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Aquafaba and Cinnamon Essential Oil for the Production of Biodegradable and Compostable Wine Vineyard Waste-Based Material

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Abstract: (1) Grape stalks and aquafaba (Aq) from chickpeas are promising agricultural byproducts with potential applications in the development of sustainable biocomposite materials due to their ligno-cellulose and protein content. (2) This study aimed to evaluate the incorporation of Aq and cinnamon essential oil (CEO) into grape stalk-based materials to enhance mechanical properties and prevent microbial contamination. Four formulations were prepared, and their mechanical, physicochemical, and antifungal properties were assessed. (3) The incorporation of CEO significantly reduced water absorption, while formulations containing Aq exhibited the highest mechanical resistance, likely due to synergistic interactions between proteins and polysaccharides that modified the microstructure of cellulose fibers. Scanning electron microscopy (SEM) images supported these findings. Additionally, CEO-treated samples showed resistance to fungal contamination by *Botrytis cinerea*, unlike untreated samples, which were colonized by the fungus. Biodegradability tests indicated slower degradation for CEO-treated samples (10 weeks) compared to those without CEO (5–7 weeks). (4) The results suggest that the combination of Aq and CEO creates a promising material for use in food packaging, though further research is needed to fully understand the reinforcement mechanisms.

Keywords: biocomposite materials; environmental sustainability; grape stalk; biodegradability

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1. Introduction

In recent decades, packaging production has heavily relied on polymers derived from fossil fuels due to their economic accessibility. However, this practice has led to significant environmental consequences, such as pollution and the accumulation of plastic waste worldwide. This situation has driven the implementation of stricter regulations in several countries, including Chile, where laws have been enacted to reduce plastic waste generation and ban the use of single use plastics [1]. In response to these challenges, bioplastics derived from plant-based sources have emerged as promising and sustainable alternatives for biomaterial production. The raw materials used include potato, sugarcane bagasse, rice, corn, jackfruit, among others [2,3]. However, plant-based bioplastics show reduced strength and stability [4], prompting innovative solutions such as adding cellulose fibers to improve their physical properties [5]. Mechanical strength, compatibility with

different matrices, and the overall performance of biocomposite material are key factors in their development and application. These aspects determine their durability, structural stability, and ability to integrate efficiently into various industries, such as biomedical, food, and sustainable packaging [6,7]. Several advances in research have been developed in this field using different cellulose sources such as plants, bacteria, algae, and fungi. Examples include grape pomace and eucalyptus pulp [8]. These sources contribute to the unique physicochemical properties and high biocompatibility of cellulose based biocomposite material, making them ideal for a wide range of applications [8–10]. Thus, the use of cellulose fibers has shown potential as a colloidal stabilizer by interfering with liquid drainage at the gas–liquid or liquid–liquid interfaces, simultaneously acting as a reinforcing agent in the polymeric structure [10–12].

A commonly discarded byproduct of the wine industry in the south-central region of Chile is grape stalks [13], making its valorization a priority for the local government. This material, rich in fibers and phenolic compounds, has been successfully used as cellulose fiber source in studies to produce biodegradable packaging [10]. Another common byproduct from agroindustry is aquafaba (Aq), which is the liquid phase resulting from the heat treatment of chickpeas in water. This component contains proteins with various functional properties, particularly its emulsifying, thickening, gelling, and foaming capacity [14–16]. The use of aquafaba has been explored in various applications, and its potential for the manufacture of biocomposite materials (material composed of two or more distinct constituent materials of plant origin) is currently being investigated. Simultaneous utilization of both materials has never been performed, as far as the researchers have found. Previous studies on protein–polysaccharide interactions suggest a possible synergistic effect, which suggests an advantage, since it improves the mechanical properties, biocompatibility and functionality [17] of biocomposite materials, making them more resistant, versatile and suitable for various applications [18]. Additionally, it could significantly contribute to the transition towards a circular economy in agricultural regions, revitalizing the local economy [13,19,20].

On the other hand, essential oils (EOs) are organic compounds with biological properties, including antifungal, antimicrobial, and antioxidant properties. These EOs can reduce the hydrophilicity of materials such as starch, by interacting with water molecules, decreasing their availability for physicochemical and microbiological reactions [21,22]. The inclusion of essential oils in biocomposite materials has proven effective in protecting against fungi, contributing to the quality and safety of the final products. Some essential oils possess antifungal properties that, when incorporated into biocomposites, act as natural protective agents against fungal growth [21], which improves the durability of the material. Furthermore, these essential oils can reinforce the structure of biocomposites, preventing their biological degradation and ensuring the preservation of their aesthetic and functional characteristics over time [23]. Finally, it is highlighted that, in addition to their ability to prevent fungal proliferation, essential oils contribute to product safety by preventing the colonization of pathogenic microorganisms, thus guaranteeing the safety of the final materials for the consumer [24]. In fact, the incorporation of cinnamon essential oil (CEO) has shown high effectiveness in combating molds (*Aspergillus niger* and *Penicillium citrinum*) and yeasts (*Candida albicans*), due to its cinnamaldehyde content [22].

This study aims to evaluate the effect of incorporating aquafaba and cinnamon essential oil (CEO) on the physicochemical, morphological, mechanical, and biocompostability properties of an innovative biomaterial made from wine industry residues. Unlike previous research, this study introduces aquafaba as a functional biopolymer and CEO as a bioactive agent, analyzing their impact on structural stability, mechanical strength, and material degradability. The novelty of this work lies in the unique combination of these components,

which not only enhances the properties of the biomaterial but also promotes a circular economy approach by upcycling agro-industrial byproducts. This study represents a significant advancement in the search for sustainable alternatives to conventional polymers, with potential applications in biodegradable packaging, biomedicine, and other sectors that require eco-friendly and functional materials.

2. Materials and Methods

2.1. Materials

The grape stems were provided by the Guild Association of Winegrowers of the Itata Valley, Ñuble Region, Chile. The grape stalk was transported to the laboratory, washed with distilled water and dried at 65 °C for 48 h in a forced air oven (HES VC55ECO, Santiago, Chile). The dried samples were ground in a knife mill (Cromtek, TE-631/4, Santiago, Chile). Glycerol and magnesium stearate were purchased from Sigma-Aldrich/Merck (Santiago, Chile). The starch and guar gum were purchased from a local store (Furet, Chillán, Chile).

