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# Preparation of Active Food Packaging and Coating Material Based on Bacterial Cellulose to Increase Food Safety

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## *ABSTRACT*

*The use of bacterial probiotic metabolite-based active-packaging and coatings is an innovative approach that has gained widespread attention worldwide. Additionally, its utilization can lead to improvements in qualities and properties of food products. This study was aimed to develop a food spoilage prevention system using active food packaging and coating material in preventing food spoilage while increasing its shelflife. The materials used were bacterial cellulose (BC) based bioplastics fortified with fermented soymilk extracts (FSME) using Lactobacillus acidophilus as the producer of the antimicrobial and antioxidant agents. Moreover, the applications of FSME containing probiotic bacterial metabolites are discussed to highlight their efficacy in enhancing the quality and shelf life of food products.The antimicrobial test showed that the FSME could inhibit the growth of pathogenic microbial cultures at minimum inhibitory concentration (MIC) of 10% (v/v) as shown by clear zones, around colonies of E. coli (14.33±0.58 mm), S. aureus (18.33±6.03 mm), S. Typhimurium (11.67±1.15 mm), L. monocytogenes (11.33±2.31 mm), and B. cereus (13.33±3.06 mm). Meanwhile the results of $IC_{50}$ for antioxidant activity test (µg/mL) indicated that the FSME showed radical scavenging activity against DPPH at approximately 75.27±2.552 (2.5%, v/v), 55.00±0.791 (5.0%, v/v), 43.17±1.603 (7.5%, v/v) and 15.05±0.346 (10%, v/v), respectively. The shelflife of strawberries coated with the active food coating using the bioplastic fortified with FSME showed an increase in shelf life of 14 days at 4°C. The overall results indicated that the use of BC based bioplastics fortified with FSME can play an important role in preventing premature spoilage and increasing the shelf life of food products.*

***Keywords:*** *antimicrobial, antioxidant, bacterial cellulose, fermented soymilk, shelf life*

## INTRODUCTION

The use of synthetic plastics as food packaging materials has increased significantly, since plastics are considered easier and cheaper to produce compared to other materials, because they are produced from crude oil by-products. However, the widespread use of plastic products poses many problems do to involve the use of hazardous chemicals as stabilizers or dyes, thus they are difficult to decompose in the soil, resulting in impacts on human health and the environment (Ncube *et al.,* 2020). Biodegradable plastics which is then shortened to bioplastic, are proposed to be used as packaging materials to replace synthetic plastics in order to maintain quality and safety of food while decreasing waste and $CO_2$ gas emissions by providing bidding options for environmental recovery and restoration (Lagarón *et al.,* 2015).

On the other hand, the increasing demand for bioplastics as packaging materials in the food, beverage and pharmaceutical industries is expected to increase the growth of the bioplastics market over the next few years (Vignali and Vitale, 2017). The use of proper food packaging is an important consideration to prevent and protect products from contamination during transportation and storage. Therefore, it is very important to prevent these microbial contaminants using an appropriate, effective, specific, and efficient packaging system.

One of alternative solutions for the purpose in preventing microbial contamination is through the application of active food packaging and coating system. Active packaging has a wider application than conventional packaging by referring to two different concepts. The first concept relates to monitoring information regarding food quality. The second concept relates to responsive packaging, which can release bioactive compounds if food starts to spoil. Active packaging is responsive to environmental changes, so that it can release antimicrobial compounds, antioxidants, or other compounds that affect the quality of food in packaging, and thus to extend the shelf life of food, maintain product quality in

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packaging and maintain food safety (Yildirim and Röcker, 2018; Dobrucka and Przekop, 2019).

Bioplastic film application as food coatings is a simple way to improve the quality of food by increasing secure against physical, chemical, and biological changes. In addition, the visual features are increased, the nature of volatile aroma is largely preserved, thereby making the food more and more attractive for consumers (Mohamed *et al.,* 2020). In addition, bioplastics are ideal ways for combining active compounds use antioxidant, antimicrobial, flavouring or also nutraceutical performance to obtain the active packaging formulas (Ganiari *et al.,* 2017; Umaraw *et al.,* 2020). In this context, the current emphasis is on designing innovative packaging films and edible coatings integrated with active substances that can effectively inhibit oxidation and microbial spoilage (Yong and Liu, 2021). One of typical active packaging systems perform actions such as antioxidants, and antimicrobials from natural or artificial sources have been used to prevent food spoilage caused by moisture, air, bacterial growth, or adverse biochemical alterations due to oxidation (Ahmed *et al.*, 2017; Wicochea-Rodríguez *et al.,* 2019). Noteworthy innovations in edible packaging research emphasized the development of environmentally friendly and biodegradable alternatives to the traditional petroleum-based polymers used in food packaging and preservation. As thin protective layers, edible films and coatings have been extensively investigated for food packaging and can be safely consumed despite being an integral component (Ribeiro *et al.,* 2021). Films and coatings produced from various food-grade polymers, such as polysaccharides, lipids, and proteins, are being explored as environmentally friendly replacements for conventional food packaging (Kumar *et al.,* 2021).

However, most of bioplastic-films have low mechanical and thermal properties, absorb high moisture, and poor antimicrobial resistance (Cabañas-Romero *et al.,* 2020). Many efforts have been made to reduce these drawbacks by adding fillers to the bioplastic films (Noorbakhsh-Soltani *et al.,* 2018). Among of these fillers, natural cellulose fibres including bacterial cellulose (BC) have significant potential (Qasim *et al.,* 2021; Cazón and Vázquez, 2020). BC is considered as a source of pure cellulose, which is usually synthesized by the bacteria. In this experiment the bacterial species *Gluconacetobacter xylinus* (conventionally known as *Acetobacter xylinum*; Zhong, 2020) was cultured to synthesize the BC as microfibrils on a medium scale. Due to the high yield of cellulose, is considered to be a model organism for the production of BC (Keshk, 2014) for commercial fermentation. However, large-scale production of bioplastics for different applications is limited by high costs, compared to synthetic plastics derived from fossil oil, and concerns over functionality. Various biopolymers used have disadvantages such as high-water vapor permeability, oxygen permeability, brittleness, low thermal resistance, low mechanical properties, susceptibility to degradation, and low processability (Abe *et al.,* 2021). The BC based bioplastics have absorbed much attention because of their high purity cellulose content, fine nanofiber network and very low production costs, resulting in better properties rather than plant cellulose, as well as high tensile force and highly fibre content (Abral *et al.,* 2018).

