Gelatine Film Incorporated with *Clitoria ternatea*-Derived Anthocyanin Microcapsules, An Effective Food Packaging Material Against Foodborne Pathogens

Effective Food Packaging Material with Natural Antimicrobial Agent

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SUMMARY

*Research background.* Microbial contamination in food products is one of the significant causes of food spoilage and foodborne illnesses. The use of active packaging films incorporated with antimicrobial agents can be a measure to extend food quality and shelf life. Nevertheless, synthetic antimicrobial agents such as silver, copper, titanium, and zinc in the packaging films raised concerns among consumers due to toxicity issues.

*Experimental approach.* The current study aimed to develop biodegradable gelatine-based edible films incorporated with microcapsules of *Clitoria ternatea*-derived anthocyanin as a natural antimicrobial agent. The impact of anthocyanin microcapsule incorporation on the morphology, thermal, mechanical, water vapor barrier, and physicochemical properties of the gelatine films were evaluated in this study. The effectiveness of the developed films against...
Results and conclusions. The results show that incorporating anthocyanin microcapsules enhances the gelatine film physical and mechanical properties by increasing the thickness, tensile strength, Young's modulus, and elongation at break of the films. SEM analysis revealed that the films surface morphology with anthocyanin microcapsules exhibit a homogeneous and smooth surface texture compared to the control. The thermogravimetric analysis also showed a slight improvement in the thermal properties of the developed films. Agar well diffusion assay revealed that the developed films exhibit significant inhibition against a broad-spectrum of bacterium. Furthermore, the films composed of gelatine anthocyanin microcapsules significantly reduced the total viable count of microorganisms in the bean curd at the storage time interval of 12 days compared with the control films.

Novelty and scientific contribution. Increasing global awareness for healthy and safe food with minimal synthetic ingredients as preservatives has sparked the search for use of antimicrobial agents of natural origins in active food packaging material. In this study, a safe and effective active packaging film was developed using the environmental friendly biopolymer, gelatine films incorporated with microcapsules of *Clitoria ternatea*-derived anthocyanin as a natural antimicrobial agent. This study demonstrated that such a method not only be able to improve the film physical properties but also significantly prolong the shelf life of food products from microbial spoilage.

Key words: gelatine film, food packaging, anthocyanin, natural antimicrobial agent, foodborne pathogens, *Clitoria ternatea*

INTRODUCTION

Food spoilage is a complex process resulting from the microbial population's biochemical changes, which leads to the loss of nutritional value, texture, and flavor of the food (1). Despite the improvement of food processing and production with modern technology, microbial food spoilage is still a major global issue that causes excessive food waste. The food and Agriculture Organization of the United Nations reported waste of 1.3 billion tons of food per year due to food spoilage, which constitutes an enormous financial burden of USD 750 billion (2). Furthermore, ingestion of the microorganisms or the microbial contaminants is linked to foodborne illness, including common symptoms such as diarrhea, nausea, and vomiting (3).
Foodborne illness has accounted for 3% of mortality worldwide and costs more than 15.6 billion USD annually (4).

Food packaging is a solution to improve the safety and prolong the shelf-life of food products from microbial spoilage. Non-biodegradable polymers like plastics are commonly used as food packaging material. Nevertheless, petrochemical-based food packaging materials are being phased out in favor of biopolymer-based biodegradable packaging due to environmental concerns. On the contrary, upon disposal, biodegradable packaging materials including polysaccharides, protein or lipids, will be broken down by microorganisms (bacteria, fungi, and algae) through composting process to carbon dioxide, water, methane, and biomass (5). Soy protein, maize zein, sodium caseinate, wheat gluten, pea proteins, sunflower protein, and gelatins have all been utilized to make biodegradable films (6). Among these biodegradable protein films, Gelatin-based films have a significant potential for commercial use as food packaging films due to their associated and unique features. Gelatine is a natural water-soluble protein characterized by the absence of a noticeable odor and the random configuration of polypeptide chains in an aqueous solution (7). It is obtained from collagen's partial hydrolysis, a fibrous protein mainly found in certain parts of vertebrate and invertebrate animals as bones, skins, connective tissues, and tendons. Film-forming properties of gelatine have prompted considerable interest as edible food packaging films, which served as an excellent alternative to conventional plastic food packaging that causes various environmental issues.