2.2. Obtaining Aquafaba (Aq)

For aquafaba production, chickpeas were soaked in drinking water for 16 h according to the method described by Meurer [25]. The soaked chickpeas were drained, and the beans were washed three times with drinking water. Heat treatment was applied at for obtaining aquafaba is 98 ± 2 °C [26], using a 3:1 weight ratio (water:hydrated grain) for 20 min. After cooking, the water and cooked chickpeas were transferred to a glass container and left in the refrigerator at 5 °C for 24 h. The aquafaba was then separated from the cooked beans using a strainer and stored at −40 °C until further use.

2.3. Biomaterial Preparation

The preparation of the biomaterial samples was based on the methodology used by Cabanillas, Cruz-Tirado, and Debiagi [21,22,27], with some modifications. Due to time constraints for this study, only the amount of Aq and grape stems in the formulations were selected as variables, as they were of the greatest interest for achieving the research objective. A 2^2 factorial experimental design was used, based on values found in the literature [9], to evaluate the incorporation of aquafaba and vineyard residue. In the preparation procedure, Aq or distilled water was mixed with glycerol for 3 min until completely dissolved. Then, magnesium stearate, starch, guar gum, and previously ground grape stem were added. The latter two ingredients were incorporated in the final step due to the high viscosity they produce in the mixture. All ingredients were mixed for 5 min using a mixer (Stick Mixer 2616 Oster, Milwaukee, WI, USA) at maximum speed. Next, 50 g of each formulation was evenly distributed in a mold (15 cm × 15 cm) and subjected to heat treatment with a heat sealer for 20 min at 130 °C. The operating conditions of the tray sealer were previously established in earlier studies [21,22]. After the heat treatment, a CEO solution with a concentration of 7.5% *w*/*w* was applied to both sides of the samples as a surface coating, following the method described by Debiagi [22]. The sample was left to dry at room temperature for 24 h.

2.4. Experimental Design and Formulation

To evaluate the effect of the incorporation of Aq and CEO on the mechanical, antifungal and biodegradability properties of the produced biomaterial, a 2^2 factorial experimental design was used. Based on previous results, starch, grape stalk, guar-gum, glycerol, and magnesium stearate amount, as well as CEO concentration were selected. The initial formulations of the studied materials are shown in Table 1.

Table 1. Experimental factorial design.

Formulation	Water (mL)	Aq (mL)	CEO (% p/p)	Starch (g)	Guar Gum (g)	Glycerol (mL)	Mg Stearate (g)	Grape Stalk (g)
Aq-CEO-7.5%	0	100	7.5	35	1	10	0.4	20
Aq-CEO-0%	0	100	0	35	1	10	0.4	20
W-CEO-7.5%	100	0	7.5	35	1	10	0.4	20
W-CEO-0%	100	0	0	35	1	10	0.4	20

Aq-CEO-7.5% sample with aquafaba and essential oil; Aq-CEO-0% sample with aquafaba and no essential oil; W-CEO-7.5% sample with distilled water and essential oil; W-CEO-0% sample with water and no essential oil.

2.5. Physicochemical Characterization of the Material

2.5.1. Chemical Composition

The proximal analysis of the Aq, grape stem and the biomaterial was carried out by the laboratory for experimentation, control and certification of food quality (Lecyca, Universidad del BíoBío, Chillán, Chile). The content analysis of alpha cellulose, lignin and holocellulose were carried out in the Analytical Chemistry Laboratory of the Department of Industrial Processes of the Catholic University of Temuco (Temuco, Chile).

2.5.2. Water Absorption Capacity

The water absorption capacity (WAC) of samples at high relative humidity was also evaluated by a weight gain kinetics study over 4 days in a controlled atmosphere, according to Debiagi [22]. All samples were placed in desiccators at 75% and 98% of relative humidity (RH), until the equilibrium was reached. Afterwards, the sample's equilibrium moistures were determined by the gravimetric method drying until constant weight in an oven at 105 °C. Each test was performed in triplicate.

2.5.3. Mechanical Properties and Morphology

The methodology proposed by Matsuda et al. (2012) [28] was used with samples of the biocomposite material in the form of 100 mm by 25 mm test pieces. To determine the mechanical properties of the samples, a texturometer equipped with a 100 kg load cell was used. The texturometer was programmed with an initial grip separation of 80 mm and a crosshead speed of 2 mm/s. Each formulation was tested 10 times, and the reported values correspond to the average of the 10 tests performed on 10 samples. The texturometer measures the maximum strength of the material by applying a progressive load until rupture or significant deformation is reached. This parameter is expressed in Newtons (N) and is obtained by recording the maximum force applied before fracture. The measurement is performed using a probe or punch connected to a load cell, which records the force and displacement values, generating data that allow the mechanical properties of the material to be characterized under controlled conditions.

The morphology of the material was studied by SEM microscopy (JEOL JEM 6380 LV, FESEM Laboratory, Universidad Católica de Santiago, Chile) at an acceleration voltage of 6 kV. Samples were sputtered with gold for 2 min before SEM observation.

2.6. Biodegradability

The biodegradability of the samples was evaluated through a qualitative test based on a standardized methodology [10], with the aim of analyzing their disintegration under controlled composting conditions. Each sample had an initial weight of 1 g and dimensions of 5 cm × 5 cm.

For the test, the samples were placed in a composter equipped with sensors that monitored temperature and humidity, ensuring direct contact with the compost. The vessels

were kept under aerobic conditions at an average ambient temperature of 24 °C, with daily watering to maintain the optimal humidity of the system throughout the experiment.

Degradation was assessed weekly over a 10-week period through visual inspection and weight measurement [10]. In addition, the progression of the samples was documented with photographs taken at each analysis interval.

2.7. Microbiological Analysis

The zone of inhibition method was used according to Pelissari et al. [29], with modifications. The samples were aseptically cut into 10 mm × 10 mm rectangles and placed on plates containing PDA (Potato Dextrose Agar). The plates together with the rectangles of the biocomposite material were incubated at 25 °C ± 0.5 °C (24 h) prior to inoculation with the *Botrytis cinerea* strain (Strain RGM_2736), provided by the Bank of Microbial Genetic Resources, INIA Quilamapu, Chili. Subsequently, a spore suspension of the strain was diluted in a sterile solution to obtain 10^6 spores mL^{-1}, which were counted by direct counting using a Neubauer chamber. Next, 1 mL of the solution was plated on PDA medium, and incubated at 25 °C ± 0.5 °C (24 h). A 5 mm circle of the strain was planted in the center of each of the plates containing the rectangles of the biocomposite material, with the respective treatments and incubated at 27 ± 0.5 °C for 5 days. Finally, the inhibition of *Botrytis cinerea* growth was evaluated in each plate and treatment. The tests were carried out in quadruplicate for each formulation.

