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Application of antibacterial and antioxidant edible coating incorporating bacterial cellulose from sago liquid waste and garlic for preservation of tomato (*Solanum lycopersicum* L.)

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Abstract

The present work aimed to investigate the antibacterial and antioxidant properties of edible coatings derived from bacterial cellulose composites sourced from sago liquid waste and garlic, as well as their potential for preserving the quality of tomatoes (*Solanum lycopersicum* L.). The experimental approach involved the preparation of edible coatings using a mixture of bacterial cellulose (BC) slurry and garlic extract. The antibacterial and antioxidant activities were determined using the disc diffusion and DPPH method, respectively. Subsequently, tomatoes were coated using the dipping method with different treatments, namely BC/glycerol/CMC/garlic (S1), BC/garlic (S3), beeswax (comparison), and uncoated tomatoes (control). The physicochemical properties of the tomatoes were assessed, including sensory aspects by ten untrained panellists, weight loss by gravimetric analysis, vitamin C content by titration method, and pH value on days 0, 5, 10, 15, and 20 during the shelf life study at room temperature. Results showed that garlic extract applied to BC coating possessed antibacterial and antioxidant properties. BC coating containing garlic demonstrated more impact on the physicochemical qualities of tomatoes as compared to uncoated ones. Among the various treatments, S1 exhibited the most effective preservation of tomato quality. Therefore, the addition of garlic extract proved beneficial in augmenting the antibacterial and antioxidant capacity of BC coating, thus leading to enhanced quality maintenance and extended shelf life of tomatoes.

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Introduction

Edible packaging has emerged as a promising solution in recent years to reduce the reliance on plastic packaging for food products. Its primary purpose is to conceal food ingredients while protecting against mechanical or physical damage, in order to prevent a decline in the quality of the packaged items (Salehi, 2020). Among the various methods of edible packaging, one particularly suitable approach for preserving the freshness of fruits and vegetables is the use of edible coatings involving the application of a protective layer placed directly onto the surface of the product. This method offers several advantages including the ability to safeguard delicate fresh products like fruits and vegetables by slowing down the rate of respiration, enhancing texture quality, preserving volatile compounds, and reducing the risk of microbial contamination (Sharma *et al.*, 2019).

The criteria that should be met for the production of edible coatings encompass qualities such as strong adhesion, rapid drying, non-toxic, resistance to cracking, absence of flavour or colour

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interference with the product, accessibility, affordability, and compatibility with health standards (Jung and Choi, 2021). Furthermore, the effectiveness of edible coatings in maintaining the quality of packaged products is influenced by their constituent components (Yadav *et al*., 2022).

The main components in the manufacturing of edible coatings are grouped into three categories namely polysaccharides, proteins, and lipids. One of the polysaccharide derivatives with the potential to be used as edible coatings is bacterial cellulose (BC) which is produced from the metabolism of *Acetobacter xylinum* (Yanti *et al*., 2021a). Bacterial cellulose has been widely applied in the food packaging industry to increase shelf life or product safety from various causes of quality degradation such as microbial spoilage, oxygen exchange, moisture loss, and physical damage prevention (Shi *et al*., 2014). Bacterial cellulose obtained from sago liquid waste has characteristics that can be used as food packaging plastic (Yanti *et al*., 2021a), and has been applied for food packaging in the form of edible film (Yanti *et al*., 2021b; 2021c)

However, the utilisation of BC derived from sago liquid waste as edible packaging still presents certain shortcomings, specifically in terms of water resistance and limited water vapour barrier properties. Furthermore, it has to demonstrate the ability to impede the growth of spoilage microorganisms (Yanti *et al*., 2021c). All these will shorten the shelf life of fruits and vegetables due to water vapour and microorganisms entering through the coating. Efforts to minimise the entry of moisture and microorganisms can be made by adding other materials such as plasticisers, emulsifiers, and antibacterial agents.