Active food packaging films incorporating synthetic or natural antimicrobial agents are among the recent solutions in preventing microbial spoilage. Various agents, including metal, salt, organic acids, and chitosan, have been studied in antimicrobial packaging with varying degrees of success (8,9). Among all, the use of inorganic and metal nanoparticles such as silver (Ag), titanium dioxide (TiO₂), ZnO, and copper oxides I and II (Cu₂O, CuO) in food packaging has been reported with fairly good antimicrobial activity. However, concerns over the risks associated with the Ag ions potential ingestion migrated into food and drinks. Lansdown has reported that chronic ingestion or inhalation of silver can lead to the deposition of silver metal/silver sulfide particles in the skin (argyria), eye (argyrosis), and other organs (10). Thus, there is a growing interest in the development of antimicrobial packaging materials with natural antimicrobial agents. Numerous research has documented the effective production of smart food packaging materials employing natural components such as citric acids, pomegranate extract, apple peel extract, and purple cabbage extract in recent years (11,12). The use of these pigments not only improves the antioxidant and antibacterial characteristics
of the food packing materials but also allows color changes to occur, which are related to pH shifts during food deterioration (13,14).

Anthocyanins are pigments found in various flowers, vegetables, fruits, and berries (15). Traditionally the colored anthocyanin pigments have been used as a natural food colorant. These natural pigments are bioactive substances reported with strong oxidation resistance and therapeutic benefits against inflammation and infections (16,17). In recent years, anthocyanins have been gaining attention as food additives in the food industries (18). *Clitoria ternatea*, commonly known as butterfly pea, belongs to the *Fabaceae* family and native to Asian countries (19). *C. ternatea* is a flower crop with edible flowers used as a natural colorant for various local delicacies and served as tea in South East Asia. It is rich in pigments, mostly anthocyanin compounds (20). Our previous study has shown that *C. ternatea* derived anthocyanins exhibited antimicrobial effects against a broad spectrum of foodborne pathogenic bacteria (21). However, anthocyanins are sensitive to the ambient environment and can be degraded easily to reduce their bioavailability (22).

In the present study, we have developed the gelatine-based film incorporated with microcapsules of *C. ternatea* derived anthocyanin as a natural source of antimicrobial agent for application in the food packaging industry. The anthocyanin microcapsule incorporation on the film physical properties and the gelatine film antimicrobial properties against foodborne pathogens were evaluated. The antimicrobial efficiency of food packaging material developed was also investigated using a food model.

**MATERIALS AND METHODS**

**Plant materials**

Plant samples of *Clitoria ternatea* were gathered in Bandaraya Melaka, Malacca, Malaysia, on Jalan Tengkera (N 2° 12’ 3” E 102° 14’ 21”). Botanists from Universiti Kuala Lumpur had verified the material. Fungicides and pesticides are not used in the crop area. Only flowers with no evident signs of disease were collected during the sampling, which was done by hand. The materials were collected and placed in resealable plastic bags for processing within 24 h. The samples were cleaned in the lab under running tap water. The flowers were dried at 60 °C in a convection drying oven (Vac Oven 19.8L; Thermo Scientific, Massachusetts, USA) until they reached a consistent mass. A food blender was used to grind the dried flower (800G/S; mrc laboratory instruments, Easex, UK). The powdered ingredients were kept in a desiccator until they were needed again.