The fermented soymilk extract (FSME) containing active compounds derived from probiotic bacteria as antimicrobial and antioxidant agent which were incorporated into the bioplastic-based food packaging and coating has become relatively popular amongst researchers recently and, consequently, this area has been studied thoroughly in the recent years. The aims of this study were to extract active ingredients derived from FSME which were formulated into the bioplastic matrices to develop active food packaging and coating as alternative method to control pathogenic microorganisms in order to improve food stability and safety and most importantly favors consumers' health.

## MATERIALS AND METHOD

### Materials

Coconut water, bean sprouts, corn and cassava starch, glycerine, and fresh fruits were purchased from fresh market, Citraland Surabaya. Bacterial starter culture of *Acetobacter xylinum* was purchased from Shakara Shop, Surabaya while bacterial culture of *Lactobacillus acidophilus* was isolated by growing the culture derived from fermented soymilk product using commercial starter culture. Pathogenic microbial strains of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *Bacillus cereus* were honorable contribution from Universiti Malaysia Sabah. The 1,1-diphenyl-2-picryl hydrazyl (DPPH, Sigma-Aldrich, Merck, Germany) was purchased from Elo Karsa Utama Co. Ltd..

### Bacterial cellulose production

The method for making BC based bioplastic films was initiated by preparing media for bacterial culture (*Acetobacter xylinum*) growth on coconut water containing bean sprouts extract 3.0% (v/v), sucrose 7.5% (b/v) and acetic acid to adjust pH 4.5 prior to sterilize using autoclave (Hirayama Hn-85, Japan) for 15 min at 121°C and then incubated at 30°C for 10 days (Costa *et al.,* 2017). Fermentation to produce BC is carried out in static mode, since under the static condition, three-dimensional inter-connected reticular pellicles are formed. Cellulose formation under static

conditions is regulated by the supply of carbon and water into the medium. BC formation is increased with the increase in growth time and the C-H bonding. When the pellicle growth slows down and all the bacteria are entrapped, the synthesis of BC reaches its threshold (Pang *et al.,* 2020). After cultivation, the BC membranes were washed with water and soaked in 0.1 M NaOH at 80°C for 2 h to remove bacterial cells possibly attached to the BC pellicles. Then, the pellicles were washed with deionized water several times to warrant the complete remove the alkali, leaving the pellicles at neutral pH. The purified cellulose was dried at 60°C for 12 h until reaching a constant mass (Wu *et al.,* 2014).

**Preparation for antimicrobial and antioxidant agents**

Bacterial cultures of *L. acidophilus* were used to prepare FSME. The pure culture was inoculated into test tubes containing sterile MRS media for the preparation of stock culture and working culture, then was incubated in an airtight chamber at 37°C to 48 h and furthermore incubated at 37°C to 24 h. Then the fermented soymilk was centrifuged (Themo Fisher, Germany) at a speed of 5000 rpm for 15 minutes to obtain fermented soymilk extract (FSME) containing an active compound (Shihabudeen *et al.,* 2010). In this study, antimicrobial agent contained in the FSME was used for testing minimum inhibition concentration (MIC) was diluted into four concentrations, *i.e.* 10, 25, 50, and 75%, respectively, prior to dissolving into bioplastic pulp. The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disk diffusion (Kirby–Bauer) method. The highest dilution of a FSME that still retains an inhibitory effect against the growth of a microorganism is known as MIC. Selected plant extracts were subjected to a serial dilution (10% to 75%) using sterile nutrient broth medium as a diluent. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract.

The antioxidant activity of the FSME was determined against 1,1-diphenyl-2-picryl hydrazyl (DPPH) as defined by Shekhar and Anju (2014). Concisely, 1.0 mL of 0.1 mM DPPH solution dissolved in methanol was added into 3 mL of FSME at different concentrations (2.5, 5.0, 7.5, and 10% in methanol). The mixture was shaken vigorously and left at ambient temperature for 30 min. The absorbance was determined at 517 nm using spectrophotometer (Perkin Elmer, Lambda 25, USA). The $IC_{50}$ value of each concentration required to inhibit 50% of the DPPH was determined. Low absorbance was obtained from the reaction mixture determined to exhibit high free radical scavenging activity (Nazir *et al.,* 2018).

**Preparation for manufacturing bioplastic film**

The BC film formed was then formulated into a bioplastic pulp in 200 mL distilled water with the addition of 30.0% BC, 5.0% cassava starch, 3.0% glycerin, 2.0% CMC and the rest was distilled water, while 10.0% FSME solution as the active ingredient was added later after being homogenized by continuously steering at 80°C using spatula for approximately 30 min and then cooled to room temperature, and finally added with suspension of the FSME which acts as an antimicrobial and antioxidant agents. Furthermore, the activated bioplastic pulp was poured into a tray at a thickness of 1.0-2.0 mm and dried at 45°C for 8-10 h up to get dry and a thin of bioplastic film was formed (Yildirim and Röcker, 2018).

Besides that, the activated bioplastic film was then applied as a coating material for strawberry by dipping 5 fresh strawberries into bioplastic pulp and hence the entire surface of the fruits was coated with bioplastic pulp which had been activated with the antimicrobial and antioxidant agents derived from the FSME, and furthermore drained and dried in a drying oven at 37°C for 30 min and then removed from the oven to dry by airing at room temperature.

The study was conducted using 3 different formulations with differences in BC composition Formula 1 (F1, 30%), Formula 2 (F2, 20%) and Formula 3 (F3, 10%), respectively and the same concentration of FSME (10%). Bioplastic films derived from BC produced by *A. xylinum* were crushed and mixed with starch, glycerin, CMC and distilled water. The mixture was then heated and stirred continuously at 80°C for 30 min to ensure starch gelatinization. The resulting gel is cooled at 30°C and coated on strawberries and dried. The percentage of bacterial cellulose used is based on preliminary tests that have been carried out, where the minimum percentage used to coat strawberries is 10% and the maximum percentage is 30%, because if using a higher percentage will result in a coating gel that is too viscous and the coating easily peels off.