Adding natural ingredients with antibacterial and antioxidant activities is very important to improve the function of edible coatings in order to maintain the quality and safety of products coated with edible coatings (Esa *et al*., 2014). One ingredient with antibacterial and antioxidant properties that can improve the function of edible coatings is garlic. Shi *et al.* (2014) stated that adding garlic filtrate to the edible coating will increase the function of the edible coating in coating the fruit. Therefore, the antibacterial and antioxidant activity of edible coatings made from bacterial cellulose composites of sago liquid waste and garlic were studied in the present work due to their ability to maintain the quality of tomatoes.

Materials and methods

The materials included bacterial cellulose (BC) produced using sago liquid waste as a substrate in the fermentation of *Acetobacter xylinum* LKN6 (Yanti *et al*., 2017), garlic, and tomatoes. Meanwhile, the chemicals were glycerol, carboxy-methyl cellulose (CMC), NaOH, and Nutrient Agar obtained from Merck Chemical Co. (USA). The test bacteria used were *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923, obtained from the culture collection of the Microbiology Laboratory, Faculty of Mathematics and Natural Science, Halu Oleo University, Indonesia.

Bacterial cellulose production and purification

Based on previous study, BC was produced statically using sago liquid waste as a production medium (Yanti *et al*., 2017; 2018). Subsequently, the production medium was incubated for 14 d at room temperature. The BC pellicle formed was harvested and immersed in 0.3 N NaOH to remove contaminants, and sterilised at 121°C, 15 psi, for 15 min. The purified BC sheets were washed with running water to remove the remaining dead cells and media components.

Production of cellulose slurry

The bio-cellulose gels were cut into small pieces, and mechanically blended for 1 h using a blender at a ratio of BC to water of 1:4 (Yanti *et al*., 2021c), and slurry obtained was collected and stored at 4°C for further use.

Garlic juice preparation

A total of 100 g of garlic was peeled and washed thoroughly under running tap water. The peeled garlic cloves were then mashed using a mortar and pestle without any addition of water to obtain garlic essence (not garlic extract), and filtered using a sterile filter cloth. Subsequently, the filtrate was measured to yield 10% using a measuring cup. Garlic essence is preferred over solvents in order to make it safer for consumption (Hamidi *et al*., 2022).

Preparation of edible coating

Preparation of edible coating in 300 mL was carried out according to Vignesh and Nair (2019) with slight modifications. The formulation of the edible coating solution comprised 97.5% cellulose slurry, 1% carboxy methyl cellulose (CMC) as a stabiliser, 1.5% glycerol as a plasticiser, and 10% garlic filtrate as an antibacterial and antioxidant agent. Approximately, 1 g of CMC was dissolved in 25 mL of sterile distilled water, and heated on a hot plate at 70 - 75°C while stirring with 1.5 mL glycerol (0.5%). The mixture of CMC and glycerol was then added with 97.5 mL of slurry (32.5%), and stirred for 30 min until homogeneous. The solution was stirred at 48°C while incorporating 30 mL of garlic filtrate (10%) with distilled water to obtain a total volume of 300 mL. Subsequently, it was degassed for 1 h on a hot plate.

Determination of coatings' antibacterial activity

Antibacterial activity of edible coatings was determined against *Escherichia coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923 using the disc diffusion method. Tetracycline antibiotic was used as a control, and the experiments were carried out in duplicates.

Determination of coatings' antioxidant activity

Antioxidant activity of edible coatings was determined using the DPPH method according to Isopencu *et al.* (2021). Ascorbic acid was used as a control.

Fruit packaging via dipping method

The fruit packaging ability of BC/Glycerol/CMC/garlic composite coating was determined by the dipping method (Atta *et al*., 2021). Tomatoes were obtained from a local supplier (Moramo Regency, Southeast Sulawesi, Indonesia). Treatment comprised four different samples including uncoated (control), BC/Glycerol/CMC/garlic-coated (S1), BC/garlic-coated (S3), and beeswax-coated (BW) tomato samples. Commercially mature tomato fruits were washed using sterile water, and allowed to air dry. Dried tomatoes were then dipped in S1 and S3 for 2 min before air-drying for 10 min. All samples were stored in an open plastic container at room temperature (20 - 27°C) for 20 d. Each sample was analysed in triplicate. During the experiment, the uncoated fruits were used as the control, and beeswax (BW)-coated fruits as a comparison for every treatment. During incubation, the tomatoes' physicochemical properties were evaluated to assess their sensory features such as odour, texture, and colour. Additionally, measurements were recorded for weight loss, pH value, and vitamin C content to monitor the quality and shelf life of the tomatoes.

