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Edible Spray Coating with Water Hyacinth Cellulose and Coconut Husk-based Carbon Black for Food Applications

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Abstract

Food waste and microbial contamination have led to an annual increase in foodborne diseases. One potential solution is the application of an edible spray coating (ESC) as a bioplastic, incorporating antibacterial agents. The spray technique is considered most effective due to its ease of application and controllable thickness. This research evaluates carbon black (CB) derived from coconut husk waste as an antibacterial agent in ESC. Coconut husk was selected because it contains up to 54% lignin. The ESC was formulated using a mixture of hydroxypropyl methylcellulose (HPMC), k-carrageenan, water hyacinth cellulose, and glycerol. Additionally, other antibacterial agents such as silver and chitosan were included as positive controls. The CB was produced through pyrolysis of coconut husk at 700 °C, with nitrogen gas added at a heating rate of 5 °C/min. The water hyacinth cellulose was extracted using a chemical method. In this study, ten ESC formulations were tested with varying concentrations of silver, chitosan, and CB. The resulting ESC viscosities ranged from 16.8 to 46.9 cP. Antimicrobial activity against E. coli and S. aureus demonstrated that ESC with 1.5% w/v CB exhibited antibacterial activity with inhibition zones of (2.2±0.3) mm and (32±3) mm, respectively. The application of ESC on cherry tomatoes and strawberries showed that samples containing CB experienced a lower weight loss over time. This indicates the potential of CB in preventing microbial contamination.

Introduction

Food waste has emerged as a significant global issue, presenting major economic, environmental, and social challenges [1]. The sheer volume of food waste that approximately 30% of global food production, or around 1.3 billion tons, is either lost or wasted annually throughout various stages of the food supply chain [2]. According to the United Nations Environment Program's Food Waste Index Report of 2021, about 931 million tons of this waste was generated in 2019 [3]. This is largely due to the relatively short shelf-life of food and microbial contamination. This issue can be addressed through food coatings, such as edible coatings. The use of edible coatings on fruits can extend their shelf-life and maintain their qualitative characteristics [4].

Several techniques for applying edible coatings include dipping, brushing, and spraying. All these techniques exhibit multiple advantages and disadvantages. For example, in dipping techniques, the solution can effectively cover the surface of the food, however, the coating solution may become contaminated after the food has been immersed [5]. Similarly, the brushing technique offers the advantage of requiring no specialized equipment for its application. However, this method often results in a non-uniform coating finish due to streaks caused by brush strokes. The spraying technique is one of the most effective edible coating methods because it provides homogeneous coating, controllable thickness, and low production costs. The spraying system in edible spray coating (ESC) does not contaminate the coating solution and allows temperature control to keep the antimicrobial agents effective [6].

To slow down the respiration rate of the fruit and enhance the barrier properties of the coating [7], cellulose needs to be added to the ESC composition. Cellulose, the most prevalent biopolymer derived from biomass, has garnered significant interest because of its biodegradability, renewability, sustainability, biocompatibility, and ease of modification. Cellulose has densely packed fibril fibers and a crystalline structure, which creates tortuous pathways within the coating matrix. This configuration reduces the respiration rate of fruits, thereby preventing spoilage [7]. Water hyacinth is an aquatic weed that contains a high cellulose content of up to 55% [8]. Using water hyacinth as a raw material can increase the economic value of this plant while also addressing the problem of waterway blockages.

Adding antibacterial agents to edible coatings can be a potential solution for protecting food from contamination. This is because antibacterial agents can control the proliferation of bacteria that cause damage to fruits [9]. Therefore, these active substances enhance food protection against bacterial contamination, indirectly extending the shelf life of fruits. Previous studies have demonstrated that the addition of thyme essential oil, incorporated into alginate-based edible coatings, exhibited the active compound's ability to combat pathogenic bacteria such as *Listeria monocytogenes, Staphylococcus aureus, Salmonella Typhimurium, and Escherichia coli* [10]. Additionally, metal nanoparticles have garnered interest as antibacterial agents in edible coatings. For instance, incorporating starch-based coatings with ZnO nanoparticles has been proven effective in inhibiting the growth of both Gram-positive and Gram-negative bacteria, particularly *Bacillus subtilis* [11]. Given the widespread use of essential oils and metal nanoparticles as antibacterial agents, this study aims to explore the incorporation of carbon-based materials as antibacterial agents.