Referring to the first trial, the preparation of the best formulation (F1) added with FSME as much as 10%, was done after the physical analysis on the strawberries was done. A ten percent of distilled water was replaced with a 10% solution of the FSME. The percentage of this FSME solution was obtained through the minimum inhibition concentration (MIC) test against several microbial strains.

**Observation of bioplastic film using SEM and FTIR**

Morphological characteristics for biocellulose were observed using a scaning electron microscope (EVO MA 10, Carl Zeiss Microscopy, Germany). The microstructural analysis of the bioplastics was carried out using a Scanning Electron Microscope (SEM) (Kumari *et al.,* 2010) and Fourier transform

infrared spectrometer (FTIR) (Agilent Cary 630, USA), which was used to obtain infrared spectrum of absorption, emission, and photoconductivity of bioplastic films. The microstructural analysis was performed to observe and obtain the microstructure and 3D micrographs of the bioplastic film before and after supplementation with 10% FSME. For the preparation of samples to be observed using SEM, bioplastic samples were coated with gold at a thickness of 10 nm (Pereira *et al.,* 2016).

**Fruit weight loss measurement**

Fruit weight loss was recorded using an electronic analytical scale at a sensitivity value of 0.01 g according to the method of Yan *et al*. (2019) once the strawberries were coated with an edible coating fortified with FSME and dried. The strawberries used were at stage where the skin of the strawberry changed from pink to red. The fruits were stored in a cold room at 4°C as soon as the delivery was made. Strawberries that have uniformity in terms of size, shape and free from any damage were selected to obtain as many as 18 strawberries used for the experiment. The selected strawberries group coated with bioplastic film F-1, F-2, and F-3 and fortified with 10% FSME were compared to group of strawberries those were uncoated with any bioplastic film neither FSME fortification as control group to observe fruits quality dealing with fruits weight loss after storage. Each fruit group sample was weighed on the first day of storage and followed by the third, seventh, ninth, eleventh and fourteenth days. Fruit weight loss during storage is expressed in percentage (%) and compared with initial weight.

**Fruit firmness measurement**

Fruit firmness was tested according to the method of Yan *et al*. (2019). Strawberries were coated with bioplastic film F-1, F-2, and F-3 and fortified with 10% FSME were compared to group of strawberries those were uncoated with any bioplastic film neither FSME fortification as control group to inhibit the loss of fruit firmness. Fruits hardness test was carried out using a texture analyzer (TA-XT Plus; stable Micro System, UK) equipped with a P4 steel probe having a diameter measurement of 4 mm. Strawberries were tested with a depth of 7 mm and a speed level at 0.5 $mms^{-1}$. The value of the test result is reported as the peak force in Newton (N) and readings are taken from the mean values for the three samples.

**Statistical analysis**

Analysis of data for testing of antimicrobials and antioxidants activity, fruit weight loss and fruit hardness, were carried out using one-way analysis of variance (ANOVA). Post hoc Tukey's test at confidence level ($p$<0.05) and Kruskal-Wallis analysis were used at confidence level ($p$>0.05), using Statistical Package of Social Science (SPSS) version 24.0.

## RESULTS AND DISCUSSION

**MIC as antimicrobial effect of FSME**

The concentration values of antimicrobial agent to inhibit bacterial growth at the lowest concentration was obtained by conducting a minimum inhibitory concentration (MIC) test. Among of the factors could influence the MIC were microbial strain and antimicrobial agent. According to Morgan *et al.* (2022) inequality of bacterial cell load during inoculation on agar plates would affect the results. This was due to a higher bacterial concentration would provide a smaller diameter of the inhibition zone. In addition, the number of droplets of antimicrobial agent into diffusion-well could affect the value of the MIC as well. However, these two factors might not provide such a significant difference towards the values of the inhibition zone.

Based on Table 1 and Figure 1, the result showed that the MICs for five tested bacterial strains showed that there was a difference in the diameter of clear zones which were indicated to the inhibition zone exhibiting the antimicrobial activity against *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Escherichia coli*. *S. aureus* showed the highest diameter of the inhibition zone followed by *E. coli*, *B. cereus*, *S.* Typhimurium and *L. monocytegenes*. Nevertheless, *L. acidophilus* without dilution showed the highest antibacterial activity against *B. cereus* which showed diameter inhibition zone of 22.00 mm. Epidemiological surveys of fresh produce and the occasional outbreaks, demonstrate the potential for a wide range of these products to become contaminated with pathogenic microorganisms. Bacterial pathogens such as *Salmonella enterica*, *E. coli*, *B. cereus*, *Pseudomonas* spp. and *L. monocytogenes* (Oliveira *et al.,* 2019) are especially of major concern due to the environmental occurrence of these bacteria. Fresh and frozen berries are being increasingly involved as a vehicle of foodborne diseases (Palumbo *et al.,* 2013). The last reported outbreak in 2011 traced back to a farm source of contaminated strawberries and implicated with *E. coli*. Production practices, growth conditions and the location of the berries on the growing plant in combination with intrinsic and extrinsic factors as well as harvesting and processing, will affect the microbiological quality of berries at the time of consumption (Li and Wu, 2013). Since berries are a perishable fruit, they are generally consumed raw or minimally processed, as well as a frozen ingredient added to many foods (Hsu *et al.,* 2014).

Table 1. Antimicrobial activity of FSME against tested pathogenic bacterial strains

| Pathogenic Bacterial Strains | Concentration of FSME (%) | | | | |
|---|---|---|---|---|---|
| | Diameter of Inhibition Zone (mm) | | | | |
| | 1.0 | 2.5 | 5.0 | 7.5 | 10 |
| *B. cereus* | 9.33±1.15$^{a}$ | 11.67±1.53$^{a}$ | 13.67±1.53$^{ab}$ | 17.33±2.08$^{b}$ | 22.00±1.73$^{c}$ |
| *L. monocytogenes* | 9.00±1.73$^{a}$ | 10.67±0.58$^{ab}$ | 13.00±1.00$^{bc}$ | 14.67±1.15$^{c}$ | 19.67±0.58$^{d}$ |
| *S.* Typhimurium | 8.67±3.21$^{a}$ | 11.67±4.16$^{a}$ | 14.00±3.61$^{a}$ | 16.33±3.06$^{a}$ | 18.33±4.93$^{a}$ |
| *S. aureus* | 11.67±2.08$^{a}$ | 13.00±2.00$^{a}$ | 14.67±1.53$^{a}$ | 15.67±1.53$^{a}$ | 18.00±4.36$^{a}$ |
| *E. coli* | 10.67±1.15$^{a}$ | 11.67±1.15$^{ab}$ | 13.67±1.15$^{abc}$ | 14.67±1.15$^{bc}$ | 17.00±1.73$^{d}$ |

