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The Influence of Chitosan on the Physical Properties of Avocado Seed Starch-Based Biofoam

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Abstract. The global reliance on styrofoam has left us with a crisis of non-biodegradable waste. To tackle this, our study explores a sustainable alternative, i.e., converting avocado seeds, which are an overlooked agricultural byproduct, into biodegradable foam (biofoam). We focused on characterizing avocado seed starch and investigating how the addition of chitosan influences the foam's physical performance. The properties observed in this research include tensile strength testing with a universal mechanical tester, microstructure analysis using SEM, biodegradability, and water absorption. In addition, characteristics of the starch, including granule size and shape using a polarized microscope, and crystallinity using XRD, were also analyzed. Our analysis revealed that the starch granules are primarily spherical (7.2–41.7 μm) with a B-type crystalline structure. By incorporating 20% chitosan, we observed a significant structural shift from uniform globular cells to a bubble-like matrix, which tripled the material's tensile strength from 0.07 MPa to 0.263 MPa. Furthermore, the chitosan acted as a vital functional filler, reducing water sensitivity and slowing the rate of biodegradation to more practical levels. These results offer a compelling case for materials science: we can transform food waste into high-performance, eco-friendly packaging. By aligning with UN Sustainable Development Goal (SDGs) 12 (Responsible Consumption and Production), this research provides a tangible pathway toward a circular economy, proving that the future of plastics might just be hidden in our organic waste. Using avocado seed waste to produce starch, a raw material for creating eco-friendly biofoam, promotes sustainable production and consumption patterns.

Keywords: Avocado seed; biodegradability; biofoam; starch; tensile strength

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INTRODUCTION

Styrofoam has become a part of our daily lives in various applications. For example, we can find styrofoam in food packages or other industrial sectors (Annissa et al., 2023), as well as in building construction (Sulong et al., 2019). We can easily find styrofoam for food packaging, such as disposable plates, bowls, or spoons. Meanwhile, in industrial sectors, we can find styrofoam is used to pack and transport electronics or fragile products. In addition, it is used as an insulation material, aggregate in lightweight concrete, and decorative tiles and moldings in building construction.

Styrofoam becomes a problem since its raw material. It is made of monomer styrene, which is a liquid hydrocarbon from crude oil. Therefore, this makes styrofoam non-degradable. As the problem grows, the massive use of styrofoam in various applications has led to waste management problems in our environment.

While convenient, plastic foam presents a significant environmental challenge due to its lack of biodegradability and difficulty in recycling. Efforts for styrofoam recycling have been made, but considering new materials as a replacement for polystyrene might be the best way for the environmental problems it may cause. In this case, we can use starch, which is a polymer composed of sugar monomers, degradable, and abundantly available.

As the largest portion of carbohydrate in plants, starch can be produced in discrete granules that are spherical, oval, ogival, lenticular, or polyhedral forms, and can be found in a few micrometers to more than 100 micrometers (Thieme et al., 2023). The granules are composed of amylose, a linear polymer of α-(1→4)-linked D-glucose units which contain a small number of α-(1→6) linked branches, and amylopectin, a heavily branched molecule with an α-(1→4)-linked D-glucose backbone and α-(1→6) linked branches. In terms of size, the amylose chain is short with only hundreds to thousands of glucosyl units, while the amylopectin chain is shorter and has widespread branches, making it a highly complex molecular structure (Bertoft, 2017).

A lot of researchers have studied some properties of biofoam produced by starch. These include starches from corn and arrowroot (Suryaningsih et al., 2025), taro (Azizati et al., 2024), potato (Rodrigues et al., 2020; Zhang et al., 2020), sago (Gani & Kusumayanti, 2022; Saleh et al., 2022), cassava (Fatrozi et al., 2020; Sumardiono et al., 2021; Patty & Wattimena, 2023), and cassava and banana peels (Darni et al., 2023).

Previous research showed that some starches are used for food production, while the rest are essentially wastes, including the seeds of avocado, mango, cassava peels, and banana peels. Using these starches to make biofoam doubles the benefits: it reuses waste materials and produces good products that also help solve environmental problems.