*C. ternatea* derived anthocyanin extraction
C. ternatea derived anthocyanin extraction was carried out based on the method reported previously (21). Briefly, the C. ternatea flowers were dried at 60 °C until constant mass and ground into fine particles. The samples anthocyanin content was extracted by soaking the powdered plant materials in acidified ethanol (pH=4.5) for three days at a ratio of 1:20 (m/V). The extract was then dried under reduced pressure by using a rotary evaporator (Rotavapor R-100; Buchi, Flawil, Switzerland) at 50 °C. The anthocyanin test was conducted by adding the extract to equal volume (V/V) of 2M hydrochloric acid and ammonia solution. The absorbance at 520 nm of the blue-violet solution using the spectrophotometer (UV-1280; Shimadzu, Tokyo, Japan) indicates a positive result for anthocyanin.

Preparation of anthocyanin microcapsules suspension

The solid lipid microcapsules were prepared with the hot homogenization technique with slight modification (23). The lipid phase of 100 mL of 2% of pluronic F127 (Sigma-Aldrich, St Louis, MO, USA) solution was prepared in distilled water in an ice bath. Then, 200 mL of anthocyanin extract was added to the pluronic solution. The solution was then mixed with a homogenizer (Heidolph, Schwabach, Germany) at 10,000 rpm for 2 min. To prepare the solid phase, 1% dextran (Sigma-Aldrich, St Louis, MO, USA) solution was prepared at 70 °C. The solution was then gradually added to the mixture and homogenized for another 1 min at 10,000 rpm until a transparent solution was obtained. A blank control was included by replacing the anthocyanin extract with ethanol. These microcapsules suspension was then used for the preparation of the films.

Synthesis of gelatine with anthocyanin microcapsules

The gelatine films were prepared by the solution casting method as reported previously (24). The gelatin powder (Sigma-Aldrich, St Louis, MO, USA) was dissolved in distilled water at 40 °C to form a 5.0 % (m/V) gelatin solution. Then, 3% (m/V) of sorbitol (Merck, Darmstadt, Germany) was added as a plasticizer. The mixture was stirred continuously for 20 min at 100 °C. Then, the anthocyanin microcapsules were added slowly into the film solution mixtures, and the mixture was stirred for 20 min at room temperature to obtain a film-forming solution. The film-forming solution (12 mL) was transferred to a polystyrene Petri dish (10 cm×10 cm) and then placed at 35 °C until completely dried. A control gelatine film was prepared with the same method by replacing the anthocyanin microcapsules with a blank control.

Characterization of the gelatine films color parameters
The film color values were determined with a colorimeter (Minolta, Tokyo, Japan) under constant light conditions. The value for $L^*a^*b^*$ were averaged from five measurements of each sample. The total difference in the color of the films ($\Delta E$) was calculated according to the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

$\Delta L$, $\Delta a$, and $\Delta b$ are the differences between the color of the standard color plate and film samples.

**Scanning electronic microscopy (SEM)**

The microscopic surface characteristics of the films were investigated by scanning electron microscopy (Carl Zeiss SMT, Cambridge, UK). Each sample was carefully mounted into grids of dimension 3 mm×0.5 mm and coated with platinum prior to examination.

**Film thickness**

The film thickness was measured by an electronic digital micrometer (Mitutoyo Corporation, Kanagawa, Japan). Measurements were replicated six times for each film.

**Mechanical properties analysis**

The mechanical properties of the films, such as tensile strength (TS), Young's modulus (YM), and elongation at break (E) was analyzed according to ASTM standard method 828-88 (ASTM 1989) (25). Samples of 2.0 cm×10.0 cm were prepared. The tests were performed using LLYOD LR30K PLUS (AMETEK Measurement and Calibration Technologies, West Sussex, UK) operated at initial grip separation and crosshead speed set at 50 mm and 50 mm/min, respectively. Five samples were tested for each film, and the average values were presented. Durometer hardness test was done to measure the resistance of the samples to deformation induced by mechanical indentation or abrasion. The control and film incorporated with anthocyanin microcapsules were subjected to stress introduced to the samples using a Durotech Model M202 Durometer (Durotech, London, UK). Shore A was used on the 6 mm thick samples. Five measurements were carried out for each type of film.