The sensory features of odour, texture, colour, and overall acceptance were evaluated by ten untrained panellists on days 0, 5, 10, 15, and 20 of the shelf life study. The panellists were selected among the students (aged 19 to 23 years old) from the Department of Biology, Halu Oleo University, Indonesia. Ratings were presented on a scale of 1 - 2 = very bad, 3 - 4 = bad, 5 - 6 = moderate, 7 - 8 = good, and 9 - 10 = excellent, for each fruit, and 5 was the limit value of product acceptance (Atta *et al*., 2021).

Weight loss measurements were conducted using a gravimetric method which involved comparing the difference in weight, before and after the storage period (AOAC, 1995). The pH of the sample was determined using a pH meter following a calibration process with buffer solutions of pH 4 and 7 prior to measurement. To prepare the sample, approximately 10 g of tomatoes were weighed and crushed using a hand blender, along with the addition of 100 mL of distilled water. The resulting mixture was then transferred to a 250-mL volumetric flask, and diluted to the desired concentration using distilled water. The solution was subsequently filtered, and the pH was measured using the calibrated pH meter to obtain a value of 7 after measurements (AOAC, 1990). The pH value of tomatoes before storage was measured as a basis for determining the pH value during storage.

The titration method determined vitamin C content (Elgailani *et al.*, 2017). The test for vitamin C levels in 100 g of tomatoes was carried out by adding 1 mL of each freshly prepared sample solution to 200 mL of distilled water. Approximately 10 mL of each solution were placed into separated conical flasks. To each flask, 5.0 mL of KI solution (0.2 M), 2.5 mL of hydrochloric acid (HCl) (1.0 M), and a few drops of the starch solution were added. Each of the five solutions was then titrated against KIO_3 (0.015 M) from a burette until the appearance of a blue-black colour, thus indicating the endpoint of the reaction. The titration process was repeated three times for each sample. The results obtained were recorded, tabulated, and used to calculate the concentration of vitamin C for each sample.

Statistical analysis

All statistical analyses were calculated using independent sample *t*-test with p-value < 0.05

considered as statistical significance using IBM SPSS Statistics 22 software for PC (IBM SPSS Inc., USA).

Results and discussion

Antibacterial activity of coatings

Foodborne diseases associated with crop commodities have been increasing. Fresh fruits can become contaminated with pathogenic bacteria during various stages, from pre- to post-harvest. The most common pathogens associated are *E. coli* and *S. aureus*. These pathogenic bacteria can contaminate fresh fruits, and lead to foodborne diseases (Kothe *et al*., 2019). Therefore, these bacteria are frequently employed as indicator bacteria in inhibition tests. In the present work, disc diffusion results demonstrated that S1 and S3 exhibited clear inhibition zones. Conversely, BC coating without garlic extract did not display any inhibition zone (Figures 1A and 1B). The formation of a clear inhibition zone suggested the inhibitory effect of the tested sample.

Inhibition zones against *E. coli* and *S. aureus* (Table 1) showed that the BC coating without garlic extract (S2 and S4) had no inhibitory activity, while BC coating incorporated with garlic extract had inhibitory activity. These results indicated that the garlic extract had an antibacterial effect on the BC coating. Albeit BC possesses numerous favourable qualities for packaging material, its limited effectiveness against microbial pathogens hinders its direct application in food packaging. Consequently, the incorporation of antibacterial chemicals becomes necessary to enhance BC's antibacterial properties for such purposes (Thongsrikhem *et al*., 2022).

