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Allium Sativum as Antimicrobial Agent in Thermoplastic Sago Starch (TPSS) Films for Dermal Wound Healing

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Abstract:

Wound healing takes precedence as wounds can cause serious harm to humans. Most wound dressings developed nowadays were derived from non-biodegradable sources but there were several issues such as dehydration and bacterial infection. The purpose for this study is to investigate the significance of garlic effects (Allium sativum) as a vital source of allicin, which acts as an antimicrobial agent in thermoplastic sago starch (TPSS) dermal wound healing films. The TPSS films were fabricated from 6.5 wt. % of starch and 3.5 wt. % of glycerol, along with 0, 0.25, 0.50, 0.75, 1.00 and 1.25 wt. % of garlic contents via solvent casting method. The samples named as TPSS/G00, TPSS/G25, TPSS/G50, TPSS/G75, TPSS/G100 and TPSS/G125, respectively. Based on the analysis, the TPSS/G25 film had achieved maximum tensile strength at 2.33 MPa with good thermal stability. This result was supported by the smooth surface from the micrograph of the film observed under scanning electron microscope (SEM). Yet, the allicin compounds were found missing as proven from the Fourier Transform Infrared (FTIR) spectra that showed the absence of antimicrobial properties for all films produced, as fabrication processes involved heating. Therefore, these films were unable to inhibit bacterial growth, as evidenced by the Kirby-Bauer test. It is concluded that the allicin in the garlic plays important roles as antimicrobial properties in fabricated films to meet the requirement as wound dressings.

Keywords: *garlic, allicin, biodegradable, dressing, antimicrobial*

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I. INTRODUCTION

Wound healing is vital since untreated wounds can lead to infection, fever and corruption of internal organs [1]. Most wound dressings developed nowadays are from non-biodegradable sources and have issues like dehydration and immune rejection [2]. It is hoped that with the development of thermoplastics and biodegradable wound dressings, these issues can be addressed to further improve wound healing. In light of these studies, biopolymers from natural starch such as sago have been developed to ameliorate the situation of current dermal patch [3], [4]. Wound healing patches based on biopolymer materials are economical, renewable, biodegradable and have good biocompatibility with the human body [2], [5]. Thermoplastic biopolymers however have high stiffness, which can produce dermal patches that are less flexible and brittle [6]. As such, plasticizer needs to be introduced to the thermoplastic film to increase its flexibility. Glycerol was chosen due to its availability and good properties in increasing the flexibility of thermoplastic thin films [7]. This plasticizer blends well with sago starch through the bonding of hydroxyl groups from both glycerol and sago starch [8]. The addition of antimicrobial agents can effectively inhibit any microbial growth in thermoplastic sago starch (TPSS) thin films. Garlic has shown a promising potential in inhibiting bacterial growth with the presence of organic sulfur compounds like allicin in its constituent. Thus, garlic was added into the composition as an antimicrobial agent. Moreover, the allicincompound has been proven to successfully inhibit bacterial culture such as *E. coli, S. aureus and L. sakei* as reported by

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Kuorwel and team [9]. Allicin is the result of enzymatic reaction from enzyme *alliinase* and *alliin* compounds. This compound however, is very sensitive to degradation during processing and storage [10]. The degradation of allicin can be attributed to the disruption of its precursor, the deactivation of enzyme *alliinas*e and also through mechanical means [11]. This study aims to investigate the effectiveness of allicin in garlic as an antimicrobial agent in the development of TPPS thin films for dermal wound healing.

II. MATERIALS AND METHODS

A. Materials and Film Preparation

Four main materials were used in this study, which are sago starch powder, garlic powder, glycerol and distilled water. Sago starch powder with the density of 0.571 g/cm^3 was obtained from Hup Seng Heng Sdn. Bhd. Malaysia. Glycerol with the purity of 86% - 89% and density of 1.232 g/cm^3 was purchased from Merck Sdn. Bhd., Malaysia. Garlic powder was obtained from a local supermarket. Six samples of thermoplastic film were prepared with different compositions of garlic powder. The film was prepared using the solvent casting method. Based on a previous study [12], the optimum composition for film preparation was defined as 6.5% starch, 3.5% plasticizer and 90% distilled water. The glycerol, sago starch and distilled water mixture made up the foundation of the film. The samples were prepared with 0.00 wt. % to 1.25 wt. % of garlic, designated as TPSS/G00, TPSS/G25, TPSS/G50, TPSS/G75, TPSS/G100 and TPSS/G125, respectively. The foundation mixture was first mixed and magnetically stirred at 700 rpm for 30 min. For the addition of garlic powder, the desired weight of the garlic powder was added into 20.0 g of90.0 g distilled water and stirred for 1 h at room temperature. The garlic solution was then poured into the sago starch mixture solution with the remaining 90% of distilled water and stirred until gelatinization occurs. Once gelatinization occurred, the temperature was kept constant within the range of 65^{o}C – 70^{o}C for 20 min with constant stirring. Then, the mixture was casted in a mold and dried in an oven at 40^{o}C for 10 h.