The addition of Carbon Black (CB) in ESC has the potential to act as an antibacterial agent, thereby extending the shelf-life of food. In previous studies, CB exhibits antibacterial activity against *Pseudomonas aeruginosa* [12]. The pores and hydrophobic properties of CB can adsorb microorganisms via Van der Waals or electrostatic interactions due to the charge difference between the CB surface and the microorganisms [13]. CB is an amorphous porous material containing 90–99% carbon, produced through the partial combustion of hydrocarbon compound [14]. Therefore, CB can be produced from biomass waste containing lignin, because it consists of 60% carbon [15]. Coconut husk is an agricultural solid waste that contains up to 54% lignin [16]. Currently, the utilization of CB as an antibacterial agent is still limited, while biomass waste such as coconut husks is abundant. Utilizing coconut husks to produce CB is expected to increase the economic value of this waste.

Therefore, this study evaluates CB derived from coconut husk waste to determine the effective concentration of CB in the application as an antibacterial agent in ESC. This study also evaluates the antibacterial activity, toxicity, and analyzes the impact of CB application compared to silver and chitosan in ESC suspension. Characterization through Scanning Electron Microscope-Energy Dispersive Spectroscopy (SEM-EDS) was conducted to provide information on the surface morphology and elemental composition of CB derived from coconut husks. Characterization through Fourier Transform Infrared Spectroscopy (FTIR) was also conducted to provide information on the functional groups of cellulose extracted from water hyacinth. The ESC suspensions that were made were characterized through FTIR and SEM-EDS to obtain properties that demonstrated cohesiveness in the suspension. Several tests on the ESC suspension were then conducted, including antibacterial assay, toxicity assay, viscosity test, and shelf-life test. From the results of this study, the direction and efficiency of the experiment were determined and discussed through statistical analysis and compared with previous studies.

Experimental Method

Preparation and Characterization of Coconut Husk Carbon Black

The coconut husk was washed using aquadest to remove the impurities from it. The sample was then dried and ground until it passed through a 100-mesh sieve. Then, the sample was pyrolyzed for 3 h with the addition of nitrogen gas at a rate of 5 °C/min at a temperature of 700 °C [17]. The CB obtained from pyrolysis was subjected to ball milling treatment to reduce its particle size. The CB was analyzed using SEM-EDS (Thermoscientific Prisma E) to examine its morphology and particle size.

Preparation and Characterization of Water Hyacinth Cellulose

The water hyacinth stems were washed using aquadest to remove the impurities from it. The sample was then dried and the ground passed through a 100-mesh sieve. Then, delignified it with 10% sodium hydroxide (NaOH) and heated at 80 °C for 1 hour. It was subsequently washed using aquadest to achieve a neutral pH and dried at 100 °C until a constant weight was attained. The dried sample was bleached with 1% sodium chlorite ($NaClO_2$) at 75 °C for 1 hour and adjusted to pH 4 using acetic acid (CH_3COOH). The sample was then washed using aquadest to achieve a neutral pH and dried at 60 °C to obtain solid cellulose [18]. The cellulose was further hydrolyzed with 50% sulfuric acid (H_2SO_4) at 60 °C for 30 minutes. At the expiration of the time, aquadest is added in an amount equal to twice the total volume of the acid used. The solution is then centrifuged at 6000 rpm for 10 minutes, and the supernatant is decanted. This process is repeated until a neutral pH is obtained [18]. The cellulose was characterized using FTIR (Bruker Tensor 37) with a wavenumber of 4000–500 cm^{-1} and SEM (Thermoscientific Prisma E) to analyze its functional groups, morphology, and particle size.