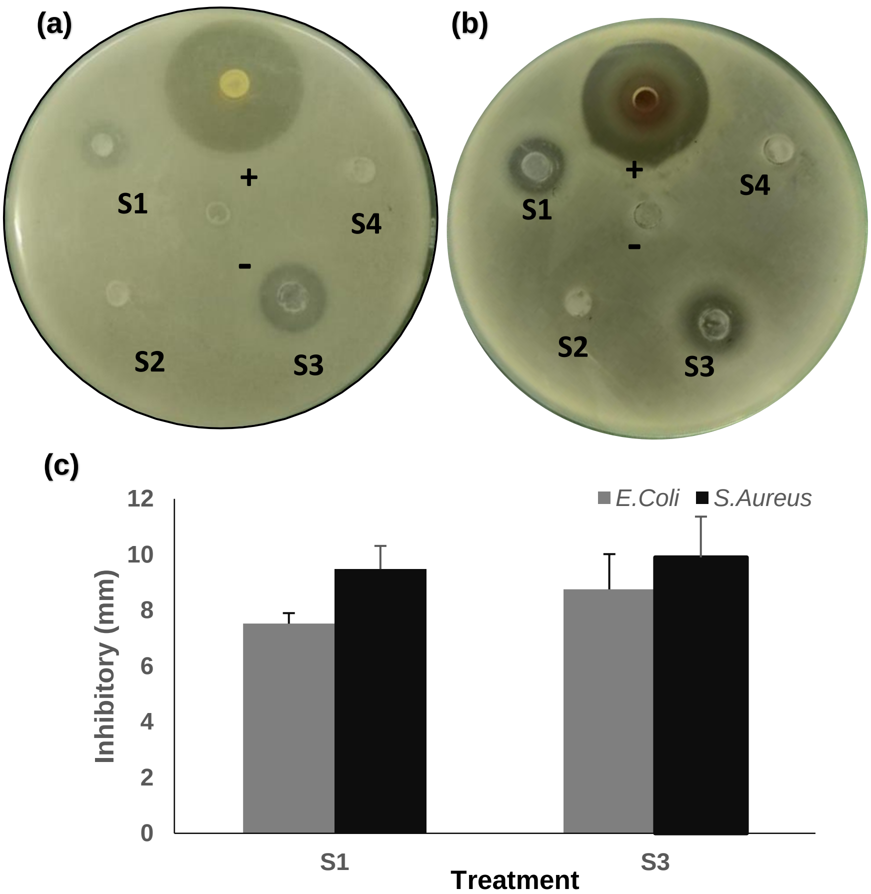

Figure 1. Antibacterial activities against **(a)** *Escherichia coli* and **(b)** *Staphylococcus aureus* on Nutrient Agar. (+): positive control (tetracycline); (-): negative control (distilled water); S1: BC/glycerol/CMC/garlic; S2: BC/glycerol/CMC; S3: BC/garlic; and S4: BC. **(c)** Significance of antibacterial activity. S1: BC/gly/CMC/garlic coated, and S3: BC/garlic coated ($p < 0.05$). Means are average of triplicate ($n = 3$).

Table 1. Inhibition zones of coating against *Escherichia coli* and *Staphylococcus aureus*.

Sample	Inhibition zone (mm)	
	Escherichia coli	***Staphylococcus aureus***
S1 (BC/gly/CMC/garlic)	7.52 ± 0.38	9.48 ± 1.27
S2 (BC/gly/CMC)	0	0
S3 (BC/garlic)	8.75 ± 0.83	9.91 ± 1.46
S4 (BC)	0	0

Means are average of triplicate (n = 3).

Based on Figures 1A and 1B, BC coatings garlic could inhibit Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*), thus indicating that garlic had a broad spectrum of antibacterial activity. The antibacterial activity of the garlic extract could have been influenced by the presence of allicin. According to Wolde *et al*. (2018), allicin is formed from organosulphur compounds in garlic by the enzyme alliinase. Leontiev *et al*. (2018) added that allicin had the capacity to interfere with the growth of Gram-positive and Gram-negative bacteria by inhibiting the production of RNA and bacterial lipid synthesis.

Results in Table 1 showed that S1 had lower antibacterial activity than S3. Although the statistical analysis (Figure 1C) showed both S1 and S3 treatments had no significant antibacterial activity (p-value > 0.05), this indicated that the addition of glycerol and CMC slightly decreased the antibacterial activity of BC edible coating. These results were consistent with the study conducted by Utama *et al.* (2022) in which the inclusion of glycerol decreased the inhibitory activity of edible films against bacteria. This could have been attributed to the enrichment of the edible composition by glycerol that restricted the presence of antibacterial substances, thus diminishing the inhibitory efficacy of the edible film against bacteria.