**Moisture content**

The film samples of the size 2 cm×2 cm were placed in an aluminium dish and dried at 90 °C for 24 h in a hot air oven (24). The initial and final mass of the films were measured. The moisture content was calculated according to Eq. 2.
\[ w(\text{moisture}) \% = \left( \frac{m_i - m_f}{m_i} \right) \times 100 \]

where \( m_i \) is the initial mass of the film (g), and \( m_f \) is the mass of the film when dried (g).

**Water vapor permeability (WVP)**

Water vapor permeability (WVP) was determined gravimetrically according to the standard method E96-95 (ASTM, 1995) (26). The films were placed on the container with distilled deionized water in a chamber with controlled temperature and relative humidity (23 °C and 15 % RH). Each container mass loss covered with the films was measured at 1 h intervals for 8 h. The WVP in g/(m²·Pa·s) values of the films were calculated according to Eq. 3.

\[ \text{WVP} = \frac{\text{WVTR} \cdot L}{\Delta p} \]

where \( \text{WVTR} \) = measured water vapor transmission rate (g/m²·s) through a film, \( L \) = mean thickness of the film (m), and \( \Delta p \) = partial water vapor pressure difference (Pa) across the film. For each measurement, at least five repetitions were made.

**Thermal properties**

The thermal properties of the films were determined based on the standard ASTME1131, ISO 11358 (27,28). Thermo-gravimetric analysis (TGA) was performed on a Q-5000IR (TA Instruments, New Castle, DE, USA) coupled with a differential thermal analyser (DTA) Each sample film (>5 mg) was heated from 30°C to 600 °C with a heating rate of 20 °C/min under nitrogen flow (40 mL/min). The melting point (\( T_m \)) was recorded from the temperature at which the endothermic peak appears in the thermograms, and three samples were measured for each film type.

**Fourier transform infrared (FT-IR)**

FT-IR spectra of the gelatine powder, gelatine control film, anthocyanin extract, and gelatine/anthocyanin microcapsules film were analyzed using FT-IR spectroscopy, Thermo Scientific Nicolet IS10 (Thermo Fisher Scientific, Waltham, MA, USA) operated at a resolution of 4 cm⁻¹. The spectrum of each sample was recorded in the wavelength range of 500-4000 cm⁻¹.

**Antimicrobial assay against foodborne pathogens**

Test microorganisms

Three Gram-positive and 6 Gram-negative foodborne pathogenic bacteria were used to evaluate the antimicrobial effectiveness of films developed in this study; *Staphylococcus*
aureus, Bacillus subtilis, MRSA, Escherichia coli, Proteus mirabilis, Yersinia enterocolitica, Salmonella typhimurium, Pseudomonas aeruginosa and Acinobacter antratus. The test microorganisms were previously isolated from contaminated food sample, verified by its 16s rRNA gene and maintained at Upstream Processing Laboratory, Universiti Kuala Lumpur, Malaysia.

Agar diffusion assay

The film antimicrobial properties were determined by the agar diffusion method, as reported previously with minor modification (29). Briefly, the bacterial inoculum was prepared based on the 0.5 McFarland standard and spread on the nutrient agar (Merck, Darmstadt, Germany). as the lawn. Wells of 1 cm diameter were made on the nutrient agar using agar well borer, and 80 μL of each film solution was loaded into the well and allowed to solidify before incubated at 37 °C. After incubation of 24 h, inhibition zones were determined by the clear zone diameter around the wells.

Application of the gelatine-anthocyanin films as active packaging for bean curd (tofu)

One hundred grams of bean curd (tofu) were cut and wrapped with the control, and anthocyanin microcapsules incorporated gelatine films. The samples were stored for 12 days at two different temperatures; 27 °C and 4 °C. Sampling was done at an interval of 4 days for microbiological analysis. The bean curd was homogenized in stomacher (Seward Stomacher 400 Lab System, Norfolk, UK) with 225 mL of sterile peptone water, followed by serial dilution, and then spread on nutrient agar. After incubation of 24 h at 37 °C, the number of colonies on each plate was counted, and total viable cells (CFU/mg) were calculated.