Preparation of Edible Spray Coating

The ESC suspension was prepared by mixing k-carrageenan and hydroxypropyl methylcellulose (HPMC) (3:1) and diluting with aquadest to a total volume of 50 mL at 40 °C for 2 h. During the mixing process, 10% wt glycerol and 1% wt cellulose were added [5]. To evaluate the effectiveness of CB against the antimicrobe, we tested several ESC compositions

(Table 1). ESC without CB was selected as a negative control, while ESC with silver particles or chitosans were as positive controls. These were selected as silver particles and chitosans were shown to have antibacterial activity against *E. coli* and *S. aureus* [19], [20]. The suspension was then ultrasonicated for 10 minutes.

Table 1. Design of ESC Suspension Variations

Variation	**k-carrageenan (% wt/v)**	**Glycerol (% wt)**	**HPMC (% wt/v)**	**Cellulose (% wt)**	**Carbon Black (% wt/v)**	**Silver (% v/v)**	**Chitosan (% wt/v)**
V0	0.2	10	4	1	0	0	0
V1A	0.2	10	4	1	1	0	0
V1B	0.2	10	4	1	1.5	0	0
V1C	0.2	10	4	1	2	0	0
V2A	0.2	10	4	1	1	0.5	0
V2B	0.2	10	4	1	1.5	0.5	0
V2C	0.2	10	4	1	2	0.5	0
V3A	0.2	10	4	1	1	0	0.5
V3B	0.2	10	4	1	1.5	0	0.5
V3C	0.2	10	4	1	2	0	0.5

Characterization of Edible Spray Coating Variation

The variations of the ESC before and after the addition of CB were characterized using FTIR (Bruker Tensor 37) with a wavenumber of 4000–500 cm^{-1} to analyze the characteristic functional groups of CB and cellulose. Additionally, the variations of ESC were characterized using SEM-EDS (Thermoscientific Prisma E) to examine the surface morphology and chemical elements, in order to determine the percentage of the predominant elements present in the solution.

Toxicity Assay

Toxicity assay was conducted using the Brine Shrimp Lethality Test (BSLT) [21]. The test organisms used were *Artemia salina*. A total of 17 g of sea salt was dissolved in 500 mL of aquadest. One gram of *Artemia salina* cysts was soaked and left to hatch and developed into larvae (nauplii) within 48 h [22]. The ESC solutions were diluted with aquadest to obtain five concentrations (10; 100; 1,000; 10,000; and 100,000 ppm). Each concentration was aliquoted into a microplate containing 10 nauplii. Toxicity was measured by calculating the percentage of nauplii mortality after 24 h, and a linear regression equation was established [23]. ESC solutions were considered non-toxic if the lethal concentration (LC50) was greater than 1,000 ppm [21].

$$\%\, larval\ mortality = \frac{Number\ of\ deaths\ larvae}{Number\ of\ test\ larvae} \times 100\%$$

Antibacterial Assay

Antibacterial activity was tested using the disk diffusion test (DDT) against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria. The bacteria were rejuvenated in nutrient broth media and then diluted to the standard 0.5 McFarland turbidity. Chloramphenicol 0.1% was used as positive control and V0 served as the negative control. Tryptone soy agar media was poured into petri dishes to a depth of 15 mL and allowed to solidify. A bacterial suspension of 70 μL was added to the solidified media [24]. Sterile paper discs were then placed on the surface of the plates and 20 µL of the ESC variations were injected onto the discs. The plates were incubated at 37 °C for 24 h. The test was conducted in a laminar air flow (LAF) cabinet, where the inhibition zones on the media were observed and measured using Fiji ImageJ software.

Viscosity Test

The viscosity test aims to determine the thickness and flow rate of each ESC variation. The viscosity test is conducted using a viscometer (Viscometer NDJ-5S) set at rotor 1 and speed 60 rpm.

Shelf-Life Test

All variations of the ESC were applied to the surface of cherry tomatoes and strawberries using a biospray device. The pressure of the biospray was set to 30 psi. The fruits were exposed to the biospray system and sprayed until 3–10 mL of the suspension was used for each fruit. The fruit then stored and observed for 8 days at room temperature. The shelf-life test aimed to evaluate the effect of ESC coating on the shelf-life of the food. Parameters measured included changes in color and weight loss.