Antioxidant activity of coatings

Antioxidant substances found in garlic have been shown to be effective in diminishing lipid peroxides, reactive oxygen species (ROS), and low-density lipoprotein (LDL) oxidation (Jang *et al*., 2018). Garlic contain alliin and its equivalents, including allyl cysteine, allicin, and allyl disulphide, each of which has a distinct pattern of antioxidant action to protect against free radical damage (Jang *et al*., 2018). Alliin, an antioxidant molecule, is the main organosulphur compound found in garlic. Alliin will be converted into allicin by alliinase when the structure of the garlic cell is compromised, and this allicin is known to have antioxidant properties (Bhatwalkar *et al*., 2021). This is evidenced by BC coating fortified with garlic having a higher antioxidant activity than BC coating without garlic fortification (Table 2), which further confirmed that garlic contains natural antioxidant compounds, and can increase antioxidant activity in BC edible coatings. BC coating without garlic demonstrated weak antioxidant activity (Table 2), thus indicating that BC had a very small antioxidant activity. Several studies have also reported that pure BC had little antioxidant activity (Sari *et al*., 2020; Nowak *et al.*, 2021; Zmejkoski *et al.*, 2021). Edible coating with antioxidant activity can maintain fruit quality during storage. Eça *et al*. (2014) stated that adding antioxidants to the coating formulation could improve the preservative function, inhibit browning, and reduce the undesirable effects of nutrient oxidation.

Table 2. Antioxidant activity expressed as the IC_{50} (ppm) of coating BC fortified with garlic.

Sample	Antioxidant activity IC_{50} (ppm)	Category*
S1 (BC/gly/CMC/garlic)	55.43 ± 0.64	Strong
S2 (BC/gly/CMC)	290.93 ± 1.05	Weak
S3 (BC/garlic)	54.67 ± 3.75	Strong
S4 (BC)	379.71 ± 2.09	Weak
Control (ascorbic acid)	36.66 ± 0.52	Very strong

Means are average of triplicate (n = 3); *Molyneux (2004).

Effect of edible coating on tomato physicochemical quality preservation

Fruits treated with edible coatings are intended to preserve their physicochemical attributes, and prolong their shelf life. In the present work, a range of parameters associated with the physicochemical quality was measured including sensory attributes, weight loss, pH value, and the content of vitamin C. These measurements were conducted to assess the efficacy of the edible coatings in maintaining the desired quality characteristics of tomatoes throughout their storage period.

Sensory quality of tomatoes

Based on preliminary study on the antibacterial activity of edible coating materials, it was observed that BC coating without garlic had no antibacterial activity (Table 1). Therefore, only S1 and S3 were evaluated. The appearance of differently coated tomatoes are shown in Figure 2. It was observed that coated tomatoes lasted longer than those without coating. BW and S3 shrivelled on day 15 of storage, while control rotted on day 20. However, S1 was still smooth (not wrinkled) until day 15. These results indicated that S1 could maintain the shelf life of tomatoes as compared to S3 and BW. However, S3 had a superior average inhibition in the *in vitro* inhibitory test data as compared to S1. This was due to the absence of glycerol and CMC in S3 which could hamper the diffusion of the antibacterial chemicals by increasing the viscosity of the solution. Isopencu *et al.* (2021) stated that one of the water-soluble cellulose derivatives, CMC, is employed because of its high solubility, capacity to change the viscosity of solutions, and create flexible films and lack of toxicity. In contrast to direct tests conducted on fruits, the inclusion of the two additional chemicals might have provided a protective barrier against excessive water loss, thereby extending the shelf life of fruits and preventing wrinkles. The observations made regarding these tomatoes were aligned with the sensory characteristics outlined in Table 3. Based on Table 3, tomatoes treated with S1 exhibited sensory values for odour, texture, and colour that remained favourable even after 5 to 20 d. Therefore, the inclusion of garlic in the fortified BC coating maintained the quality of the tomatoes as compared to control and BW.