2.8. Statistic Analysis

The means of the results were evaluated by analysis of variance (ANOVA) and compared by Tukey's test with a significance level of 5% ($p < 0.05$), using the Jamovi software (version 2.3.28 solid, macOS, The Jamovi Project, UK) [30].

3. Results

3.1. Characterization of Mechanical and Physicochemical Properties of the Biomaterial

Table 2 presents a summary of the composition of grape stalk, aquafaba (Aq), and biomaterial formulations, Aq-CEO and W-CEO. Grape stalk shows high levels of fiber (54.5%), holocellulose (43%), alpha cellulose (18%), lignin (19%), and hemicellulose (25%). Protein, lipids, and nitrogen-free extract (NFE) are prominent in Aquafaba, with 21% protein, 8.57% lipids, and 65% NFE, respectively. In contrast, protein content is identical in both Aq-CEO and W-CEO formulations at 1.03%. However, the lipid, ash, fiber, and NFE contents show slight variations between them (Table 2).

Table 2. Chemical composition of the raw material and formulations.

	Grape Stalk	Aquafaba	Aq-CEO	W-CEO
Protein (%)	3.82 ± 0.04	20.99 ± 0.11	1.03 ± 0.01 [a]	1.03 ± 0.02 [a]
Lipids (%)	0.95 ± 0.01	8.57 ± 0.05	0.74 ± 0.06 [a]	0.77 ± 0.01 [a]
Ash (%)	3.26 ± 0.03	1.14 ± 0.03	0.69 ± 0.01 [a]	0.65 ± 0.04 [a]
Fiber (%)	54.54 ± 0.03	1.77 ± 0.06	4.24 ± 0.11 [a]	3.58 ± 0.09 [b]
NFE (%)	26.82 ± 0.02	64.61 ± 0.20	46.61 ± 0.05 [a]	47.1 ± 0.09 [b]
Holocellulose (%)	43 ± 0.12			
Alpha Cellulose (%)	18 ± 0.03			
Lignin (%)	19 ± 0.09			
Hemicellulose (%)	25 ± 0.14			

Values are presented with standard deviations. Different letters in the columns indicate significant differences ($p < 0.05$) in the analyzed results. Aq-CEO corresponds to the formulation with aquafaba and cinnamon essential oil; on the other hand, W-CEO corresponds to the formulation with distilled water and cinnamon essential oil.

Table 3 presents the water absorption of the biocomposite material, where it can be observed that the samples containing cinnamon essential oil (W-CEO-7.5% and Aq-CEO-7.5%) absorb less water compared to the samples that do not contain CEO. On the other hand, the same table also presents the maximum strength of the different formulations, highlighting the formulation with aquafaba and CEO, which achieved the highest maximum strength value.

Table 3. Water absorption (WAC) at different relative humidity and maximum strength of biomaterial samples.

Formulation	WAC-75%R.H (%)	WAC-98%R.H (%)	Maximum Resistance (N)
W-CEO-7.5%	5.0 ± 0.625^{a}	5.84 ± 0.358^{a}	22.1 ± 1.925^{a}
W-CEO-0%	11.0 ± 0.957^{b}	12.5 ± 0.625^{b}	10.4 ± 0.182^{b}
Aq-CEO-7.5%	5.4 ± 1.30^{a}	6.04 ± 1.30^{a}	44.7 ± 2.493^{c}
Aq-CEO-0%	10.8 ± 1.30^{b}	11.7 ± 1.58^{b}	33.7 ± 3.254^{d}

Values are presented with standard deviations. Different letters in the columns indicate significant differences ($p < 0.05$) in the analyzed results. WAC-75% R.H. represents the water absorption capacity at 75% relative humidity, while WAC-98% R.H. reflects the water absorption capacity at 98% relative humidity.

3.2. Biomaterial Morphology and Microstructure

The microstructure of all samples can be observed in the SEM micrographs (Figure 1). Inserts in the micrographs show a lower magnification photography of the corresponding formulation. Figure 1A clearly shows the formation of the cellulose fiber network. While Figure 1B, shows abundant globular structures along with cellulosic fibers. On the contrary, samples without Aq showed an heterogenous surface where cellulosic fibers are not visible.

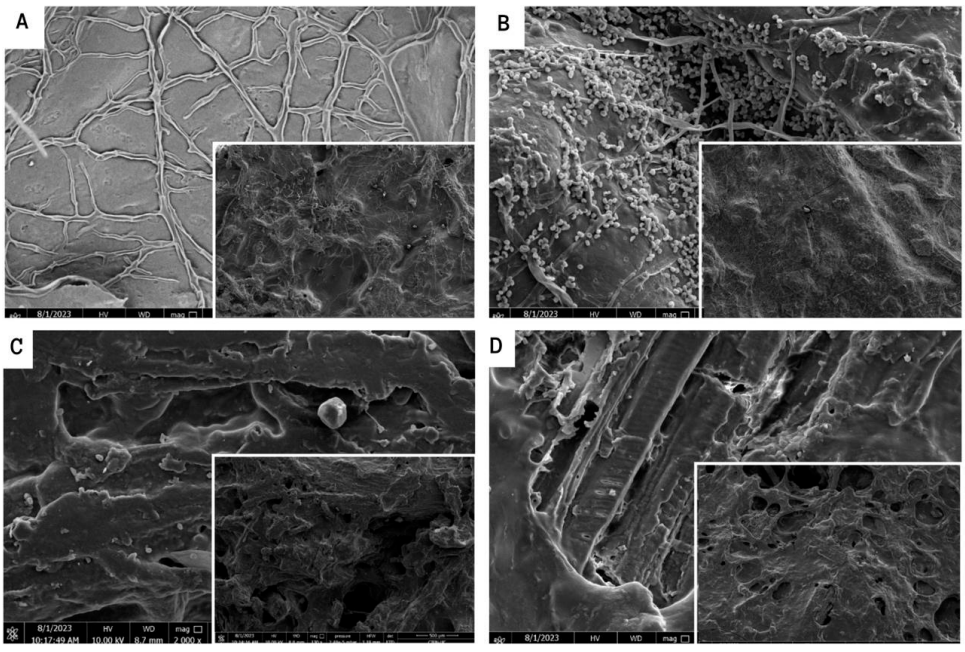

Figure 1. SEM images of: (**A**) Aq-CEO-0%, magnification: 2000×/250×. (**B**) Aq-CEO-7.5%: magnification: 2000×/250×. (**C**) W-CEO-0%. magnification: 2000×/130×. (**D**) W-CEO-7.5% magnification: 2000×/130×.