Statistical analysis

All tests were repeated at least three times, and data were analysed with SPSS software v. 17.0 (30). One-way analysis of variance (ANOVA) was used to evaluate the significance by Duncan's test (p<0.05) between samples. Origin v. 8.0 (31) and Excel v. 2010 (32) were used for statistical analysis.

RESULTS AND DISCUSSION

The film total color difference and surface morphology were observed to study the anthocyanin microcapsules incorporation's physiological effect. As shown in Table 1, the incorporation of anthocyanin microcapsules in the films increased the blueness values (b*)
value significantly while redness ($a^*$), lightness ($L^*$), and $\Delta E$ values decreased. The results obtained are parallel with the findings of Pourjavaher et al. (33), whereby changes of $\Delta E^*$ value of cellulose nanofibers films was observed after the addition of anthocyanin from red cabbage. The control gelatine film had a thin, yellowish, and homogenous surface, while the gelatine film incorporated with anthocyanin microcapsules appeared to be darker and greenish, attributed by the anthocyanin extract color at pH=6 (Fig. S1). The color attribute of anthocyanin is a unique feature of this natural pigment. Anthocyanin at different pH will go through a structural transformation associated with color changes (34). In recent years, there has been emerging interest in developing smart packaging material using anthocyanin to detect food spoilage based on the pH changes due to the organics acids or ammonia produced (35). In their study in assessing the organoleptic performance of edible flowers, Benvenuti et al. reported that the anthocyanins in edible flowers significantly improved the sensory profiles and organoleptic performance of food products (36).

SEM micrographs of the gelatine film surface morphology were shown in Fig 1. The control gelatine film was observed with a relatively rough surface embedded with a substantial presence of pores. However, a smooth and homogeneous surface with lesser pores and cavities was observed on the gelatine film surface along with the anthocyanin microcapsules incorporations. Such observation might be contributed by the differences in the film thickness, which resulted in lesser wrinkles formed on the surface. Similar film surface morphology was reported by Rawdkuen et al. (37) and Wu et al. (38). Grover et al. (39) reported that a water-soluble carbodiimide added during the chemical crosslinking of the gelatine scaffolds resulted in the activation of the carboxylic groups on the gelatine, so as reduce the number of side reactions and therefore induce crosslinking with free primary amine groups. Such chemical modification leads to a less porous microstructure and as well as physicochemical changes of the gelatine scaffolds. Similar events may take place during the cross-linking process of the gelatine films with the presence of anthocyanin microcapsules.

The mechanical properties of films such as tensile strength (TS) and elongation at break (EAB) are key indicators of the film integrity and resistance to environmental stress during applications such as packing (40). The TS, EAB, and Young modulus (YM) of the gelatine films are listed in Table 1. The TS indicates the film's strength, while EAB represents the film's flexibility prior to breakage. The TS of the control gelatine film is 31.0 MPa, close to the value of mammalian gelatine film reported previously (41). With the addition of the anthocyanin microcapsules, the TS increased 20 % to 37.4 MPa. The EAB of the control film was 92.83 %, while the gelatine/anthocyanin films are slightly higher. The produced film was
strong but flexible, which is an ideal material for food packaging. In overall, incorporating the anthocyanin microcapsules had improved the gelatine film mechanical properties, including the hardness and YM of the films. The thickness of the gelatine films also increased from 0.2 mm to 0.3 mm with the addition of the anthocyanin microcapsules. The results obtained are in good agreement with the findings reported by Chin et al. (42) that antimicrobial agents like Aloe vera extract would improve the plasticity and flexibility of gelatine films. The mechanical properties of the films depend on the intermolecular interaction of the film-forming matrix during the establishment of the protein network in the gelatine film. Thus, incorporating the anthocyanin microcapsules may increase the hydrogen bonds formed between the anthocyanin microcapsules and the polypeptides present in gelatine, which in turn stabilize the protein network. Furthermore, the heterogeneous film structure might increase the resistance to the film breakage, as previously reported by Libanori et al. (43). The overall improvement of the gelatine film mechanical properties suggests that anthocyanin microcapsules had a plasticizing effect on the film.