Note: The value is the average of the three readings with standard deviation. Mean with different alphabets in the same column showed significant differences ($p$<0.05)

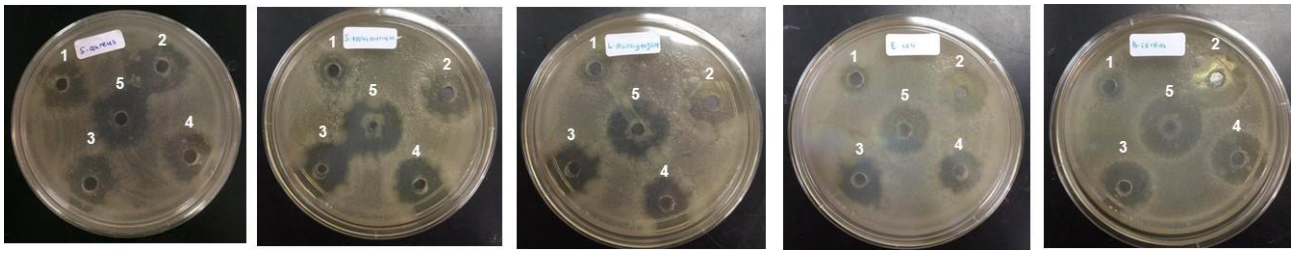



Figure 1. MIC as a result of antimicrobial effect due to antimicrobial substance contained in fermented soymilk extract (FSME) that was applied at various concentrations were A, 10%; B, 25%; C, 50%, D, 75% and E, 100%, respectively, against respective tested pathogenic bacterial strains were *S. aureus* (1), *S.* Typhimurium (2), *L. monocytogenes* (3), *E. coli* (4) and *B. cereus* (5)

Probiotics bacteria including *Lactobacillus* spp. reported to have inhibitory activity towards bacterial pathogens in the human body by means of their ability to yield antibacterial substances such as bacteriocin that has potency effect for use in therapy as well as food biopreservatives. In addition, *L. acidophilus* was also found to have antibacterial activity against many Gram-negative bacterial pathogens and micro-organisms that cause food spoilage like *E. coli* and *S.* Typhimurium (Denev *et al.,* 2015). Furthermore, *L. acidophilus* also exhibits antibacterial activity against a variety of bacteria gram-positive, including necessary pathogens like *B. cereus* and *Clostridium perfringens*, *S. aureus* and organic acid bacteria, *L. monocytogenes* (Dinev *et al.,* 2017).

A study was conducted to evaluate the shelf life of fish fillets after using probiotic *L. acidophilus*. Moreover, the probiotic reveals high antimicrobial activity by production of antimicrobial substances or competition with spoilage microorganisms (Pereira *et al.,* 2016). Among the probiotic bacteria has gained much attention owing to the high probiotic activity because of high to low pH tolerance and antimicrobial activity (Brachkova *et al.,* 2011). There are evidence regarding antimicrobial activity of probiotics with agar coatings to prolong shelf life of fillets (Pavli *et al.,* 2018). *L. acidophilus* is considered the main tools for controlling pathogens, improving food safety and prolonging shelf life (Rastall *et al.,* 2000).

**Radical scavenging activity against DPPH**

Free radical scavenging activity of FSME against DPPH was determined. As shown in Figure 2, it showed that FSME exhibited antioxidant capacity according to the concentration of FSME. It exhibited free radical scavenging activity increased with increasing the concentration of FSME. The $IC_{50}$ value of fortified bioplastic pulp with different concentration of FSME from 1.0 to 10% showed the decreased of $IC_{50}$ value. It was found that the addition of FSME at the lowest concentration (1.0%) resulted the $IC_{50}$ was 75.27 $\mu$g/mL where the addition of FSME at the highest concentration (10.0%) gave the result of $IC_{50}$ was 15.05 $\mu$g/mL. The $IC_{50}$ value is the amount of the effectiveness of a compound in inhibiting a certain biochemical or biological function. This quantiable measure indicates how much of a specific inhibitor or antioxidant is needed to scavenge a given biochemical process by half. $IC_{50}$ indicates the concentration of the substance required for 50% inhibition *in vitro*; the lesser value of $IC_{50}$ determines a greater radical scavenging activity. DPPH is described as a stable free radical, due to the delocalization of the spare electron throughout the molecule. Thus, DPPH does not dimerize, as in the case with most free radicals (Gangwar *et al.,* 2014; Aykul and Martinez-Hackert, 2016). Through this study, the FSME solution (5.0-10%) containing metabolites derived from probiotic culture *L. acidophillus* were the most suitable and effective to use in the formulation of bioplastic pulp compared to the FSME solution of 1.0-2.5% due to

this amount was the most efficient amount and showed antioxidant properties that may be applied into the bioplastic films for use as active packaging and food coating materials.

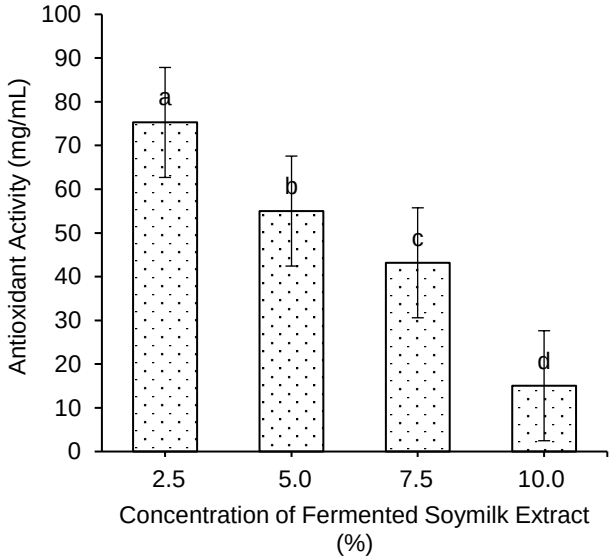
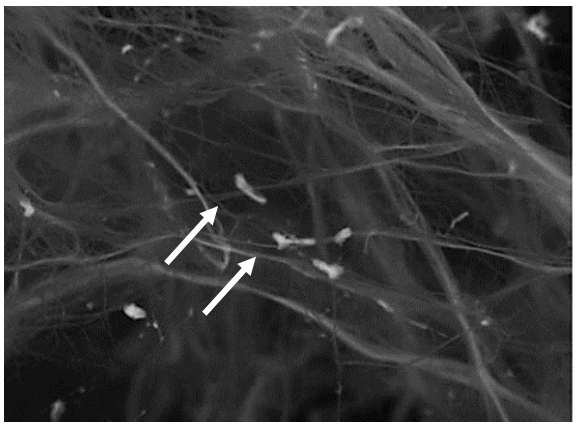