3.3. Biodegradability

Photographic evidence from the biodegradability test of the samples is illustrated in Figure 2A–H. It clearly shows the effects of the essential oil (CEO) on the structural stability of the materials. The images specifically present the Aq-CEO-7.5% and Aq-CEO-0%

samples, allowing a visual comparison between samples treated and not treated with CEO. The analysis of weight change is represented in Figure 3. The samples without CEO rapidly lost weight during the first four weeks of the experiment in comparison with samples with CEO (Figure 3). They also showed rapid fungal colonization starting from the second week, as illustrated in Figure 2D. This colonization was accompanied by a characteristic color change from brown to light gray, indicating typical fungal growth and demonstrating its proliferation throughout the material that did not contain CEO. Meanwhile, samples with CEO experienced only slight fungal presence in the fourth week, while the samples without CEO were completely colonized and showed advanced signs of decomposition by the second week (Figure 2D). The weight of the samples with CEO showed variations from the second week, with some samples even showing an increase in weight (water absorption capacity). The samples without CEO exhibited cracks and fragile structures by the sixth week, making them difficult to handle and weigh (Figure 2H). By the seventh week, these samples without CEO had decomposed and were integrated into the compost.

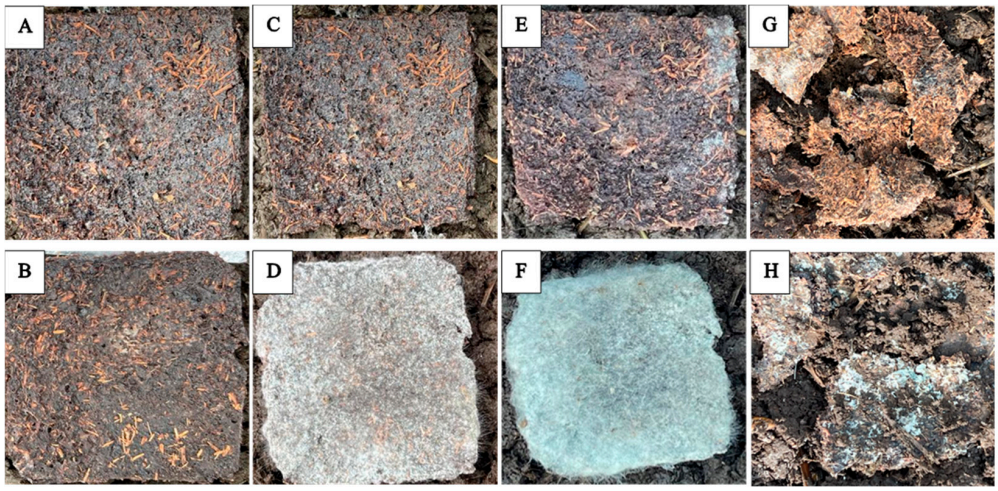

Figure 2. Images of the biodegradability test of biocomposite material at different composting times. (**A**,**C**,**E**,**G**): Aq-CEO-7.5% samples at week 1, 2, 4, and 10. Photos (**B**,**D**,**F**,**H**) are Aq-CEO-0% samples at week 1, 2, 4, and 10.

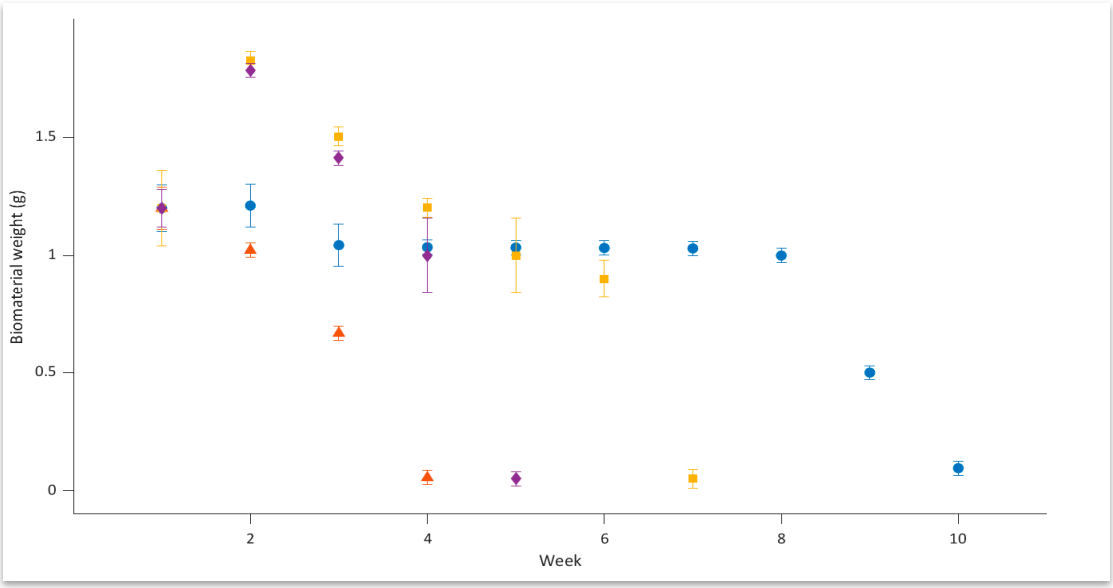

Figure 3. Biomaterial weight change over time while composting (blue circle: Aq-CEO-7.5%, purple diamond: Aq-CEO-0%, yellow square: W-CEO-7.5%, and red triangle: W-CEO-0%).

3.4. Microbiological Analysis

The results of the microbiological analyzes support the biodegradability results (Figure 4). Samples treated with cinnamon essential oil inhibited the growth of the fungus (*Botrytis cinerea*). In Figure 4C,D, it is observed that the fungus could not advance or colonize the samples, which demonstrates a wide zone of inhibition in each sample treated with the oil. On the other hand, the samples that were not subjected to cinnamon essential oil were completely colonized by the fungus.