As shown in Table 1, the Moisture content (MC) of the control film is 12.5 % and has lowered to 10.4 % by incorporating anthocyanin microcapsules into the gelatine films. In contrast, WVP for the anthocyanin microcapsules /gelatine and control films are $2.84 \cdot 10^{-9}$ and $2.14 \cdot 10^{-9}$ g/(m²·Pa·s), respectively. Hamilton (44) reported that low-density polyethylene's water permeability is $2 \cdot 10^{-8}$ g/(m²·Pa·s). The commons plastics films have higher water permeability than gelatine films as gelatine hydrophilicity contributes to the low barrier properties towards the water. However, in the current context, incorporating anthocyanin microcapsules into the gelatine film improved the water permeability and led to the film better breathable performance.

The thermal properties of the gelatine films are shown in Fig. 2. TGA results show two-step thermal decline patterns for both gelatine and gelatine/anthocyanin films. The first decomposition started around 90 °C for both gelatine and gelatine/anthocyanin film. The beginning of the second decomposition is observed at 300 °C due to the evaporation of water remaining in the film, and the second heat decomposition is due to the decomposition of gelatine. The temperature for the thermal destruction of gelatine/anthocyanin was slightly delayed compared to the gelatine control film. This result indicated that the addition of anthocyanin increased the thermal stability of the film. After the final thermal destruction, the residual percentages of the gelatine film and gelatine/anthocyanin film mass were 15.91 % and 16.44 %, respectively. Gelatine/anthocyanin indicated a higher residual than gelatine control
film due to the anthocyanin element in the film. A similar observation was reported by Kanmani and Rhim (45) with the addition of AgNPs to the gelatine film.

FT-IR spectroscopy was carried out to identify the chemical structure changes of gelatine and the incorporation of anthocyanin. Fig. 3 shows the FT-IR spectra of the pure gelatine, gelatine (control) film, anthocyanin, and anthocyanin incorporated gelatine film. All spectra show the range peaks at 3275 cm\(^{-1}\) to 665 cm\(^{-1}\). The broad and robust absorption peaks observed at 3275 cm\(^{-1}\), was assigned to hydroxyl strain vibration group (O-H) (Table 2). Whist the intense peak at 1633 cm\(^{-1}\) shows the presence of the carbonyl (C=C) stretching frequency (Table 2). The peaks appearing at 1540 cm\(^{-1}\) are due to the stretching vibration of the ester group. The peaks observed in the FT-IR spectra of the gelatine powder and gelatine control film show same bonds and wavenumbers indicating there were no major changes in the functional group, whereas the gelatine/anthocyanin film spectra showed a stretching vibration when the anthocyanin was added. Hence, it indicates the presence of anthocyanin within the gelatine network of the developed film.

The gelatine film antimicrobial activity incorporated with different anthocyanin microcapsules concentrations was tested against nine foodborne illness-related microorganisms, including Gram-positive and negative bacteria. The well diffusion assay shows that 6 out of the 9 test microorganisms are susceptible to the anthocyanin incorporated gelatine film (Table 3). The developed film exhibited a significant and broad spectrum of antimicrobial activity against both Gram-positive and negative bacteria, including the common foodborne illnesses causing bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Yersinia* sp. *etc.* The broad spectrum antimicrobial activity of free form anthocyanin was also reported by Aly *et al.* (46) and Rashid *et al.* (47). The size of the inhibition zone increased in a concentration dependant manner. With the highest concentration of anthocyanin, the inhibition zone size on well diffusion assay varied among all microbes ranging from 10 to 14.2 mm, indicating different susceptibility of the test microorganisms to the anthocyanin. The result obtained agrees with findings of Werlein *et al.* (48) that anthocyanin has better antimicrobial activity against Gram-positive organisms than Gram-negative bacteria. This might occur due to an outer membrane's presence in the Gram-negative bacteria that acts as a permeability barrier.