Avocado seeds are a source of starch that is often discarded as waste. The properties of avocado seed starch have been researched previously. It has been found that the amylose content in avocado seeds ranges from 35.34% to 48.19% across eight different cultivars (Wang et al., 2022). The granule size of avocado seed starch varies from 10.1 to 42.5 nm (Macena et al., 2020). The extract from avocado seeds has been examined for its anti-microbial and antioxidant properties (Villarreal-Lara et al., 2019; Soledad et al., 2021). Avocado seed starch has been used to make bioplastics (Merino et al., 2021), edible films (Quispe-Sanchez et al., 2026), and has been found to influence the properties of cassava-based biofoam (Aguilar & Tapia-Blacido, 2023). However, to the best of our knowledge, few studies have focused on the properties of biofoam made from avocado seed starch.

Due to some limitations of starch-based biofoam, such as high-water absorption and low tensile strength, certain fillers are necessary. One of the fillers used in producing starch-based biofoam is chitosan, a linear polysaccharide composed of randomly arranged β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine. Studies have demonstrated that chitosan can improve the physical and mechanical properties of starch-based biofoam (Zhang et al., 2020). Chitosan has also been found to affect leaf growth (Rahayu et al., 2025).

This study aims to create a biofoam using avocado seed starch, evaluate its physical properties, including microstructure, mechanical strength, water absorption, and biodegradability, and examine the effect of chitosan on these properties. The findings are significant for advancing science, particularly in materials science. Furthermore, plant wastes, including avocado seeds, can be used to make biodegradable foam, while chitosan might act as a filler for foam properties enhancement. Finally, the finding can be used as preliminary data for further research on biofoam production from starches, which act as an alternative to styrofoam.

METHODS

Starch and Biofoam Preparation

To prepare the starch, we began by grating the avocado seeds and adding water for thorough crushing. This mixture was then squeezed and filtered through a fine cloth, a manual process repeated until the remaining pulp was essentially depleted of liquid. We allowed the resulting filtrate to sit undisturbed for approximately an hour, giving the starch sediment sufficient time to settle at the bottom. To ensure the purity of the sample, we decanted the water, washed the sediment with fresh water, and repeated the settling process. The final step involved drying the saturated sediment in an oven at 70°C for a full 24 hours, yielding a stable, dry starch ready for biofoam production.

For biofoam preparation, chitosan was dissolved (0%, 5%, 10%, and 20%) in 100 mL of 1% acetic acid, and the solutions were stirred until each became a completely homogeneous solution. To each of the chitosan solutions, 5% magnesium stearate (w/w starch), 2% carrageenan (w/w starch), glycerol 16% (w/w starch), 29% pure protein isolate (w/w), and 40% polyvinyl alcohol (PVOH) (w/w starch) were added. The mixtures were then stirred with a mixer at high speed for 10 minutes until the dough expanded. Starch (36 g) was added to each dough, and the doughs were stirred at a low speed for 10 minutes. Then, each dough was poured into a mold and placed in an oven at 125°C for one hour. Afterward, the foams were cooled and stored at room temperature for four days.

Starch crystalline property analysis

The crystalline properties of the starch were analyzed using an XRD spectrophotometer, the XRD Rigaku MiniFlex 2. The monochromator employed was Cu-Kα radiation with λ=1.5405 Å, operated at 30 kV and 15 mA, with an angle of 2θ ranging from 3° to 145° at an interval of 0.02°. The 2θ data were truncated between 14° and 30° and smoothed using a Savitzky-Golay filter with a polynomial degree of 3 and 40 points for clarity.

The crystallinity index, I_C, of the starch was determined following the protocol of the previous study (Patty & Wattimena, 2023; Wattimena & Patty, 2025a). It was calculated using

$$I_c = \frac{A_C}{A_C + A_A} \quad (1)$$

where A_C and A_A are the areas of the crystalline and amorphous parts, respectively. The A_C and $A_C + A_A$ were determined by integrating the area under the peaks and the total area of the XRD data, respectively.