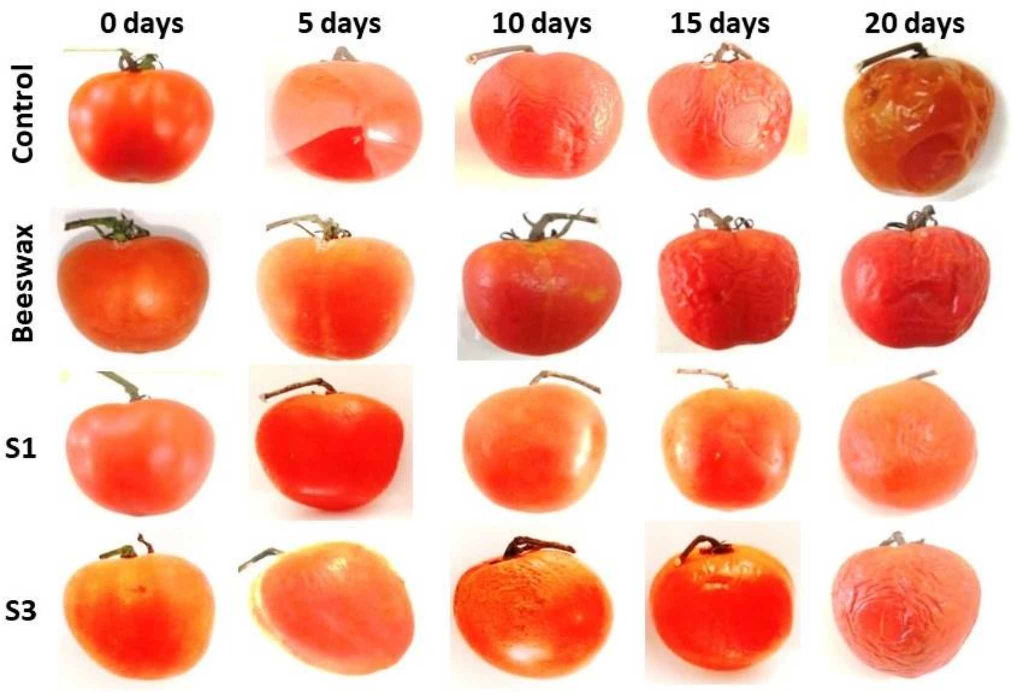

Figure 2. Fruit packaging performance of BC/glycerol/CMC/garlic (S1) and BC/garlic (S3). Uncoated tomato served as control, and beeswax-coated tomato as coating comparison.

Table 3. Sensory features of coated tomatoes.

Time (d)	Odour				Texture				Colour			
	C	**BW**	**S1**	**S3**	**C**	**BW**	**S1**	**S3**	**C**	**BW**	**S1**	**S3**
0	8.6	8.6	8.6	8.6	8.6	8.6	8.8	8.6	8.6	8.6	8.6	8.6
5	8.2	8.2	8.8	8.4	7.8	8.2	8.6	8.4	7.6	7.6	8.4	8.2
10	6.8	7.8	8.2	7.6	6.6	7.4	8.6	6.8	7.2	6.2	8.2	6.8
15	5.6	6	8.0	6.8	4.4	4.8	8.3	4.8	6.2	5.4	8.2	6.8
20	1.2	3.6	8.0	6	1.2	4	7.4	4.4	1	4.6	8.0	6.4

C: control; BW: Beeswax; S1: BC/glycerol/CMC/garlic; and S3: BC/garlic. *n* = 10 untrained panellists.

Weight loss of tomatoes

Weight loss is important in assessing the quality of fruits as it indicates the extent of moisture loss. Generally, higher weight loss corresponds to lower quality fruits. Figure 3A illustrates that all treatments exhibited an increase in weight loss as the storage time progressed. Specifically, the weight loss of tomatoes demonstrated a significant rise after 10 d of storage (Figure 3A). This contributed to the development of wrinkles and soft texture in the tomatoes as depicted in Figure 2. This is supported by Yadav *et al.* (2022) that weight loss is a crucial parameter affecting textural properties. The increase in weight loss in tomatoes occurs due to water loss due to transpiration.