Figure 2. Antioxidant activity ($IC_{50}$) of FSME against oxidator DPPH

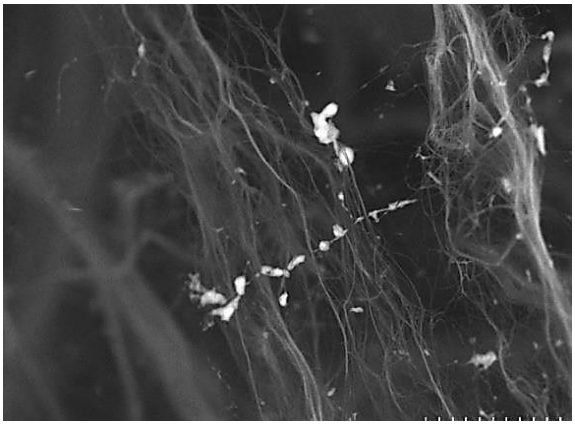
Research on lactic acid bacteria (LAB) is being actively conducted due to increasing interest in their antioxidant ability. For example, *L. acidophilus* showed DPPH, hydroxyl radical, superoxide, and reducing power activities as well as strong antioxidant activity and capability of inhibiting linoleic acid (Thibessard *et al.,* 2004). Many Lactobacillus strains show hydrogen peroxide antioxidant activity. Exopolysaccharides of *L. lactis* have exhibited promising antioxidant activity (Almalki, 2020). Certain Lactobacillus species have also degraded superoxide anions and hydrogen peroxidase (Liu and Pan, 2010). A crude sample extracted from LAB strains showed antioxidant activities because of the presence of non-enzymatic substances and intracellular antioxidant enzymes (Wojcik *et al.,* 2010). In LAB, antioxidant enzymes, such as NADH oxidase, glutathione reductase, glutathione S-transferase, catalase, glutathione peroxidase, and feruloyl esterase, counteract oxidative stress (Arasu *et al.,* 2014). The intracellular enzymes extracted from the bacterial cells by cell disruption showed antioxidant properties.

**Microstructure of bioplastic films using SEM**

Based on the observation of bioplastic film through electron microscopy (SEM) scan images, Figure 3 showed that it has a nanofiber and ribbon structure that makes bacterial cellulose based bioplastic seems to be intact and strong. The biocellulose chains join and form subfibrils. The width of the subfibril is in the range of 1.5 nm which is the thinnest natural fiber. Biocellulose subfibrils undergo a process of crystallization and become microfibrils *i.e.* in numerous bonds and subsequently turn into ribbons (Lin *et al.,* 2013). Based on several studies, the dimensions of ribbons are varied. Therefore, in this study, bacterial cellulose was used as one of the materials in the manufacture of active packaging and coating film because it could provide strengtheness to the film. Bacterial cellulose also has a high chemical purity compared to plants cellulose due to it does not contain lignin and hemicellulose. Thus, the addition of active components such as antimicrobials and antioxidants or other substances becomes easier due to its purity (Krishnamurthy and Amritkumar, 2019; Azeredo *et al.,* 2019).



**A**



**B**

Figure 3. SEM observation through the surface of bioplastic film showed bioplastic film at 3400x magnification (A) and bioplastic films with addition of FSME at 4700x magnification (B). Arrows show some bacterial cells carried into the pulp of bioplastic

As mentioned previously, the bioactive packaging materials may contain bioactive agents that are eventually released into the food product (Lopez-

Rubio *et al.,* 2006). In the particular case of edible coatings containing probiotics, the release is not even required, since the coating itself is supposed to be eaten with the food (Espitia *et al.,* 2016). The application of probiotic edible films in food matrices is a tool for effective probiotic delivery and enhancing food stability and food safety by controlling the growth of spoilage microorganisms through antimicrobial substances produced by probiotics (Pavli *et al.,* 2018).

Soukoulis *et al*. (2017) investigated the stability of probiotic *L. rhamnosus*, incorporated in films of different biopolymers with the addition of whey protein concentrate. The population of the strain one hour after the inoculation was high, showing no acute toxic effects of the biopolymer type or of the whey protein concentrate on its cell viability, whereas the compositional characteristics of the film-forming solution were found to be influential.

In another study of Kanmani and Lim (2013), edible films with probiotic supplemented bacteria were studied. *L. rhamnosus*, *L. acidophilus* and *L. reuteri* were incorporated into pullulan, starches and their combination films. At room temperature, all of the films showed similar cell viabilities, whereas the maximum viability was observed in pure pullulan films. However, all the films, except of starch, maintained cell viabilities up to 20 days. After extended storage time (30-60 days), no viable cells were detected, probably due to increased bacterial metabolism. The pure pullulan film and the pullulan/ starch film retained cell viability higher than 80% even after 30 days of refrigerated storage, probably due to decreased bacterial metabolism (Soukoulis *et al.,* 2017).