The shape and size of the granules, as well as their birefringence properties, were characterized using a polarized microscope, an Olympus BX-53M Trinocular Polarizing Microscope.

Characterization of biofoam microstructure

Characterization of the biofoam surface was performed using scanning electron microscopy (SEM). The SEM model used was Zeiss EVO 10. Before measurement, a thin layer of gold was applied to the surface of the sample to prevent electron buildup caused by the non-conductivity of the sample.

Tensile strength measurement

Tensile strength measurements were performed using a Mechanical Testing Universal Machine. The sample dimensions were 50.0 mm x 5.0 mm, with the thickness averaged from measurements at 7 different locations on the sample using a micrometer screw. The pulling rate was 10 mm/min.

Water Absorption Measurement

Water absorption, which is the amount of water absorbed by a material, is defined as the ratio of the weight of water absorbed to the weight of dry material. Mathematically, water absorption (W_A) can be expressed as

$$W_A = \frac{W_1 - W_0}{W_0} \times 100\% \quad (2)$$

where W_0 and W_1 are the initial and final weights, respectively, so $W_1 - W_0$ is the weight of water absorbed. Analysis of water absorption was conducted by cutting each sample into a size of 2.50 x 5.00 cm. Each sample was weighed to determine the initial dry weight. The samples were then immersed in water for 1 minute, 3 minutes, and 5 minutes, then dried with a tissue to remove any remaining water on the surface. Each sample was weighed again, and the weight gain was calculated to determine the percentage (%) of water absorption using Equation 2.

Biodegradability measurement

The soil burial test method was used to measure the level of biodegradation of the foams. Each foam sample was cut to a size of 2.50 x 5.00 cm and then immersed in water for 1 minute. The samples were weighed as the initial weights and then planted in soil for 14 days. After 14 days, the samples were cleaned of any adhering soil and weighed again as the final weight. The percentage of weight loss was calculated using the following formula:

$$W_l = \frac{W_0 - W_1}{W_0} \times 100\% \quad (3)$$

where W_0 and W_1 are the initial and final weights, respectively, and W_l is defined as the index of biodegradability in this study. For convenience, it is referred to as biodegradability in the text.

Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA) and the Tukey test to identify significant differences in tensile strength, water absorption, and biodegradability index of biofoam with varying concentrations of chitosan. One-way ANOVA was applied for tensile strength and biodegradability analysis, while two-way ANOVA was used for water absorption analysis.

RESULTS AND DISCUSSION

Avocado Seed Starch Properties

Figure 1 shows avocado seed starch under a polarized light microscope at 100x magnification, shown without polarizing filters (left) and with polarizing filters (right). The figure illustrates starch granules, which are mostly spherical or oval in shape. The observed granule diameters range from 7.2 to 41.7 μm, with an average diameter of 19.1±7.7 μm. These sizes are similar to previous studies, where the diameter of avocado seed starch ranged from 10.1 to 42.5 µm (Macena et al., 2020) and from 5 to 30 µm (Wang et al., 2022).

A Maltese cross with a dark center and bright quadrants, shown on the right of Figure 1, results from placing starch between two crossed polarized filters. It indicates the birefringent property of starch. Light passing through the first polarizer is polarized in one direction, and because starch is birefringent, it can split the polarized light into two components with different refractive indices. If the polarization direction is changed, the components can pass through the second polarizer. The dark center occurs where the polarization is unchanged, while the bright quadrants appear where the polarization is altered. The Maltese cross pattern reflects alternating amorphous and crystalline rings, starting from the hilum at the center. The crystalline rings are composed of branches of amylopectin forming double helices, whereas the amorphous rings are junctions of amylopectin branches interacting with long amylose (Bertoft, 2017).

Figure 2 displays the XRD data of avocado seed starch. The observed 2θ peaks are 15°, 17°, 19°, 22°, and 24°. According to XRD data, starch can be classified as type A with peaks at 15°, 17°, 18°, and 23°, type B at 5.6°, 17°, 22°, and 24°, and type C at 5.6°, 15°, 17°, and 23°, or a combination of types A and B (Xie et al., 2017). Based on these criteria, avocado seed starch is classified as a B-type starch. It has been found that for B-type starch, such as avocado starch, the arrangement of double helices in a crystalline ring is hexagonal with 36 water molecules per unit cell (Bertoft, 2017).