Results and Discussion

Carbon Black

Biomass-based CB is produced from lignin compounds undergoing pyrolysis. The results of the lignin content test on coconut husk in this research showed a lignin content of 29.93%. This result is like previous research, which was 29.8% [25]. The physical process occurring in coconut husk turning into CB is presented in Figure 1. The yield of CB obtained from pyrolysis reached 36.5%. SEM images at 1000x magnification showed a porous surface morphology (Figure 1d). The pores on the CB surface are formed because volatile compounds are eliminated during the heating process [26]. These pores can adsorb many bacteria due to their affinity with microorganisms [13]. Based on the EDS analysis (Table 2), the obtained CB contained carbon with an atomic percentage of 92.88%. It can be assumed that most of the volatile compounds in the CB have been removed [26].

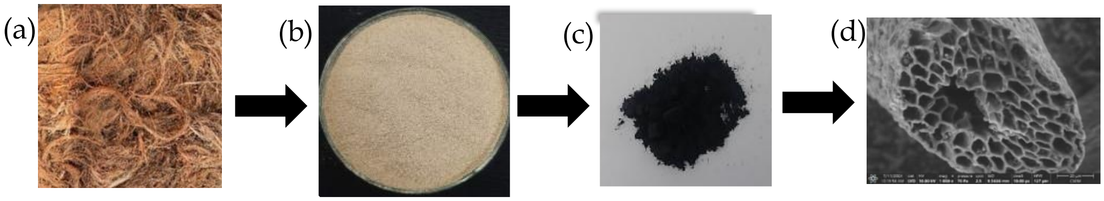

Figure 1. (a) Coconut Husk, (b) Chopped Coconut Husk, (c) Carbon Black, and (d) SEM Image of Carbon Black

Table 2. Element Composition of Coconut Husk Carbon Black

Element	Atomic %
C	92.88
O	6.85
Mg	0.07
Al	0.20

Cellulose

Cellulose was achieved through delignification, bleaching, and acid hydrolysis stages. The chemical process occurring in water hyacinth turning into cellulose is presented in Figure 3. The cellulose extraction from water hyacinth yielded 46.27%, similar to previous research which reported a yield of 46.26% [27]. The mass reduction is presumed to be due to the disruption of lignocellulosic bonds, causing lignin and hemicellulose to degrade easily during the cellulose extraction process [27]. The FTIR spectra of water hyacinth cellulose (Figure 2) showed -OH stretching and C-H stretching peaks at 3399 cm^{-1} and 2903 cm^{-1}, respectively. Other absorption peaks at 1159 cm^{-1} and 895 cm^{-1} correspond C-O-C pyranose ring and β-glycosidic linkages, which are characteristic functional groups of cellulose [28].