**Molecular structure of bioplastic films using FTIR**

Based on the observation through Fourier transform infrared spectroscopy (FTIR) on bioplastic film samples, as showed in the Figure 4A and 4B, that the main transmission peak was located in the bands 800-1150 $cm^{-1}$ of glycerol transmission group; while the bands 1200-1350 $cm^{-1}$ was a combination of NH in the bending region with CN vibration stretching (amide III), 1400-1550 $cm^{-1}$ NH bending (amide II), 1600-1700 $cm^{-1}$ stretching vibration group of CO and CN (amide I); 2850-2980 $cm^{-1}$ stretching CH and 3000–3600 $cm^{-1}$ independent groups of OH and NH bonds (Ramos *et al.,* 2013; Bagheri *et al.,* 2013). According to the observations of FTIR on the control bioplastic film sample (Figure 4A), indicated that the main peak was at 3266.11 $cm^{-1}$ and the bioplastic film sample fortified with FSME (Figure 4B), the main peak was at 3269.84 $cm^{-1}$. This was due to the O-H group stretch bond. Both bioplastic film samples showed absorption bands at 2923.09 $cm^{-1}$ indicated there was C-H stretching while at peaks 1014.13 to 993.63 showed there was C-O stretching (Lubis *et al.,* 2020).

Moreover, with the addition of antimicrobial FSME to bioplastic film, the structure of the prepared bioplastic films did not change significantly due to the very low loading of FSME in the prepared bioplastic films as present in Figure 4B. Furthermore, Figure 4A displays the FTIR spectrum of pure bioplastic films without the adding of FSME. By the addition of FSME to the bioplastic gel to prepare bioplastic film, there is no variation in the characteristic bands of the prepared coating films because of the large symmetry in the structure between bioplastic film with adding and without adding of FSME.

**Physical characteristic of weight loss and fruit hardness**

Based on Figure 5, among of the coating formulations, the F1 was found to have the lowest percentage of weight loss followed by F2 and F3 upon the $14^{th}$ day of storage. Edible coating extends postharvest life of fresh fruits. It is used to improve food appearance and provide safety to the food by its environmentall friendly properties. It acts as a barrier for moisture and gases during processing, handling and storage. It reduces food spoilage and enhances safety by their activity or by incorporation of antimicrobial compound. Other advantages of using edible coating is to extend shelf life of fresh and minimally processed product and protect it from harmful environmental effect by maintaining the transfer of oxygen, moisture, and flavour compound in a food system (Vaishali *et al.,* 2019).

Strawberries have a short shelf life and they tend to suffer mechanical injuries, rapid texture softening, physiological disorders and infections caused by food pathogens thus resulting in deterioration of fruit quality is a challenge for the industry. According to the shelf life for fresh strawberries stored at 0°C is usually around 2 weeks, while for room temperature around 20°C strawberries have a shelf life of around only 3-4 days (Khodaei *et al.,* 2021). The longevity of strawberry fruit is often associated with fungal infections that cause fruit damage to occur. In addition, the addition of antibacterial agents into edible coating compounds also has the potential to increase the shelf life of fruits by inhibiting microbial growth (Jadhav and Gurav, 2018; Błaszczyk *et al.,* 2022; Shahat *et al.,* 2020; Salha and Gedanken, 2021).

According to Figure 6, uncoated control strawberries had undergone quality deterioration due to fungal growth on the surface thus causing significant quality deterioration in terms of color and texture loss on days 11 and 14 when compared to the coated ones of F1. Deterioration quality is often hampered by a decrease in fruit hardness as it is considered as indication of the quality degradation or spoilage caused by microorganisms.

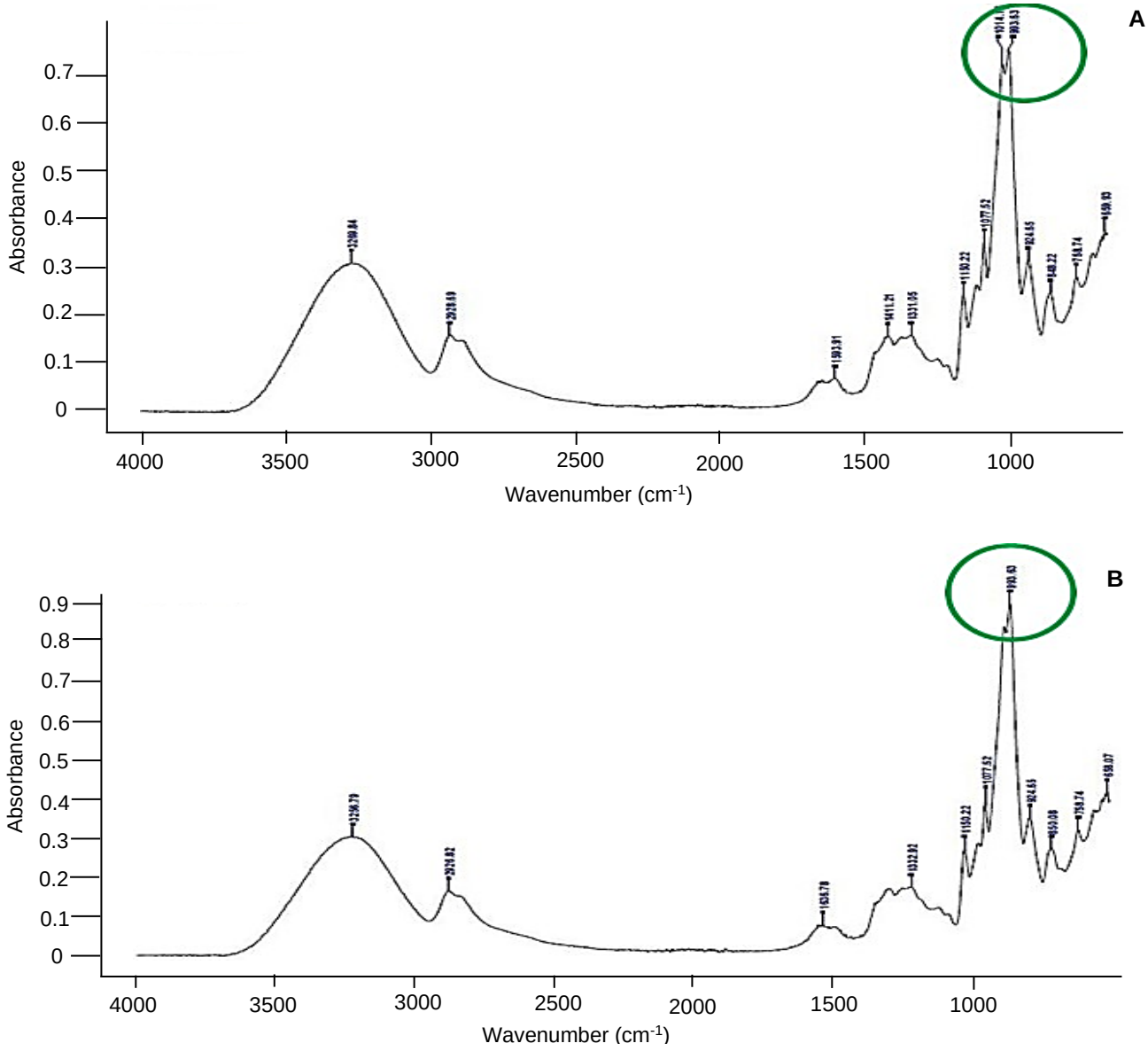



Figure 4. Fourier transform infrared spectroscopy (FTIR) chromatogram of bioplastics control (A) and bioplastic fortified with FSME (B)