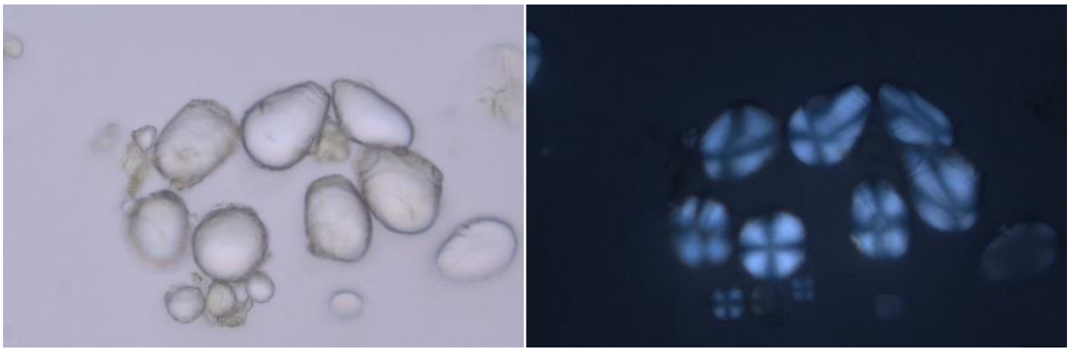

Figure 1. Avocado seed starch viewed under a polarized microscope (Olympus BX-53M Trinocular Polarizing Microscope) at 100x magnification: without polarizing filters (left) and with polarizing filters (right).

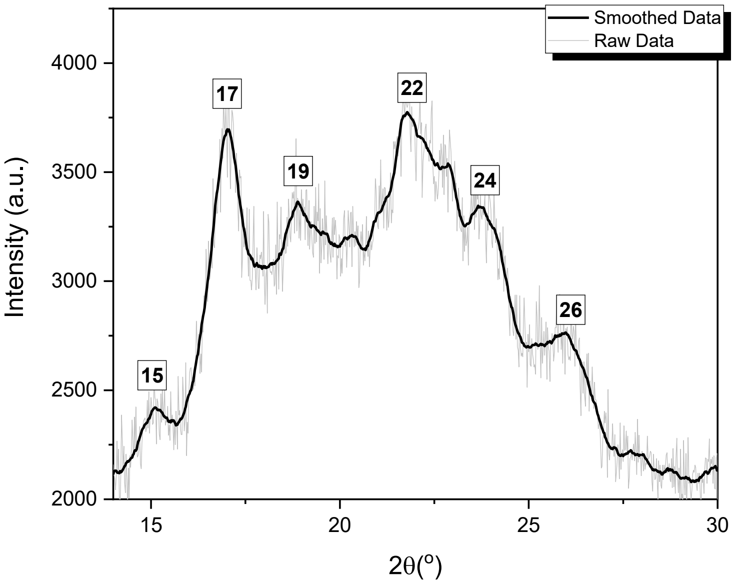

Figure 2. XRD analysis of avocado seed starch

The crystallinity index of avocado seed starch was calculated using Eq.1, where A_C was obtained by integrating the area under the peaks labeled 15, 17, 19, 22, 24, and 26 in the XRD data of Figure 2, and A_C+A_A was the total area under the entire curve of the same data. The crystallinity index was found to be 27%. Interestingly, this value is similar in magnitude to that of durian seed starch, another B-type starch, which is 22% (Wattimena & Patty, 2025b).

Biofoam Properties

Figure 3 displays the surface of avocado seed starch biofoam without chitosan (A) and its SEM micrographs at different magnifications: 100x (B), 200x (C), and 500x (D). The micrographs show uniformly distributed globular cells in the biofoam, formed by water vapor during preparation. The figure also indicates that avocado seed starch-based biofoam has a globular cell structure, similar to the cell structure found in potato and cassava starch-based biofoams, but unlike the cells in corn biofoam, which appear irregular (Bergel et al., 2017).