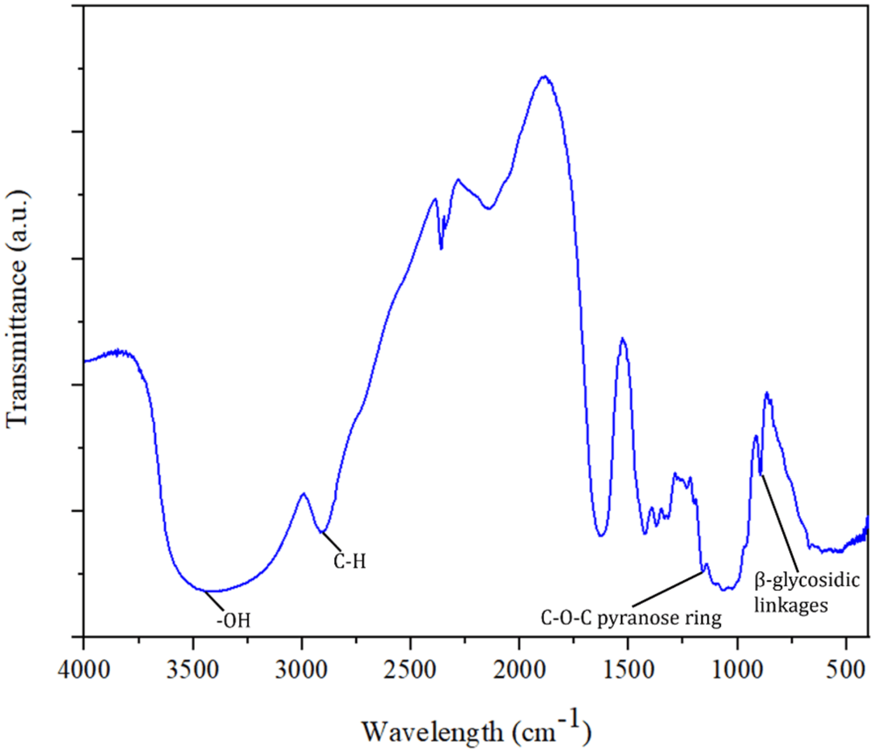

Figure 2. FTIR Spectra of Cellulose Water Hyacinth

The morphology of the cellulose particles is granular with an average particle size of (1.23±0.97) µm (Figure 3d). The smaller the cellulose particle size, the better its effectiveness in enhancing the barrier properties of the coating [5]. After the cellulose underwent acid hydrolysis treatment, it was observed that the water hyacinth cellulose largely remained aggregated and was not fully separated. This is because cellulose particles tend to agglomerate easily [29].

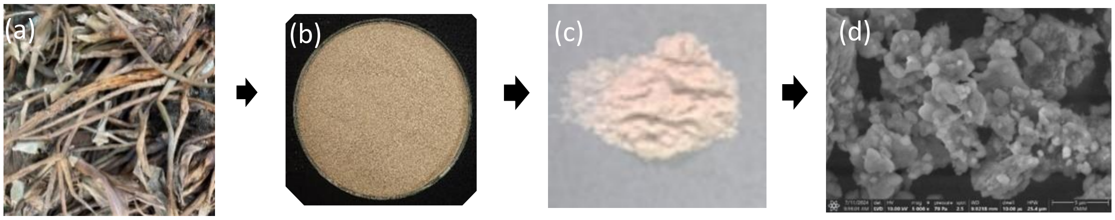

Figure 3. (a) Water Hyacinth, (b) Chopped Water Hyacinth, (c) Cellulose, and (d) SEM Image of Cellulose

Characterization of Edible Spray Coating

The FTIR spectra of ESC before the addition of CB (Figure 4a) and after the addition of CB (Figure 4b) showed peaks at 3200–3570 cm^{-1} and 2840–3000 cm^{-1}, indicating a decrease in -OH stretching and -CH stretching groups. This decrease suggests that the hydroxyl groups were replaced by hydrophobic groups from CB, thereby reducing the hydrophilic nature of the ESC [30]. The presence of $-CO_2$ groups around 2349 cm^{-1} is associated with the porous nature of CB [31].