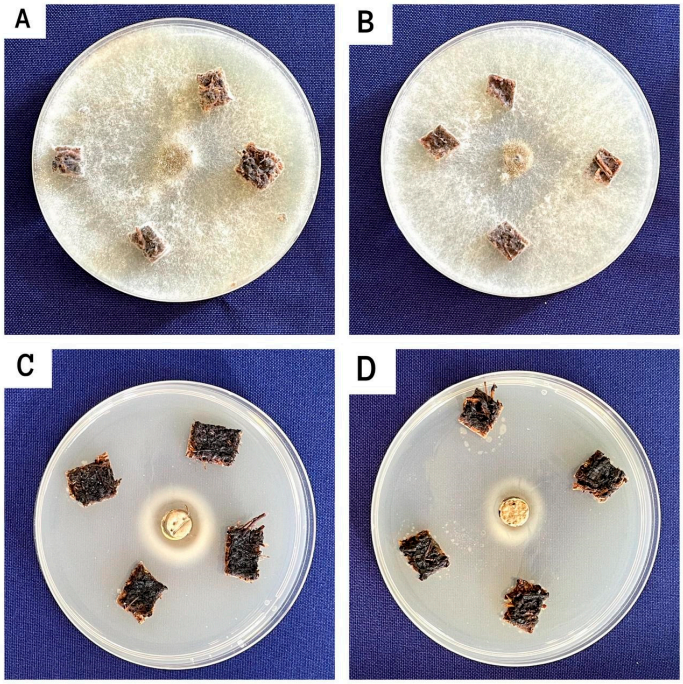

Figure 4. Inhibition zones of samples of (**A**) W-CEO-0%, (**B**) Aq-CEO-0%, (**C**) W-CEO-7.5%, and (**D**) Aq-CEO-7.5% for *Botrytis cinerea*.

4. Discussion

4.1. Characterization of Mechanical and Physicochemical Properties of the Biocomposite Material

Consistent with the typical composition of this byproduct, the grape stalk exhibits a high content of holocellulose and alpha cellulose, along with a significant amount of lignin (Table 1) [31]. Similarly, aquafaba contains 20% protein, aligning with findings from other studies [32,33]. Regarding biomaterial composition, the presence of Aq does not increase the protein content in the final biomaterial. This is consistent with previous studies showing that incorporating Aq into formulations for biocomposite manufacturing does not significantly enhance protein levels in the material [32,33]. Aq primarily consists of water and dry matter, including carbohydrates, low molecular weight proteins, saponins, and Maillard reaction products, with a protein content ranging from 0.95% to 1.5% in its liquid state [32]. Thus, while aquafaba offers several functional benefits in food formulations, it does not serve as a significant protein source when used in biocomposite material.

Regarding the water absorption capacity of the samples (Table 2), it is observed that the samples that incorporate CEO (W-CEO-7.5% and Aq-CEO-7.5%) exhibit significant differences when compared with the samples without CEO (W-CEO-0% and Aq-CEO-0%) in both RH conditions. Thus, the water absorption in the biomaterial decreased with the incorporation of CEO. The reduction in moisture absorption values in the samples with CEO is possibly due to the hydrophobic nature of the essential oil and the potential formation of covalent bonds between its functional groups and those of starch. These bonds

may form when reactive compounds in the essential oil, such as phenols, aldehydes, or ketones, chemically interact with the hydroxyl groups of starch, modifying the material's structure, reducing its water absorption capacity, and enhancing its stability [21,22]. It is likely that this phenomenon reduced the availability of hydroxyl and amino groups, thus limiting polysaccharide water molecular interactions through hydrogen bonds [34–36]. Similar results have been documented in other biocomposite materials that incorporate essential oils of clove, cinnamon or thyme [28,35,36]. Furthermore, it has been observed that biocomposite materials with essential oil have more compact structures and less porosity in the cross section, factors that can contribute to a reduction in water absorption, especially on the surface [22]. These findings underline the positive influence of cinnamon essential oil in modulating the water absorption properties of the biomaterial.

The analysis of the mechanical properties of the biocomposite material samples revealed notable differences between the different types of samples and their specific compositions. The Aq-CEO-7.5% sample exhibited the highest maximum strength (44.7 N), standing out significantly compared to other samples such as Aq-CEO-0%, W-CEO-7.5%, and W-CEO-0%, which showed considerably lower strengths [21]. This result suggests that the incorporation of essential oils (CEO) positively impacts the mechanical strength of biocomposite materials. The presence of CEO seems to enhance the strength of biocomposite materials, possibly due to the formation of a protective layer on the surface of the samples that increases their load-bearing capacity [22]. This finding is consistent with previous studies indicating that essential oils act as plasticizers, reducing fracture stress and increasing material flexibility [22,34,36–42]. However, the maximum strength achieved in this research is lower than that reported by Chiellini [37], who observed higher values in foamed materials based on potato starch and corn fibers. The analysis of recent scientific literature reveals that the incorporation of essential oils into biocomposite material significantly alters their mechanical properties, a phenomenon that critically depends on the concentration and type of oil used, as well as the polymer matrix of the biomaterial [43]. Studies such as the one by Agencia SINC, which explores antimicrobial bioplastics, emphasize how the choice of essential oil (cinnamon, clove, white thyme) and its dosage are determining factors in the final properties of the bioplastic. Similarly, research on biopolymer films made from polysaccharides and plant proteins confirms that strength and elongation vary substantially with oil concentration [44]. Additionally, the plasticizing effect of essential oils is a recurring finding in the literature. Studies such as the one by Gheorghita et al. [45] demonstrate how these compounds increase the flexibility of biocomposite material. Although the formation of emulsions has not been extensively explored in all studies, it emerges as a key strategy to improve the dispersion of oil in the matrix, which is crucial for achieving uniform mechanical properties. However, the literature also highlights the complexity of predicting the exact effect of essential oils on mechanical properties [46]. While tensile strength and elongation are the properties most affected, the magnitude and direction of these changes (whether an increase or decrease) depends on various factors [47]. Therefore, it is emphasized that specific studies are needed for each combination of essential oil and biomaterial matrix, as results can vary significantly. The addition of essential oil in emulsions also seems to have a plasticizing effect, increasing the flexibility and mobility of polymer chains in biocomposite materials [48]. This aspect is fundamental when designing materials for food packaging, where both strength and flexibility are essential to maintain product integrity during storage [49–54].