The dimension of the cell, its longest length, was measured in 115 cells and ranged from a minimum of 31.6 μm to a maximum of 420.0 μm. The average cell size was 126.3 ± 81.3 μm. The distribution of cell sizes is shown in the histogram in Figure 4, where most sizes fall between 70 and 120 μm. These are comparable to the cell sizes of biofoam made with a chitosan-wheat gluten mixture, which are 70–80 μm (Chen et al., 2015), and biofoam made with plywood sawdust waste, which ranges from 50 to 400 μm (Nofitasari et al., 2022).

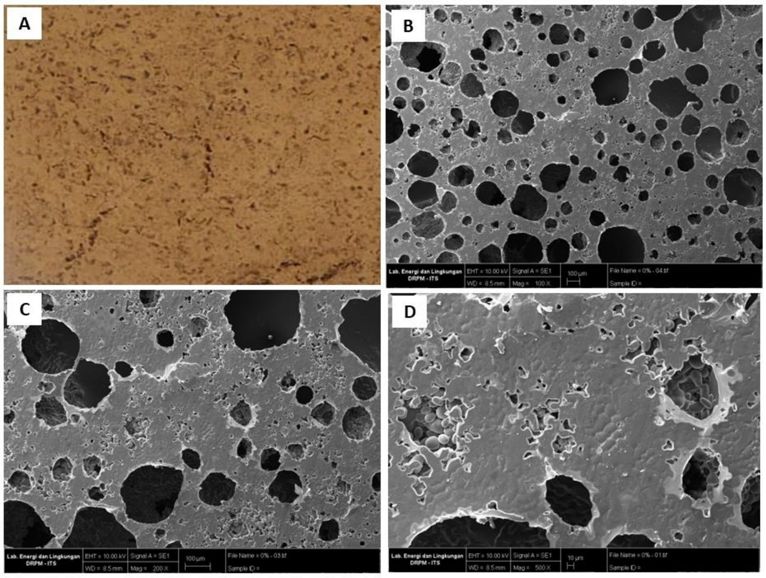

Figure 3. The surface of avocado seed starch biofoam (A) and its SEM micrographs at different magnifications: 100x (B), 200x (C), and 500x (D).

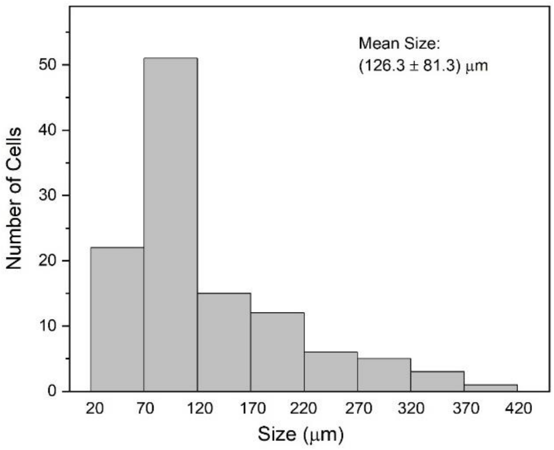

Figure 4. Size distribution of the globular cell in the biofoam formed by water vapor during preparation.

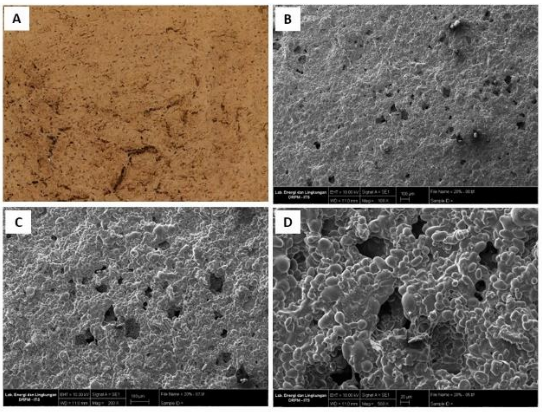

Figure 5. SEM micrograph of the surface of avocado seed starch biofoam containing chitosan (A) and its SEM micrographs at different magnifications: 100x (B), 200x (C), and 500x (D).