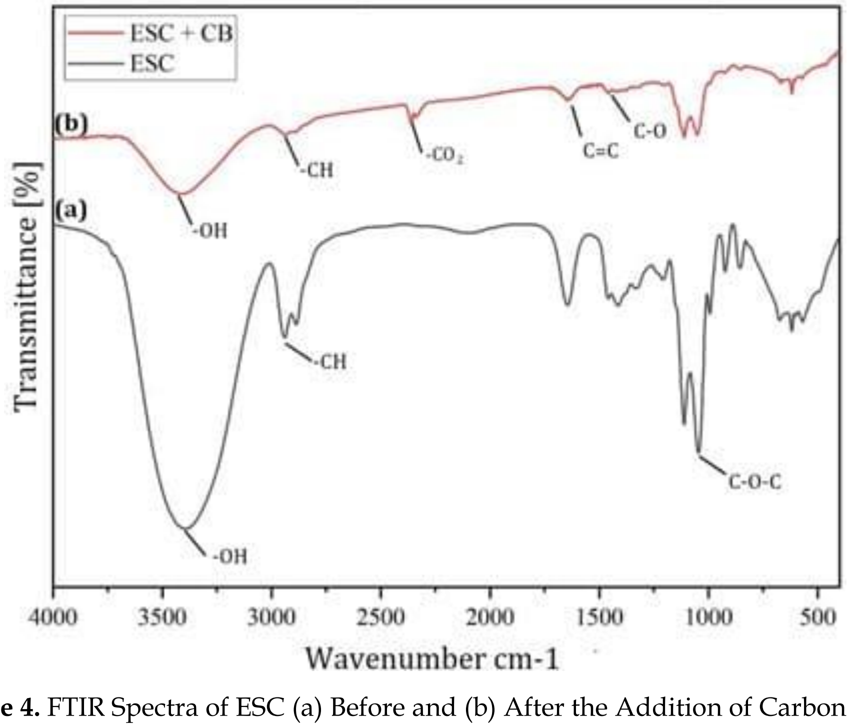

Figure 4. FTIR Spectra of ESC (a) Before and (b) After the Addition of Carbon Black

The surface morphology of V1B (Figure 5) showed a relatively uniform distribution of particles across the surface. The detected elements included carbon (C), oxygen (O), sodium (Na), sulfur (S), and aluminum (Al), with mass percentages of 53.26%, 32.06%, 7.24%, 5.27%, and 2.17%, respectively (Table 3). The abundance of C and O elements corresponds to the addition of CB, which is rich in carbon, and cellulose, which contains a high amount of oxygen [14], [28].

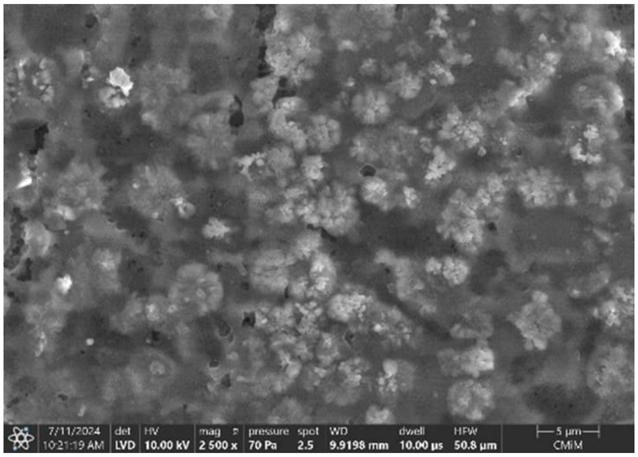

Figure 5. SEM Image of ESC After the Addition of Carbon Black

Table 3. Element Composition of ESC After the Addition of Carbon Black

Element	**Weight %**
C	53.26
O	32.06
Na	7.24
Al	2.17
S	5.27

Toxicity of Edible Spray Coating

Toxicity assay using the BSLT method was conducted with concentration intervals ranging from 10 to 100,000 ppm for each variation of the ESC solution. A solution is considered non-toxic if the LC_{50} > 1,000 ppm [23]. In this research, it was found that for the concentration range of 10 to 100,000 ppm, the mortality rate was less than 50%. This indicates that the variations of the ESC solution in are non-toxic.

Antibacterial Activity of Edible Spray Coating

Antibacterial assay data exhibit that variation V0 as negative control (K-) showed no inhibition zone on the medium (Figure 6), indicating that V0 had no antibacterial activity. Variation V1B exhibited the largest inhibition zone, measuring (32±3) mm against *S. aureus*. This value suggests that variation V1B has significant antibacterial activity against *S. aureus*. Variation V1B also produced the largest inhibition zone against *E. coli*, measuring (2.2±0.3) mm, indicating that variation V1B possesses antibacterial activity against *E. coli*. The addition of silver and chitosan did not contribute to enhancing the antibacterial activity of CB against *S. aureus* and *E. coli*. This was further corroborated by antibacterial testing of ESC solutions with the addition of silver and chitosan alone, which did not yield any inhibition zones against either bacterium. Therefore, CB demonstrates antibacterial activity against both *S. aureus* and *E. coli*.