The use of essential oils in active food packaging has been shown to improve various properties of biopolymeric materials, including antimicrobial, antioxidant, mechanical, thermal, and barrier characteristics [55]. Active films and coatings incorporating essential oils can extend the shelf life of materials and maintain their quality [55]. In particular, the

incorporation of essential oils such as cinnamon has shown improvements in the tensile strength and elasticity of starch films, which is consistent with the observed plasticizing effect [56]. These results are comparable to those of other studies that also found improvements in tensile strength and mechanical properties through the addition of essential oils [57]. In contrast, the incorporation of essential oils into polyhydroxybutyrate (PHB) films has shown a reduction in tensile strength (TS) at higher oil concentrations, which can be explained by the replacement of polymer–polymer interactions with weaker polymer–oil interactions [58–61]. However, an improvement in elongation at break was observed with a 5% by weight load of essential oils, attributable to the increase in free volume and mobility of polymer chains [57]. This behavior is consistent with other studies that have reported improvements in mechanical characteristics and a decrease in tensile strength of biopolymers with the addition of essential oils [21,22,45]. The increase in elasticity at 5% loads can be beneficial for packaging applications that do not require high tensile strength but do require good stretchability [35,37,38]. The incorporation of oils influenced the mechanical properties, increasing tensile strength and Young's modulus but reducing elongation at break [57]. The use of essential oils has shown significant advantages in extending shelf life and improving sensory and safety properties of materials, as evidenced by Rolim and Ramalho [59], who compared the mechanical properties of materials with and without oil incorporation. However, the effectiveness of essential oils is not uniform, since, as Castro et al. (2021) [60] demonstrate in tea tree oil (TTEO) biocomposite, mechanical properties vary considerably depending on the amount of oil incorporated. This variability is confirmed in recent studies exploring various essential oils and biopolymer matrices, where texturometer measurements are found to be sensitive to oil concentration [44]. Furthermore, the ability of essential oils to act as antimicrobial and antioxidant agents, as detailed in reviews on active food packaging [55], underlines their potential to improve material safety. However, optimizing its use requires a deep understanding of the interactions between the oil, matrix and application conditions to ensure consistent and safe results. Evaluations carried out by Castro et al. (2021) [60] revealed that biocomposite materials formulated with tea tree essential oil (TTEO) showed variations in their mechanical properties depending on the amount of TTEO incorporated. Although an increase in tensile strength was observed with a low TTEO load, higher concentrations led to a decrease in strength and deformation [60]. This behavior is consistent with the flocculation effect of TTEO in the CS matrix [60]. The increase in Young's modulus with a low TTEO load can be attributed to the formation of new hydrogen bonds, while higher loads cause discontinuities that weaken the material [60]. The increase in the Young's modulus with a low load of TTEO can be explained by the formation of new hydrogen bonds, while at higher concentrations, discontinuities are created that weaken the material [60]. Hernández et al. [61], points out that the addition of pine essential oil and chitosan to PLA/PBAT fibers significantly influences the mechanical properties, reducing tensile strength and Young's modulus, while increasing plasticity [2,3,7,58,61]. This complexity in the interaction between the oil and the matrix aligns with the findings of Engel et al. [10], who demonstrate that essential oils act as plasticizers at low concentrations. The variability of the effects of essential oils, depending on the type of oil and matrix used, underscores the need for more in-depth and specific research in this field.

4.2. Biomaterial Morphology and Microstructure

Regarding the morphology of the samples, the analysis of the SEM micrographs (Figure 1) revealed heterogeneity on the surface of the material when comparing samples with and without Aq. This result contrasts with previous research, specifically the studies by Debiagi [22] and Engel et al. [10], where the cassava bagasse–PVA biocomposites exhib-

ited remarkable uniformity. The observed disparity could be attributed to the manipulable nature of cassava bagasse, which can be brought to a creamy state without persistent fibrous (lignified) structures in the mixture. Comparison with the findings of Cruz-Tirado [21], where cracks and deformations were observed in sweet potato starch-based materials, highlights the differences in the behavior of materials derived from different starch sources [62]. These differences can be attributed to the specific composition of each starch source, as well as the processing processes used [63]. The absence of cracks in the cassava bagasse-based material suggests greater structural integrity, possibly due to the homogeneity of the matrix [63]. Aquafaba acts as a structural support in mixtures due to its foaming, emulsifying, and gelling properties, which prevent the formation of surface defects. Its content of low molecular weight proteins, polysaccharides, and saponins stabilizes air bubbles by reducing interfacial tension and promoting the formation of a uniform structure that prevents foam collapse [64]. Additionally, its gelling capacity, derived from water-soluble carbohydrates and proteins, contributes to the cohesion of the material, minimizing the appearance of cracks on the surface. These characteristics improve the stability and quality of the final product [65].

On the other hand, other authors [9] described the formation of a dense surface layer ("crust") in starch-based biocomposite material, a phenomenon like what was observed in this study. This layer could form due to the rapid gelation and drying of the starch paste upon contact with the hot mold, which would explain the high surface density obtained [47].

However, as in the research by Salgado et al. [9], cavities were identified on the surface, possibly caused by air or steam bubbles that expand with heat and then contract and rupture during cooling, leaving microscopic voids [10]. In contrast, Debiagi et al. [22] documented different results, obtaining a surface without cracks or cavities.

Understanding these processes of gelation, drying, and bubble formation is crucial to controlling the structure and properties of the biomaterial, such as its surface density and integrity, thus optimizing its processing. As in the study carried out by Engel et al. [10], Figure 1A,B showed a homogeneous matrix where the cellulose fibers are firmly fixed and impregnated. This homogeneous matrix can be attributed to a phase rich in proteins that, together with cellulose fibers, play a crucial role in reinforcing the overall structure of the biomaterial [9,34,39,40]. This pattern is a recurring characteristic in thermoplastic materials that have been produced by thermal expansion and that contain starch in their composition [9]. Additionally, globular structures in 1B could correspond to pollen particles of cinnamon [39]. Microcracks that are not visible without the use of scanning electron microscopy (SEM) can also be seen. In contrast, in Figure 1C,D [W-CEO-0% and W-CEO-7.5%), a smooth structure is not observed as in the previous samples, as has been documented similar results in others research [21,23,24,27,49].