To investigate the developed gelatine film's efficiency as an active food packaging, we used tofu (soybean curd) as a food model system. Tofu is a versatile protein-rich food prepared from soybeans. With relatively high moisture content (80–88 %), moderate pH (pH=5.8–6.2), and rich protein (6–8.4 %), tofu is a perishable food product with short shelf life (49). The
detection of spoilage microorganisms such as Lactic acid bacteria, Pseudomonas, Enterobacteriaceae, Bacillus spp., Klebsiella spp., and Staphylococcus spp. have been reported in numerous studies (49). TVC (total viable count) value of the soy curd using different gelatin films as a packaging system over the storage of 14 days at room temperature and 4 °C was shown in Fig. 4. The TVC of bacteria is a vital indicator of the quality of food products. At room temperature, the initial TVC value was 5.0·10² CFU/g that suggested the soy curd quality was low and acceptable. The TVC value of the soy curd increased significantly during storage. The soy curd TVC value packed in the control and gelatine/anthocyanin film was 2·10³ and 1·10³ CFU/g, respectively, on day 4, and was close to the maximum microbiological acceptability limit of 7 log CFU/g for bean curd. It is suggested that their microbiological shelf-life was about 5-6 days. The reported shelf life is longer compared to Shihang et al. where they utilized calcinated shell powder from Corbicula fluminea as a natural antimicrobial agent for tofu preservation (50). No study has been conducted to apply anthocyanin as packing system for tofu. TVC value of the samples packed with control gelatine film increased to 1.3·10⁴ and 2.8·10⁴ CFU/g on days 8 and 12, respectively. In contrast, the TVC value of samples packed with gelatine film incorporated with anthocyanin microcapsules was significantly lower than that of the control sample on the same day. The bacterial growth inhibition is particularly significant on day 12 storage with the TVC value of the control gelatine film to be two-fold of the film incorporated with anthocyanin microcapsules. These results showed that the sample bacterial growth was inhibited for the packaging system containing gelatine films incorporated with anthocyanin microcapsules. Unlike storage at room temperature, the TVC value decreased during the 12 days of storage at 4 °C. Nevertheless, the TVC value of samples packed with gelatine film incorporated with anthocyanin microcapsules is continually lower with significant growth reduction.

CONCLUSIONS

In conclusion, the incorporation of the anthocyanin microcapsules into the gelatine film significantly improved the mechanical and thermal properties of the film. The developed gelatine film also showed significant bacterial growth inhibition against the common foodborne pathogens. Using soy curd as a food model demonstrates that the gelatine film developed can suppress microbial growth, leading to food spoilage. This study shows that the gelatine/anthocyanin microcapsules film is an effective biodegradable food packaging material with a natural antimicrobial agent that has excellent potential to enhance the shelf life of food products.
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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest with this manuscript.

**SUPPLEMENTARY MATERIALS**

All supplementary material is available at: www.ftb.com.hr.

**AUTHORS’ CONTRIBUTION**

Chean Ring Leong contributed to the conception or design of the work and preparation of the manuscript, Nurul Shahida Daud and See Yuan Cheng did the data collection, Woei Yenn Tong and Khairul Faizal Pa'ee contributed to the data analysis and interpretation, Nurhanis Syafiqah Mohd Nor Hamin and Wen Nee Tan contributed by performing the analysis.