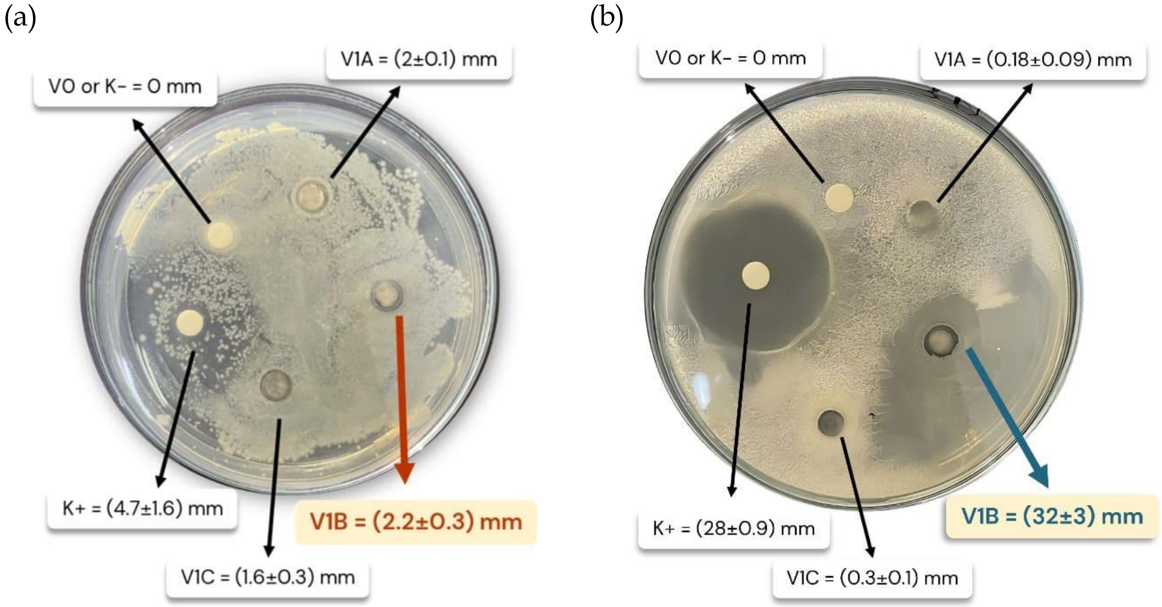

Figure 6. Antibacterial Activity of ESC against (a) *E. coli* and (b) *S. aureus*

Viscosity of Edible Spray Coating

The viscosity test shows that the concentration of CB and the addition of silver and chitosan have a significant effect on the solution's viscosity ($p < 0.05$). The viscosity of the ESC solution variations increases with the addition of CB concentration (Figure 7). The V0 variation has the lowest viscosity value of (16.8±0.2) cP, as no antibacterial agent was added to V0. Meanwhile, the V3C variation has the highest viscosity value of (46.9±0.1) cP. This is due to the addition of chitosan to V3C, which has a viscosity of 150–500 cP [32].