Figure 5 displays the surface of avocado seed starch biofoam with 20% chitosan (A), and its SEM micrographs at different magnifications: 100 x (B), 200 x (C), and 500 x (D). Although there is no significant difference in the appearance of the biofoam surface without (Figure 3.A) and with chitosan (Figure 5.A), a clear difference exists between the microstructure of the biofoam without chitosan (Figure 3 B-D) and with chitosan (Figure 5 B-D). The addition of chitosan caused the uniformly distributed cells in the biofoam to disappear and be replaced by bubbles. This agrees with previous research showing that chitosan in the biofoam creates bubbles or close pores on the top surface of a chitosan-wheat gluten mixture-biofoam (Chen et al., 2015). Chitosan has also been reported to cause the globular structure of the cells to become irregular (Bergel et al., 2017).

Figure 6 shows the tensile strength of avocado seed starch-based biofoam as a function of chitosan content. The letters in the graph indicate the results of one-way ANOVA and the Tukey test, with different letters signifying significant differences. There is a trend of increasing tensile strength as chitosan content rises. The tensile strength of biofoam made from avocado seed starch ranges from 0.07 MPa without chitosan to 0.263 MPa with 20% chitosan, representing a 201.3% increase. Chitosan concentration significantly affects the biofoam's tensile strength ($p < 0.05$). Biofoam containing 20% chitosan has the highest tensile strength, which is significantly different from the other samples. These results demonstrate that adding chitosan enhances the biofoam's strength, aligning with previous findings from biofoams produced with potato, cassava, and corn starches (Bergel et al., 2017). In amylose and amylopectin molecules, hydroxyl groups at C2, C3, and C6 can form hydrogen bonds with water molecules at suitable temperatures, weakening the mechanical strength and leading to lower tensile strength. When chitosan is present, its amine group (NH_2) becomes protonated to NH_3^+, forming electrostatic bonds with the hydroxyl groups of amylose and amylopectin. This interaction replaces water molecules, reducing hydration and resulting in stronger mechanical properties and higher tensile strength. One reason for the increase in tensile strength with chitosan is its ability to reduce water interaction and strengthen the biofoam.

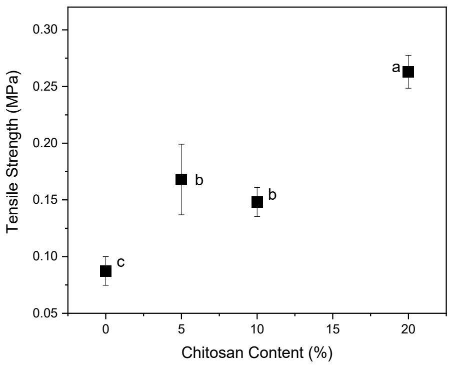

Figure 6. Tensile strength of avocado seed starch-based biofoam depending on chitosan content. Different letters among the data indicate significant differences.

The tensile strength values of avocado seed starch-based biofoam, ranging from 0.07 to 0.263 MPa, are in the same order of magnitude as those of biofoam made from potato starch, which range from 0.24 to 0.42 MPa (Rodrigues et al., 2020), and sago starch, varying from 0.07 to 0.4 MPa (Gani & Kusumayanti, 2022). However, these values are quite low compared to other biofoams, such as potato starch biofoam, which range from 1.09 to 1.27 MPa; cassava starch biofoam, from 1.01 to 1.26 MPa; and corn starch biofoam, from 1.34 to 1.61 MPa (Bergel et al., 2017), as well as chitosan/wheat gluten biofoam, which ranges from 28.6 to 43.6 MPa (Chen et al., 2015).