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Fig. 1. SEM micrographs showing the surface micromorphology of gelatine film: a) control at 250×, b) film with anthocyanin microcapsules at 250×, c) control at 500× and d) b) film with anthocyanin microcapsules at 500×.
Fig. 2. Thermal characterization: a) thermogravimetric analysis curves of gelatine and anthocyanin microcapsule incorporated gelatine films, b) derivative thermogravimetry (DTGA) analysis of gelatine and anthocyanin microcapsules incorporated gelatine films
Fig. 3. FT-IR spectra of anthocyanin extract, gelatine with anthocyanins, control (gelatine film) and gelatine powder
Fig. 4. Total viable count changes of the food model with different packaging during storage of 12 days at a) room temperature and b) 4 °C
Table 1. Physical properties of the gelatine films

<table>
<thead>
<tr>
<th>Properties</th>
<th>Control (Gelatine film)</th>
<th>Gelatine film with anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td>L/mm</td>
<td>0.21±0.03</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>TS/MPa</td>
<td>31.0±1.9</td>
<td>37.4±1.1</td>
</tr>
<tr>
<td>YM/MPa</td>
<td>607.43±16.32</td>
<td>704.26±11.24</td>
</tr>
<tr>
<td>E/®</td>
<td>92.83±4.23</td>
<td>102.67±1.63</td>
</tr>
<tr>
<td>Hardness/N</td>
<td>28.4±0.7</td>
<td>33.6±1.7</td>
</tr>
<tr>
<td>w(moisture)/%</td>
<td>12.48±0.6</td>
<td>10.39±0.5</td>
</tr>
<tr>
<td>WVP·10^{-9} (g/m²·Pa·s)</td>
<td>2.14±0.005</td>
<td>2.84±0.004</td>
</tr>
<tr>
<td>L*</td>
<td>45.13±0.16</td>
<td>38.12±0.23</td>
</tr>
<tr>
<td>a*</td>
<td>-7.31±0.11</td>
<td>-10.12±0.32</td>
</tr>
<tr>
<td>b*</td>
<td>8.11</td>
<td>12.51</td>
</tr>
<tr>
<td>ΔE</td>
<td>31.00 ± 0.24</td>
<td>26.47 ± 0.82</td>
</tr>
</tbody>
</table>

L=Thickness, TS=Tensile strength, YM=Young’s modulus, E=elongation at break, w(moisture)= Moisture content and WVP=water vapour permeability, L*=lightness, a*=redness, b*=yellowness, ΔE=total color difference

Table 2. Comparison of the relevant transmission peak of gelatine (control) film, gelatine/anthocyanin film, gelatine powder, and anthocyanin extract with the standard transmission peak from the literature

<table>
<thead>
<tr>
<th>Type of bond</th>
<th>Standard transmission peak/cm⁻¹</th>
<th>Experimental transmission peak/cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C=C of benzene ring</td>
<td>1629.97</td>
<td>1634.36</td>
</tr>
<tr>
<td>Carbonyl group</td>
<td>1524.92</td>
<td>1541.53</td>
</tr>
<tr>
<td>Aliphatic hydrogen</td>
<td>2934.98</td>
<td>2934.74</td>
</tr>
<tr>
<td>OH group</td>
<td>3429</td>
<td>3275.97</td>
</tr>
</tbody>
</table>
Table 3. Antibacterial activity of gelatine and gelatine film incorporated with different mass of anthocyanin microcapsules.

<table>
<thead>
<tr>
<th>Test microorganism</th>
<th>d(inhibition)/mm</th>
<th>Gelatine with anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (gelatine film)</td>
<td>m(anthocyanin) =30 mg</td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
<td>8.8 ± 0.34</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>MRSA</em></td>
<td>-</td>
<td>8.9 ± 1.79</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>8.3 ± 0.57</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Acinobacter antratus</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>7.6 ± 0.6</td>
</tr>
</tbody>
</table>

(-) = No inhibitory zone
SUPPLEMENTARY MATERIAL

Fig. S1. The physical appearances of the gelatine films: control (left) and film with 50 mg of anthocyanin microcapsules (right)