In addition, other factors that influence the decrease in fruit hardness may also be associated with fruit weight loss due to loss of water content. According to Jadhav and Gurav (2018) and Błaszczyk *et al.* (2022) the fruit weight loss is often associated with the occurence of loss of water content resulting in softening of the texture of fruit. However, the coated strawberries with F1, F2 and F3 showed a slight increase in hardness on 11$^{th}$ day of storage. Velickova *et al.* (2013) stated that the occurrence of increasing in hardness of the coated fruit was likely due to the influence of coating applied on the surface of the fruit that may reduce the metabolic activity of the fruit.

Based on the Figure 6, there was a significant decrease in hardness of strawberries of the control group compared to the coated group with F2 and F3 on the first day of storage. The strawberries of coated group with F1 were found to have the highest increase in fruit hardness compared to coated groups with F2 and F3. While the group coated with F3 was found to have no significant difference in hardness when compared to the group coated with F2. This proved that the coating could function effectively as a layer that is able to be a barrier against the respiration process and the loss of water vapor quickly at the same time, thus increased the shelf life and maintain the quality of the fruits. Maqbool *et al*. (2011) reported that the weight loss percentage of the coated

strawberries has a low percentage compared to control strawberries due to the composition of the coating material which acts as a semi-permeable layer capable of preventing the loss of oxygen gas, carbon dioxide and moisture while reducing respiration rate.

**Shelf life of strawberries during storage**

Strawberries have a short shelf life and tend to suffer from mechanical injuries, texture softening, physiological disturbances and microbial spoilage and thus resulting in decreasing of fruit quality that may be a big challenge for industry. According to Jadhav and Gurav (2018) and Blaszczyk *et al.* (2022) the shelf life of fresh strawberries stored at 0°C is around 2 weeks, while at a room temperature it shows at around 3-4 days. The life span of strawberry is often associated with fungal infections that cause fruit spoilage (Shalat *et al.,* 2020).