Figure 7 illustrates the water absorption of avocado seed starch-based biofoam depending on chitosan content at immersion times of 1, 3, and 5 minutes. The letters on the graph indicate the results of two-way ANOVA and the Tukey test, where different letters between data points signify significant differences. The results reveal that chitosan concentration and immersion time significantly affect the biofoam's water absorption ($p < 0.05$), and an interaction between these two factors is also significant ($p < 0.05$). Water absorption decreases with higher chitosan content, aligning with previous studies of biofoam made from potato, cassava, and corn starches (Bergel et al., 2017), as well as cassava peel and banana peel starches (Darni et al., 2023). The high-water absorption of biofoam is due to starches being hydrophilic molecules because of hydroxyl groups. When water is present, hydrogen bonds form between hydroxyl groups and water molecules, leading to high water uptake. However, with chitosan present, as explained above, the NH_2 group of chitosan becomes protonated to NH_3^+, which electrostatically bonds with the hydroxyl groups of amylose and amylopectin. This bonding prevents interaction between water and starch molecules, thereby reducing the biofoam's water absorption.

The results also show that the water absorption of biofoam depends on immersion time; the longer the immersion time, the more water is absorbed. However, as the chitosan content in the sample increases, water absorption becomes less dependent on immersion time. Statistically, for samples without chitosan, there is a significant difference between water absorption at 1-, 3-, and 5-minute immersion times, which is also true for samples with 5% chitosan. For a sample with 10% chitosan, water absorption at 3- and 5-minute immersion times is not significantly different, but both differ from the 1-minute immersion value. Finally, for the sample with 20% chitosan, the only significant difference is between the 1- and 5-minute immersion times. Based on this trend, adding more chitosan might result in water absorption values at 1-, 3-, and 5-minute immersion times not being statistically different, suggesting that at higher chitosan concentrations, water absorption could become independent of immersion time.

The water absorption of the avocado seed starch-based biofoam after a 5-minute immersion is about 40%, which is lower than that of potato, cassava, and corn starch, roughly 70% (Bergel et al., 2017), and lower than cassava and banana peel starches, about 80% (Darni et al., 2023). However, this water absorption level is still high for biofoam applications; adding 20% chitosan reduces it, making it suitable for use.

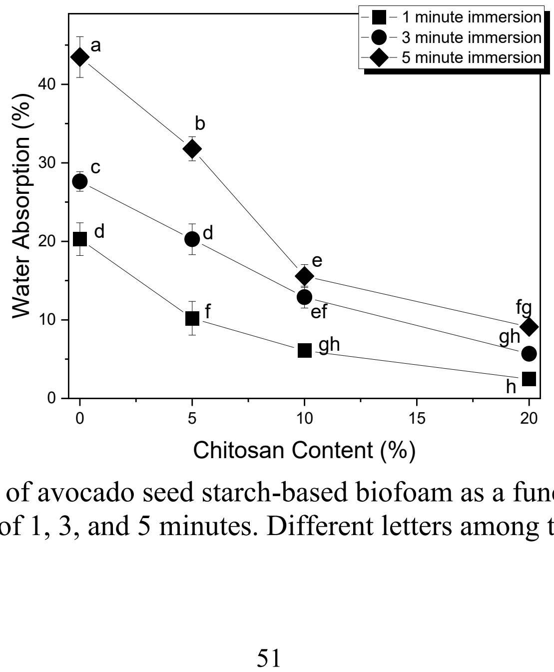

Figure 7. Water absorption of avocado seed starch-based biofoam as a function of chitosan content at immersion times of 1, 3, and 5 minutes. Different letters among the data indicate significant differences.

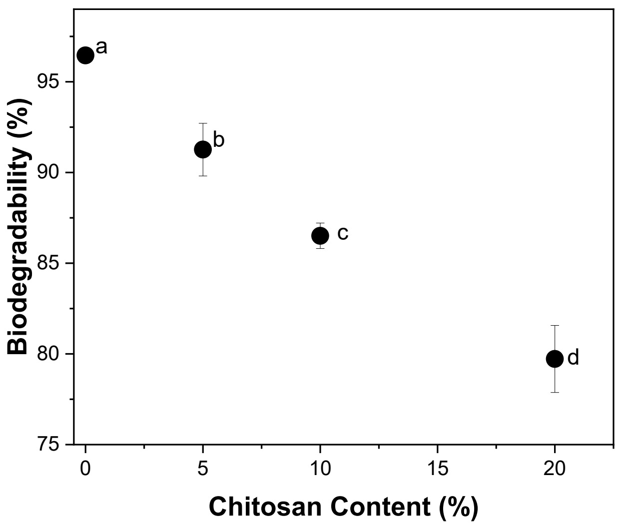

Figure 8. The biodegradability of avocado-seed starch-based biofoam as a function of chitosan content. Different letters among the data indicate significant differences.