Cruz-Tirado [21] and Salgado [9], where control samples with the presence of porosities on the surface were obtained. In these studies, the presence of the essential oil and the cellulose fiber network are not evident. This could be due in part to the protein present in aquafaba causing the cinnamon to remain attached to the surface of the material [21]. In these samples, holes are also observed on the surface that can be attributed to the lack of protein (Aq). These samples are initially composed of an emulsion of starch, guar gum and distilled water, which, upon contact with temperature, gels, forming foams and microdroplets that give rise to holes on the surface of the material, which agrees with previous research [4,23,27].

It is crucial to highlight that the incorporation of cinnamon essential oil gave the biocomposite material a shine like plastic coating, without inducing visible changes in the color of the material. This contrast with other studies, such as those carried out by

Cruz-Tirado [21] and Debiagi [22], where alterations were observed in the initial color of the material, underlines the uniqueness of the effects observed with the addition of cinnamon essential oil in this study.

These observations suggest that the biocomposite material benefits from the presence of proteins (Aq), since, although it does not add a significant amount of protein to the biocomposite material, it does contribute to improving its stability and structural resistance. This is because, despite its low protein content, the compounds present in Aq, such as low molecular weight proteins, carbohydrates and other functional components, can influence the formation and consolidation of the biomaterial matrix [66]. These findings support the importance of understanding the microstructure of these materials and its influence on the final properties of the biomaterial, which can be of great relevance in various fields, from the food industry to the manufacture of biodegradable packaging.

4.3. Biodegradability

Photographic evidence of the biodegradability testing of the samples is illustrated in Figure 2A–H. This evidence clearly shows the effects of the essential oil (CEO) on the structural stability of the materials. The images specifically present the Aq-CEO-7.5% and Aq-CEO-0% samples, allowing a visual comparison between the samples treated with and untreated with CEO. The weight change analysis is represented in Figure 3. The samples without CEO lost weight rapidly during the first four weeks of the experiment compared to the samples with CEO (Figure 3). They also showed rapid fungal colonization starting in the second week, as illustrated in Figure 2D. Meanwhile, samples with CEO only showed a slight presence of fungi in the fourth week, while samples without CEO were fully colonized and showed advanced signs of decomposition by the second week (Figure 2D). The weight of samples with CEO varied from the second week onwards, with some samples even showing an increase in weight (water absorption capacity). Samples without CEO exhibited cracks and brittle structures by the sixth week, making them difficult to handle and weigh (Figure 2H). By the seventh week, these samples without CEO had already decomposed and integrated into the compost. The physical stability of the essential oil-treated samples is reflected in their ability to maintain their structure and strength during the biodegradation process [22,31]. Samples with CEO demonstrated greater structural integrity compared to samples without CEO, which underwent rapid, visible decomposition starting in the second week, evidenced by advanced fungal colonization and significant physical collapse. In contrast, the samples with CEO showed only a slight presence of fungi in the fourth week and retained their structure for a longer period. Furthermore, while the samples without CEO exhibited cracks and fragility by the sixth week, the samples with CEO showed greater resistance to crack formation and maintained superior structural cohesion. This behavior can be attributed to the essential oil's ability to slow decomposition and promote slow water retention, which contributes to preserving the structure and physical stability of the samples for longer compared to untreated samples. This phenomenon is particularly notable, given that the samples with CEO experienced only slight fungal presence by the fourth week, while the samples without CEO were completely colonized and showed advanced signs of decomposition. This phenomenon is consistent with previous observations. Fogasova et al. (2022) [67] reported similar structural integrity during the first four weeks, suggesting that water absorption does not necessarily lead to premature disintegration of the material. This finding is supported by recent research indicating that the antimicrobial terpenoid content in essential oils may play a crucial role in inhibiting biodegradation, favoring material stability. Fayyasbakhsh et al. (2022) [54] also highlight how antimicrobial terpenoids can effectively control the biodegradation of polyhydroxybutyrate, reinforcing the idea that CEO acts as a stabilizing agent. In contrast, the rapid colonization of samples without

essential oil aligns with the results of Röhl et al. (2023) [53]. He observed that the absence of antimicrobial additives in bio-based plastics led to a higher rate of disintegration and microbial colonization.

By the other hand, the difficulty in handle samples without CEO when separating these samples from the soil in the sixth week underscores the advanced degradation and loss of structural integrity. This also highlight the accelerated decomposition observed by the seventh week in the absence of biological inhibitors. These results are similar to those obtained by Engel et al. [10], where the samples (without CEO) completely disintegrated within 7 weeks. On the other hand, samples with essential oil (AQ-CEO-7.5%) took up to ten weeks to decompose and integrate into the compost, indicating that the incorporation of CEO acts as a protective agent against fungi and yeasts [22,42,68] slowing decomposition rate. These results have been observed in other studies such as that of Cruz-Tirado [21] and Debiagi [22], showing a slower degradation compared to samples without CEO. However, the slower decomposition rate is not as much as synthetic plastics that can take more than 50 years to degrade. These results provide valuable insights into the biodegradability of materials, highlighting the crucial role of essential oils in regulating the degradation process. The data suggest that their incorporation not only slows down the biodegradation rate but also helps preserve the structural integrity of the materials over extended periods [69]. This finding is key to understanding how the physical and chemical properties of biodegradable materials can be optimized to enhance their durability in various applications. Furthermore, the slower degradation observed in materials with essential oils may reduce the frequency of waste management interventions, promoting more sustainable practices and aligning with plastic waste reduction efforts, as discussed in the work of Sanhawong [34]. By delaying decomposition, these materials could be more suitable for applications requiring structural stability before complete biodegradation.

Comparing the research by Engel et al. [10] with the present study highlights the effectiveness of essential oil in delaying material degradation. They reported that by the fifth week of evaluation, their samples already exhibited signs of accelerated decomposition and structural loss, indicating an advanced degradation process. In contrast, in this study, materials incorporating cinnamon essential oil remained intact and showed no signs of fungal colonization during the same period, demonstrating greater structural stability.

These findings underscore the potential of essential oils to extend the lifespan of biodegradable products, particularly in applications where durability is crucial before final decomposition. Furthermore, it is important to note that the biodegradation rate of materials with essential oils is also influenced by environmental factors such as temperature, humidity, and microbial activity in the surroundings [46].

