition on the thickness of the bioplastic produced. The thickness of the bioplastic in this study varied from 0.05 to 0.122 mm. Increasing the weight ratio of chitosan/starch reduces the thickness of the bioplastic produced. There is a tendency that the addition of extracts has the effect of increasing the thickness of bioplastics with a Chitosan/starch ratio of 4:1, but decreases the thickness of bioplastics with a Chitosan/starch ratio of 1:1. This can be caused by the strong interaction between glycerol and starch in the form of hydrogen bonds. However, excess glycerol in the chitosan/starch weight ratio of 4:1 causes a reduction in the tensile strength of bioplastics. Glycerol can facilitate the migration of water vapor molecules, causing the distance between bonds to increase, resulting in an increase in the thickness (Budiman *et al.*, 2022). The average thickness in this study has met the standards set by the Japanese Industrial Standard (JIS 1707:1975) with a good bioplastic thickness of ≤ 0.25 mm.

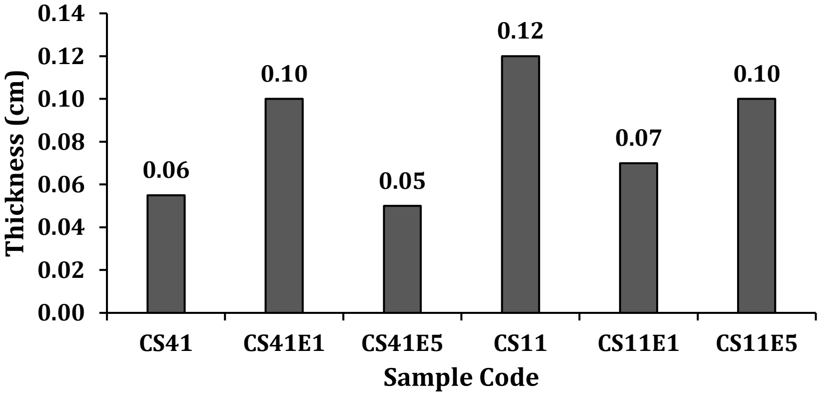

Figure 5. Bioplastic thickness test results.

Biodegradation

Biodegradation testing can take place by utilizing microorganisms in the soil whose job is to assist the decomposition process, which is also known as the soil burial test technique (Folino *et al.*, 2023). In the process, samples were planted at a soil depth of 30 cm, and then physical observations were carried out every 3 days. Figure 6 shows that the bioplastic degradation time in this study varied between 3 and 12 days.

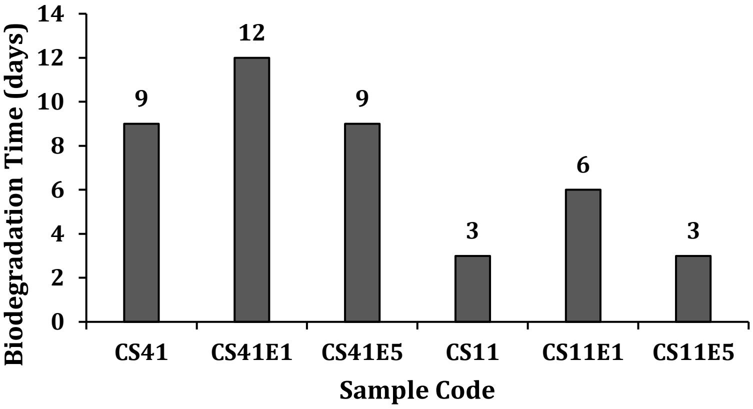

Figure 6. Results of bioplastic biodegradation tests.

A higher chitosan content (a greater chitosan/starch weight ratio) extends the biodegradation time, as does adding the extract. The extract has antioxidant and antibacterial activity, thereby protecting the polymer from biodegradation. However, adding a larger amount of extract reduced the degradation time because adding a higher extract concentration reduces the molecular interactions between polymers, making them easier to degrade. Apart from being influenced by the physicochemical properties of bioplastics (thickness, hydrophobicity, molecular weight, crystallinity, etc.), the degradation time is also influenced by the environment, namely the biotic and abiotic environment of the soil (Pooja *et al.*, 2023). Based on international plastic standards (ASTM5336), the biodegradability of polycaprolactone (PLA) plastic from Japan and polylactic (PCL) from the UK takes 60 days to decompose completely. The ability of the plastic to biodegrade has met the standards used by Japanese PLA plastic and PCL from England. Based on the criteria of tensile strength, elongation, thickness, and biodegradation, it can be concluded that the best bioplastic that meets the standards is CS11E1 with a tensile strength of 4.71 MPa, elongation of 29.33%, thickness of 0.07 mm, and biodegradation time of 6 days.

CONCLUSION

Bioplastics based on chitosan-sago starch with the addition of the ethanolic extract of Black fruit leaves have been successfully synthesized in this research. The addition of extracts affects the tensile strength, elongation, thickness, and biodegradation time of bioplastics, depending on the Chitosan/starch weight ratio. Increasing the weight ratio of Chitosan/Sago starch increased tensile strength, decreased elongation, decreased thickness, and increased biodegradation time. The addition of ethanolic extract of Black fruit leaves reduces tensile strength at a Chitosan/starch weight ratio of 4:1, increases tensile strength at a Chitosan/starch weight ratio of 1:1, tends to reduce elongation at both Chitosan/starch ratios, tends to increase thickness at a Chitosan/starch weight ratio of 4:1, decreases thickness at a Chitosan/starch weight ratio of 1:1, and increases biodegradation time. According to bioplastic standards, the best composition is CS11E1, which has a tensile strength of 4.71 MPa, elongation of 29.33%, thickness of 0.07 mm, and biodegradation time of 6 days. Optimization of the right amount and type of plasticizer needs to be done to increase the elongation of Chitosan/Sago starch-based bioplastics with the additive ethanolic extract of Black fruit leaves.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

ADNL: Conceptualization, Funding Acquisition, Investigation, Data Processing, and Writing Manuscript; ADNL: Supervision; MFKP and ES: Investigation, Data Collection, and Writing – Original Draft; DE and GSDK: Data Analysis, Manuscript Writing, and Editing.

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DECLARATION OF GENERATIVE AI

During the preparation of this work, the author(s) used Grammarly and QuillBot in order to fix the grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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