Figure 8 displays the biodegradability index of avocado seed starch-based biofoam as a function of chitosan content. It represents the percentage of biofoam degradation over a 14-day period. The letters on the graph indicate the results of one-way ANOVA and the Tukey test, where different letters between data points denote significant differences. The results show that chitosan concentration significantly affects biofoam biodegradability ($p < 0.05$). As chitosan content increases, biofoam biodegradability decreases, which aligns with previous studies on biofoam made from a mixture of cassava peel and banana peel starches (Darni et al., 2023). During biodegradation, enzymes from bacteria hydrolyze the starch in the biofoam: amylases target α-(1→4)-bonds, and β-glucosidases target α-(1→6) bonds. Since the presence of chitosan reduces water absorption—an essential factor in degradation—the degradation process slows down in biofoam containing chitosan, evidenced by the decreasing biodegradability with increasing chitosan content, as shown in the figure.

The results show that in 14 days, 96.5% of biofoam degraded, leaving only 3%, and with the addition of 20% chitosan, 79.9% degraded. This is significantly better than biofoam made from a mixture of cassava peel and banana peel starches, which degraded by less than 25% after 14 days, and the addition of chitosan further reduces these values (Darni et al., 2023). As a comparison, the biodegradability index of biofoams made from a mixture of cassava flour and corn fiber was 25% (Sumardiono et al., 2021), and from a mixture of cassava starch ranging from 20% to 80% with the addition of 6% microcrystalline cellulose (Fatrozi et al., 2020). Comparing the biodegradability index of biofoams from previous results with that of avocado seed starch-based biofoam from this study, it is clear that avocado seed starch-based biofoam demonstrates good biodegradability.

Studies showed that avocado seed starch can be used to make biofoam. They are important in scientific development, especially in materials science, focusing on the measured physical properties and their dependence on chitosan content, which can also serve as preliminary data for further research. This work supports the Sustainable Development Goals, particularly Goal (SDGs) 12: Responsible Consumption and Production. Using avocado seed waste to produce starch, a raw material for making biofoam, which is an environmentally friendly product, promotes sustainable production and consumption patterns.

CONCLUSION

This study shows that avocado seed starch is a B-type starch, composed of spherical or oval granules ranging from 7.2 to 41.7 µm in diameter, with a crystallinity index of 27%. The starch can serve as a raw material for producing biofoam, with properties that depend on the chitosan content. The microstructure of the biofoam contains uniformly distributed globular cells formed by water vapor during preparation, with sizes ranging from 31.6 µm to 420.0 µm. This structure was disrupted and replaced by bubbles in the presence of chitosan. The tensile strength of the biofoam increases from 0.07 MPa to 0.263 MPa with 20% chitosan. Chitosan also reduces the water absorption of the biofoam and extends its biodegradation time. For future research, the same experiment could involve using silver nanoparticles as a filler. Many studies have demonstrated that silver nanoparticles possess antibacterial properties. Therefore, it is important to investigate the antibacterial effects of biofoam filled with silver nanoparticles. This feature is especially important when using biofoam for food packaging products.

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AUTHOR CONTRIBUTION STATEMENT

Both authors made significant contributions to this manuscript. The work involved a literature review, experiments, data analysis, and writing. SCW primarily focused on the biology section, while PJP concentrated on the physics section. Both authors read and approved the final version and agreed to be responsible for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest related to the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the creation, analysis, or writing of this manuscript. All parts of the research, including data collection, interpretation, and manuscript preparation, were done entirely by the authors without any AI-based assistance.

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