Figure 3A shows that the highest percentage of tomatoes weight loss during 20 d of storage was in control, and the lowest in S1. Similarly, Jung and Choi (2021) showed that the weight loss of uncoated fruit was higher than that of coated fruit. Riva *et al.* (2020) stated that edible coating could cover the pores of the fruit surface, and reduce transpiration and respiration, thus reducing fruit weight loss. The edible coating acts as a barrier against CO_2 and O_2 to control the respiration rate. The percentage of weight loss of tomatoes coated with S3 was higher than the BC coating with glycerol and CMC, thus indicating that glycerol and CMC could reduce weight loss in tomatoes. Similar observations have been reported by Khodaei *et al.* (2021). Adding CMC and glycerol to edible coating composites can increase the ability of the coating material to adhere to the surface of the fruit by inhibiting and reducing the rate of water vapour transmission and migration (Yadav *et al.*, 2022). The smaller the water vapour migration in the edible coating-coated fruit, the better the reduction in the weight loss of tomatoes (Khodaei *et al*., 2021).

pH value of tomatoes

A shift in pH value in stored tomatoes serves as an indicator of changes in quality. As depicted in Figure 3B, the pH value in all coating treatments exhibited a decrease throughout the storage duration. This observed decrease suggested a potential alteration in the acidity level of the tomatoes during storage. The highest pH decrease was obtained in control from 5.09 to 4.15 after 20 d, while the lowest pH decrease was obtained in S1 from 5.07 to 4.58 after 20 d.

A comparable outcome was reported by Kumar *et al.* (2020) where the utilisation of a chitosan-pullulan composite edible coating fortified with pomegranate peel extract resulted in a significant decrease in the pH of tomatoes. Specifically, the pH of the coated and uncoated tomatoes decreased from 4.75 to 4.00, and 4.75 to 3.65 after 15 and 18 d of storage, respectively, at room temperature. This finding highlights the efficacy of the edible coating in maintaining a more stable pH level in the tomatoes as compared to the untreated samples. In contrast, Sree *et al*. (2020) reported that edible chitosan coating increased the pH of tomatoes. The study conducted by Vignesh and Nair (2019) reported that samples coated with a gelatine, chitosan, and cassava starch mixture, with or without hibiscus mucilage, led to an insignificant pH increase in tomatoes during storage. Therefore, pH of tomatoes could be affected differently depending on the type of edible coating applied, tomato varieties, and tomato maturation stages (Duguma, 2022). According to Gebregziabher *et al.* (2021), the inhibition of enzymatic breakdown of pectin, decrease in respiration, inhibition of organic acids, and inhibition of conversion to sugars were the causes of pH decrease in tomatoes during storage.