4.4. Microbiological Analysis

When evaluating the efficacy of CEO in inhibiting fungal growth through the observation of inhibition zones in culture media (Figure 4), the results obtained reveal that using CEO at concentrations of 7.5% in the formulations Aq-CEO-7.5% and W-CEO-7.5% generated significant inhibition zones, markedly contrasting with the controls without essential oil (Aq-CEO-0% and W-CEO-0%). These results provide clear evidence of the effect caused by the characteristics of the applied cinnamon essential oil. They also are consistent with previous research [29,36,50], where biocomposite materials containing essential oils were evaluated, being successful in inhibiting *Salmonella typhimurium* (Gram-negative bacteria) and *Listeria monocytogenes* (Gram-positive bacteria), molds, and yeasts.

The ability of CEO to inhibit fungal proliferation can be attributed to its bioactive compounds, which have demonstrated robust antimicrobial properties in previous studies. Hosseini et al. (2023) [36] indicate that essential oils, when incorporated into biopolymer

matrices, not only enhance the flexibility of materials but also provide significant antimicrobial and antioxidant properties. The CEO's ability to generate inhibition zones in this study may be related to its active components that interact with the fungal cell membranes, altering their integrity and function [57].

Furthermore, an important aspect to consider in CEO's antimicrobial efficacy is its volatility, which may influence its diffusion in solid media such as agar. The agar diffusion method relies on the ability of antimicrobial compounds to spread through the medium, and it has been observed that hydrophobic compounds, such as those found in essential oils, do not always diffuse efficiently in aqueous environments. However, in the present study, the observed inhibition zones suggest that CEO, despite its hydrophobic nature, exerted a clear antifungal effect. This could be attributed to the volatilization of certain active compounds, which may act in the surrounding environment and contribute to fungal growth inhibition. Previous studies [70] have suggested that volatile components of essential oils can exert antimicrobial effects by interacting with microbial membranes and interfering with cellular processes, even when direct contact is limited. Recent advancements in delivery strategies, such as nanoencapsulation, active packaging, and polymer-based coatings, have successfully tackled these challenges, enhancing the bioefficacy and controlled release of essential oils [26].

On the other hand, the results of the control images without CEO (Aq-CEO-0% and W-CEO-0%) show total fungal proliferation, confirming that the absence of essential oil allows the growth and expansion of the mycelium. This phenomenon underscores the importance of essential oils as potential antifungal agents in food packaging and other products where microbial contamination control is critical. Comparing these results with previous research, the study by Martínez et al. (2021) [71] has shown that essential oils have a significant impact on inhibiting the growth and biofilm formation of bacteria such as *Escherichia coli* and *Staphylococcus aureus*. The authors report that oils like *Lippia origanoides* and *Thymus vulgaris* possess high antimicrobial and antibiofilm activities, significantly reducing the motility capacity of bacteria [71]. Although our study focuses on fungi, the similarity in the mechanism of action of essential oils suggests that CEO could be operating under similar principles of interaction with cell membranes, resulting in the inhibition of fungal proliferation. Additionally, the antibacterial activity of biocomposites including essential oils in combination with protein polymers was investigated where it was evidenced that essential oil emulsions showed homogeneous distribution in the polymer matrix and sustained antimicrobial activity [72]. Although their study focused on bacteria, the ability of essential oils to be incorporated into polymeric matrices and maintain their activity suggests a similar possible strategy for developing antimicrobial materials in food applications.

Evidence shows that cinnamon essential oil has a notable inhibitory effect on fungal proliferation, making it a promising candidate for use in active packaging systems. The findings of this research highlight the potential of cinnamon essential oil as an effective antifungal agent, supporting the ability of essential oils to completely inhibit the growth of pathogens. This is consistent with existing literature on the use of essential oils for pathogen control. The antimicrobial properties of essential oils further emphasize their potential applications in food preservation and other areas where protection against microorganisms is essential. The antimicrobial properties of essential oils further emphasize their potential applications in food preservation and other areas where protection against microorganisms is essential.

The biomaterial analysis revealed a high concentration of holocellulose, alpha-cellulose, and lignin, with the addition of aquafaba showing no significant increase in protein content. The incorporation of cinnamon essential oil (CEO) notably improved

the material's mechanical properties, while reducing its water absorption capacity, which can be attributed to its hydrophobic nature and the formation of covalent bonds with the polymer matrix. Additionally, CEO slowed the biodegradation process, providing greater structural stability without affecting the material's compostability. Microbiological analysis demonstrated that CEO effectively inhibited fungal growth, highlighting its potential as an antimicrobial agent in biodegradable materials. These findings support the use of cinnamon essential oil as an additive in active packaging and other applications where protection against microorganisms is crucial.

5. Conclusions

The results obtained in this study demonstrate that the incorporation of aquafaba and cinnamon essential oil (CEO) in the formulation of biocomposite material from wine industry waste generates significant effects on their physicochemical, mechanical, morphological, and biodegradability properties. It was observed that the presence of aquafaba improves the cohesion and structural stability of the biomaterial, while the addition of CEO not only provides antimicrobial and antioxidant properties but also modifies the biodegradation rate under controlled composting conditions.

From a mechanical perspective, biocomposite material formulated with aquafaba, and CEO showed improved strength and greater flexibility, suggesting their viability for applications requiring materials with good structural performance and durability. Furthermore, compostability tests confirmed that the biocomposite material progressively disintegrate in an aerobic composting environment, with a significant weight reduction over the experimental period.

These findings reinforce the potential of these biocomposite material as a viable and sustainable alternative to conventional polymers, promoting the revaluation of agro-industrial byproducts and aligning with the principles of the circular economy. The combination of aquafaba and CEO allows to produce materials with improved functional properties, expanding their application possibilities in biodegradable packaging and other sectors seeking eco-friendly alternatives.

For larger-scale implementation, additional studies will be required to optimize the formulation, evaluate its performance under various usage conditions, and analyze its environmental impact in real-world environments. Furthermore, future research could focus on improving the barrier properties, thermal resistance, and long-term stability of these biocomposite material. This work significantly contributes to the development of innovative and biodegradable biocomposite material, demonstrating that the use of aquafaba and CEO allows for materials with improved structural and functional performance, greater biodegradability, and reduced environmental impact. Its implementation in various industries would not only reduce dependence on synthetic plastics but also drive the development of more sustainable and environmentally responsible solutions.

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