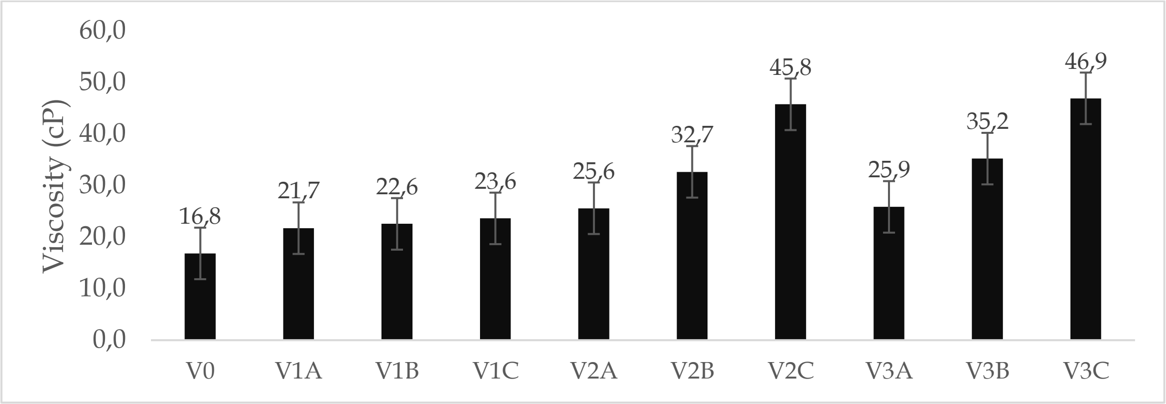

Figure 7. ESC Viscosity Graphic

Shelf life of Fruit

ESC suspension was applied to cherry tomatoes and strawberries using a biospray device. The purpose of fruits coating is to evaluate its effectiveness in extending the shelf-life of the fruits [33], [34]. In previous study, the application of edible coating utilizing the spray method demonstrated its effectiveness in food coating. In this context, the test fruit, grapes, remained preserved up to day 41, with a weight loss of less than 5% in the coated samples [5].

Both fruits, cherry tomatoes and strawberries, were observed and it was noted that the addition of CB did not significantly affect the appearance of the fruit. By the eighth day, there was weight loss due to the ongoing respiration process in the fruit (Figure 8). The concentration of CB had a significant impact on the shelf-life of the fruit. By the eighth day, V1B exhibited the least weight loss. This occurred because the coating contained cellulose, which inhibited the respiration rate of the fruit, and the addition of CB prevented bacterial contamination. The effects of CB concentration and the addition of silver and chitosan significantly influenced the fruit shelf-life ($p < 0.05$).

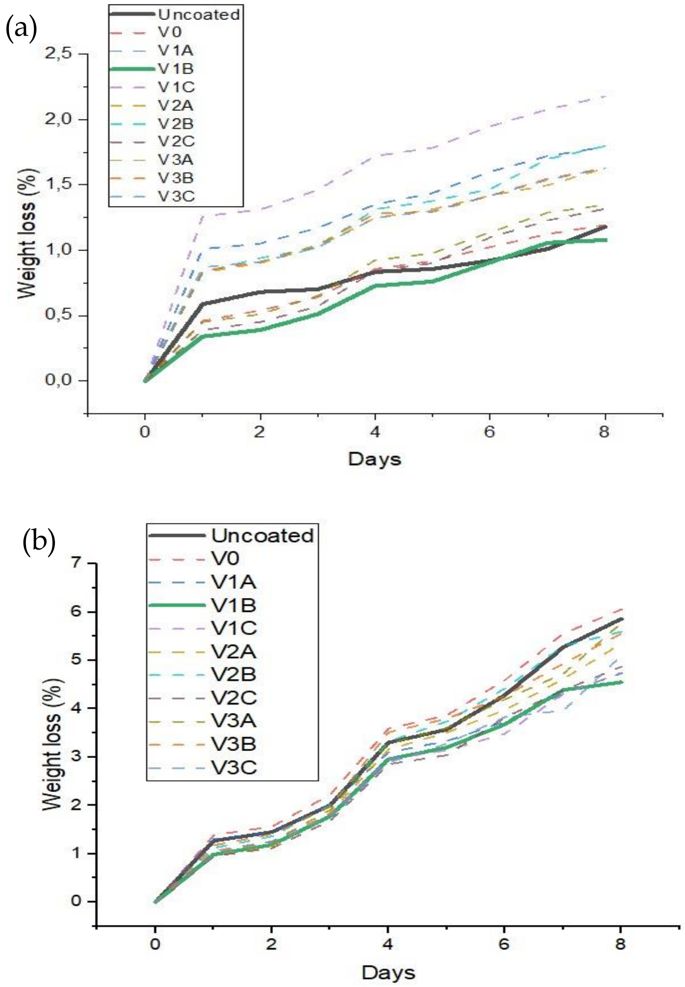

Figure 8. Weight Loss Graph of ESC Variations on (a) cherry tomatoes and (b) strawberries

Conclusion

The effective concentration of carbon black from coconut husk waste as an antibacterial agent in edible spray coating applications is achieved by adding 1.5% w/v of carbon black. All variations of the edible spray coating solutions are non-toxic and help extend the shelf life of fruits. The addition of silver and chitosan does not enhance the antibacterial activity of edible spray coating. This indicates that carbon black derived from coconut husk waste has potential as an antibacterial agent in edible spray coating applications.

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