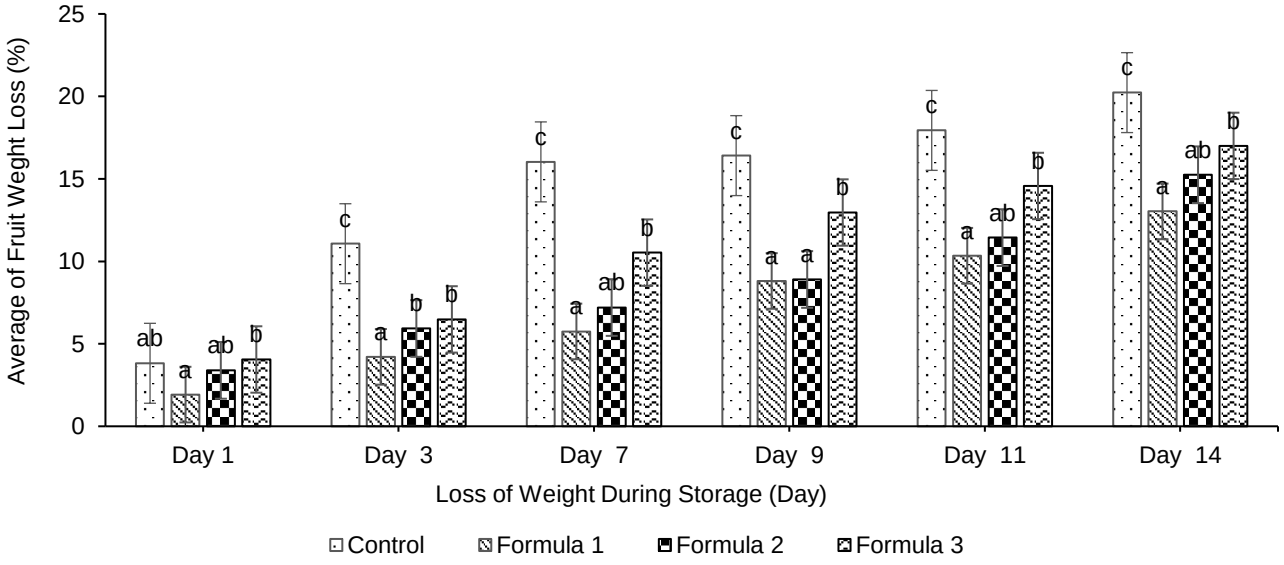



Figure 5. Average percentage of fruit weight loss during storage

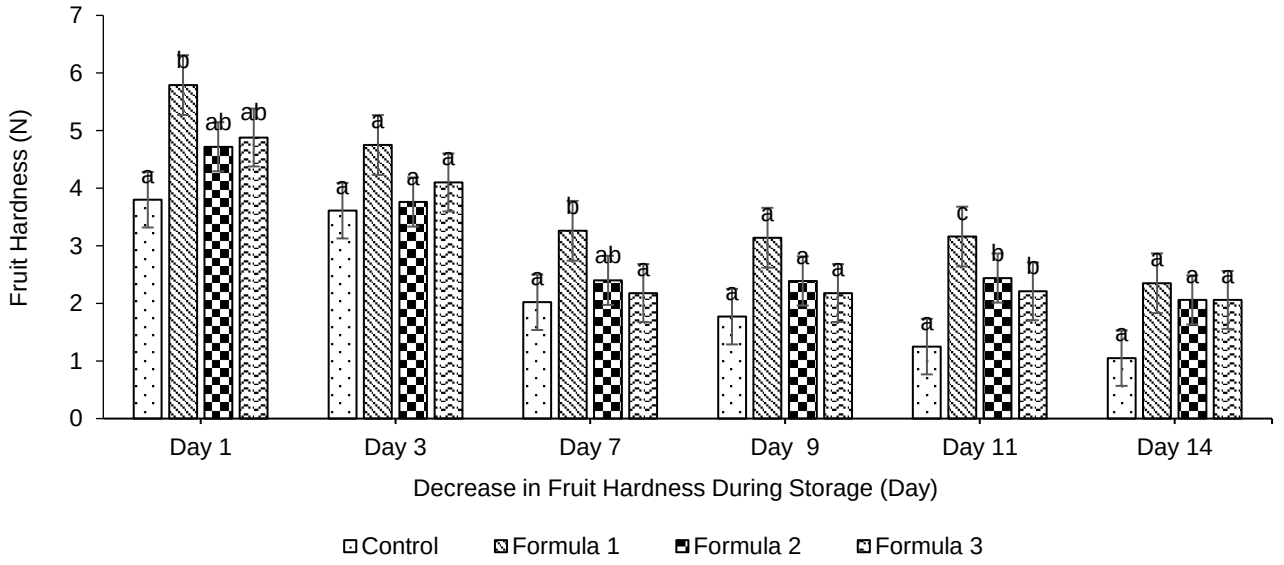
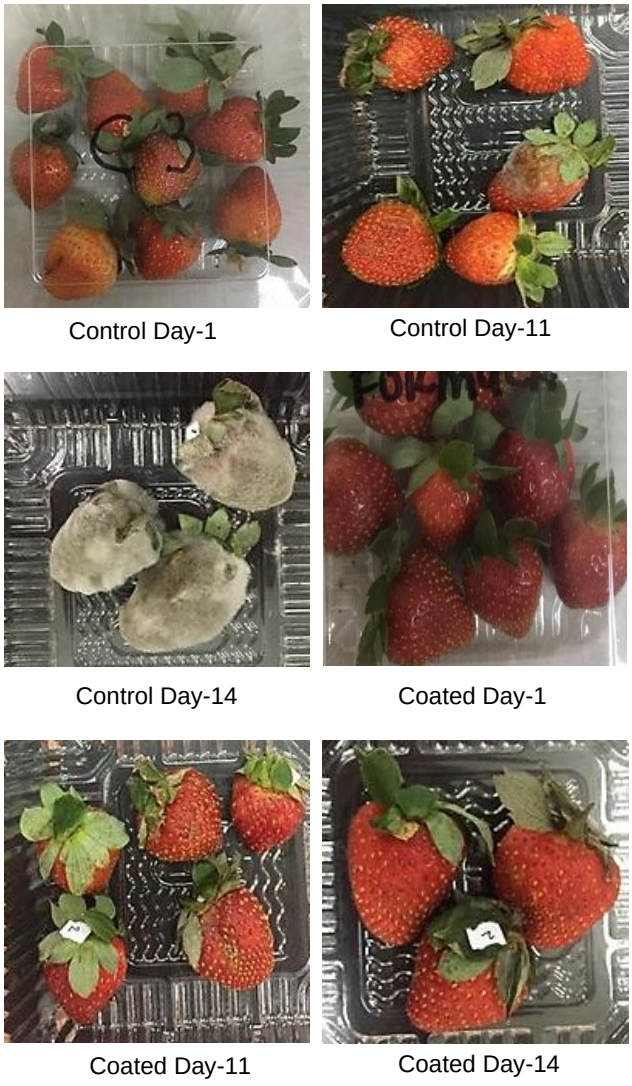



Figure 6. Decrease of fruit hardness during storage

Figure 7 showed the results of shelf life observation on strawberries that had been coated with the best formulation was Formula 1 and control samples were uncoated strawberries, and furthermore both samples were stored at 4°C. The results showed that the control samples were undergone severely spoilage due to fungal growth on their surfaces after 14 days of storage. Meanwhile, the strawberries coated with the best formulation of coating only underwent a decrease in quality in terms of color and shape. The benefit of coating on preserving fruit colour is mainly on reducing darkening and has been attributed for fresh-cut fruit due to their higher susceptibility to oxidation (Azarakhsh *et al.,* 2014).



Control Day-1 Control Day-11

Control Day-14 Coated Day-1

Coated Day-11 Coated Day-14

Figure 7. Shelf life testing of uncoated strawberries groups and coated strawberries groups with bioplastic film F-1 fortified with 10% FSME during storage and observed at day-1, day-11 and day-14

A study on the quality of strawberries coated with edible coating was reported to increase the shelf life of strawberries. Ibrahim *et al.* (2017) reported that strawberries coated with the edible coating were able to maintain their shelf life up to 4 days at room temperature and 15 days at 4°C. Whereas the uncoated ones showed their shelf life of around 2 and 8 days at room temperature and at low temperature, respectively. According to Šuput *et al.* (2015) edible coatings and films act as a semi-permeable layer capable of preventing water vapor evaporation and reducing respiration rate between fruit and environmental conditions thus slowing down the fruit damage process. These results differ from those reported by Gol *et al*. (2013) and Velickova *et al*. (2013) who showed a decrease in the total soluble solids content in strawberries, at the end of storage, and attributed it to respiration, when using other edible coatings. Firmness is an important quality parameter for fresh fruit, which decreases during storage as a result of cell wall degradation and loss of turgor.

## CONCLUSIONS

Bacterial cellulose used to produce bioplastic films has been applied to the manufacture of food coatings and packaging. The results of the functional properties test of FSME showed antimicrobial and antioxidant activity and hence it is suggested that FSME was involved in increasing the shelf life of strawberries covered with bioplastic films containing FSME during storage. The antimicrobial activity test showed that FSME could inhibit the growth of all tested pathogenic microbial cultures at MIC 10% (v/v) by showing a clear zone around the tested microbial strain colonies, while the antioxidant activity test showed that FSME showed radical scavenging activity against the DPPH. Shelf life of strawberries coated with bioplastic film containing FSME showed an increase in shelf life for for 14 days at low temperatures. The overall results indicate that the use of BC-based bioplastic films enriched with FSME plays an important role in preventing premature spoilage and increasing the shelf life of food products, thereby making them useful for application purposes as active food packaging and coating materials.

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