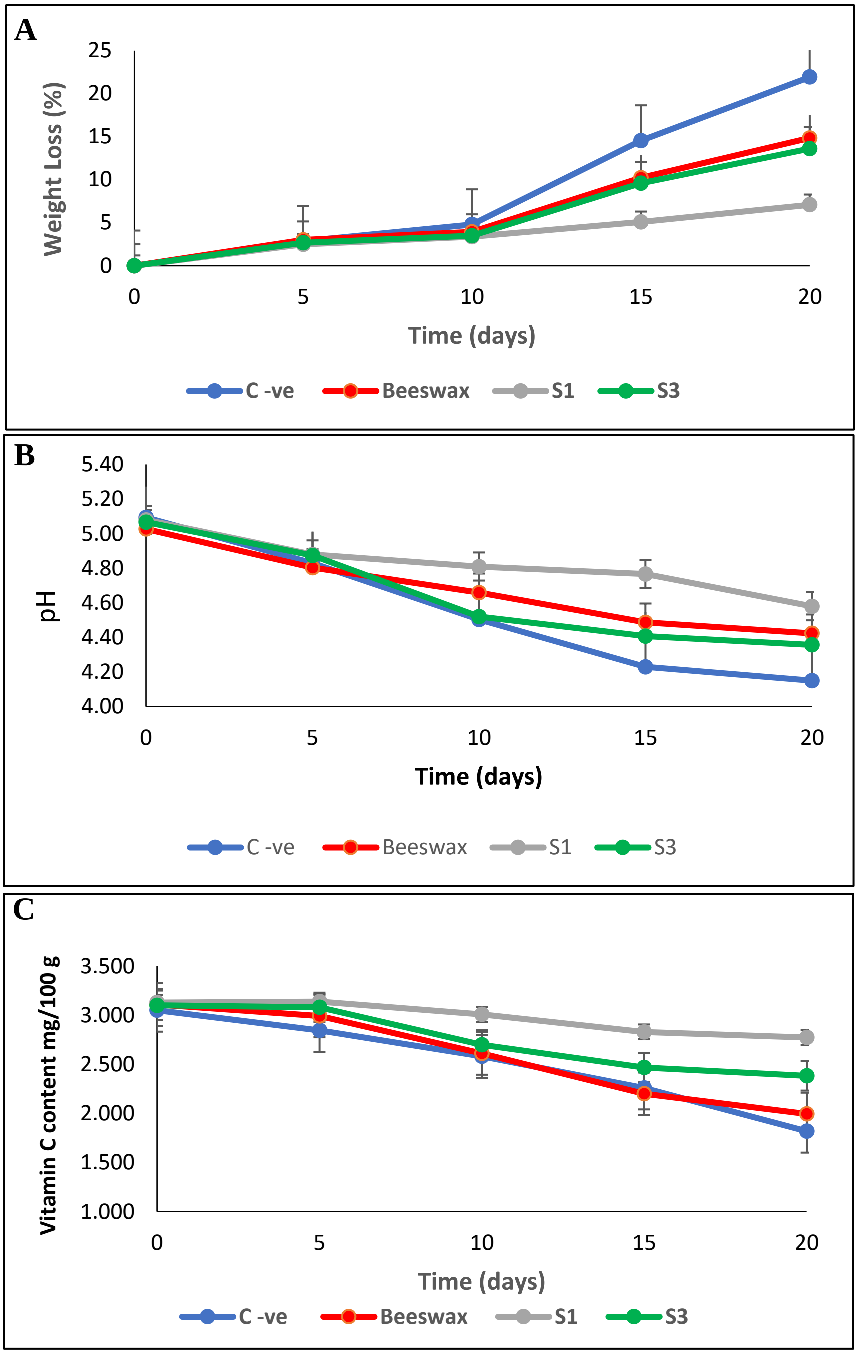

Figure 3. Quality of tomatoes upon application of coating during storage: **(A)** weight loss percentage, **(B)** pH value, and **(C)** ascorbic acid (vitamin C) content. Means are average of triplicate (*n* = 3).

Vitamin C content of tomatoes

Vitamin C is the essential nutrient most sensitive to degradation during food processing and storage (Panigrahi *et al.*, 2017). Figure 3C shows that the vitamin C contents of tomatoes decreased during storage for all coating treatments. Tomatoes coated with S1 had a minor decrease from 3.131 to 2.773 mg/100 g as compared to other treatments for 20 d of storage. These indicated that edible coating of BC fortified with garlic could maintain the levels of vitamin C in tomatoes better than those without coating during storage. Similar results were reported by Duguma (2022) that edible coating could minimise the loss of vitamin C in fruits. The ability of garlic-fortified edible coatings to retain vitamin C is due to antioxidants that can inhibit its oxidation. According to Eça *et al*. (2014), vitamin C is susceptible to oxidation due to its reaction with oxygen in the air, thus resulting in the formation of dehydroascorbic acid. Therefore, edible coatings that incorporate antioxidants can prevent a decrease in vitamin C levels in tomatoes. In contrast, uncoated tomatoes stored at room temperature were more prone to a rapid decrease in vitamin C levels.

Conclusion

Edible coating based on bacterial cellulose from sago liquid waste fortified with garlic had antibacterial and antioxidant activities, and could maintain the quality of tomatoes for up to 15 d of storage at room temperature.

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