B. Testing

Thetensile testing was done with an applied loading of 5 kN at a rate of 5 mm/min. This test will reveal the tensile strength, strain and elongation at break for the thermoplastic film. The thermoplastic thin films were cut into sections according to ASTM D882-18 [13].

Thermogravimetric analysis on the samples was done to investigate the thermal stability of the samples with a heating rate of 10^{o}C/min in the range of 25^{o}C – 500^{o}C and sampling time of 0.5 s.

Fourier Transform Infrared (FTIR) spectra of the samples were collected to study the functional groups present in the samples and the material blends by using the FTIR Spectrum 2000 spectrometer with the mode of 20 scans and 4 cm^{-1} resolution within the range of 4000 – 400 cm^{-1}.

Scanning electron microscopy (SEM) method was used to analyze the morphology of the fabricated thin films under 500X magnification with 6 kV voltage.

The antimicrobial properties of the thermoplastic films were tested using the *Kirby-Bauer* antimicrobial disk analysis. The test was done to study the susceptibility of *Escherichia coli* on the garlic thermoplastic films. The bacteria culture was grown on individual agar disc plates. The thin films were cut and dipped in distilled water before being placed on top of the grown culture. Each thin film was left for 48 h in an incubator at 37^{o}C as per human body temperature. The area of inhibition zone was measured.

III. RESULTS AND DISCUSSIONS

A. Mechanical properties

The tensile test revealed the tensile strength and elongation at break of thermoplastic thin films with six different compositions as seen in Figure 1. Tensile strength for the fabricated thin films is highly dependent on the mixing and blending of the elements in the composition. Poor blending can result in agglomerated particles and weaker C-C bonds, which in turn diminish the strength of the samples. The strengths of TPSS/50, TPSS/G75, TPSS/G100 and TPSS/G125 samples were 1.75, 1.74, 1.42 and 1.40 MPa, respectively. Moreover, when compared to other studies that reported sago starch thin films

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yielded a tensile strength of 1.64 MPa [6], the tensile strength values obtained in this study are well within range. Meanwhile, the elongation at break indicates the flexibility of thermoplastic samples. Flexibility is associated with the bonding strength of hydroxyl groups. Better bonding with the O-H group would give more flexibility to the starch rigid backbone. An increase in flexibility was observed in the samples of TPSS/G50, TPSS/G75, TPSS/G100 and TPSS/G125.

B. Thermal analysis (TGA)

The TG curves showed that thermal changes of the samples were in accordance to the typical behaviors of starch thin film degradation. The thermograms obtained showed a three-step degradation that was consistent with typical degradations for starch thin films with glycerol and garlic degradation [14], [15]. This is because the degradation of moisture content would first occur in the range of 100 – 150^{o}C. The second degradation step was in the range of 180 – 250^{o}C, which indicated the glycerol degradation. The last degradation step occurred in the range of 280 – 350^{o}C, which indicated the starch degradation and garlic decomposition. As evident in Figure 2, this can be seen in all six compositions. The weight loss at the final temperature of 500^{o}C showed that the six compositions have different thermal stabilities. To illustrate the thermal stability of the six fabricated thermoplastic thin films, the temperature at 50% weight loss of each sample was compared, as seen in Table 1. Even though thermal stability varied from one sample to another, the overall trend shows a decrease in the thermal stability of thermoplastic thin film with the addition of garlic.

C. Fourier-Transform Infrared (FTIR)

Thespectra of the thermoplastic thin films indicate that the thin films maintained the functional group of alkanes, the C-H group, since all six have peaks in the range of 3000 – 3500 cm^{-1}. The strong peaks in the range of 1400 – 855 cm^{-1} are indicative of the presence of alkanes, in which the C-H group is prevalent in the thermoplastic samples. Shifting of the O-H group and the C-H group were observed in the fabricated thermoplastic thin films, as seen in Figure 3. The O-H peaks in sago and glycerol were located at 3297.70 cm^{-1} and 3268.49 cm^{-1}, respectively. When both were blended in TPSS/G00, the peak of O-H was observed at 3277.73 cm^{-1}. This shows that the peak of O-H has shifted to a wavenumber in between the peaks of sago and glycerol due to blending. The shifting of the O-H band peak to a lower wavenumber indicated an increase in intermolecular hydrogen bond interactions between glycerol and starch [12]. The hydrogen bond interaction between glycerol and starch can occur through the C-O-C group with the C-O-H group [16]. This would usually result in a lower wavenumber of band peaks as evident from this spectrum.

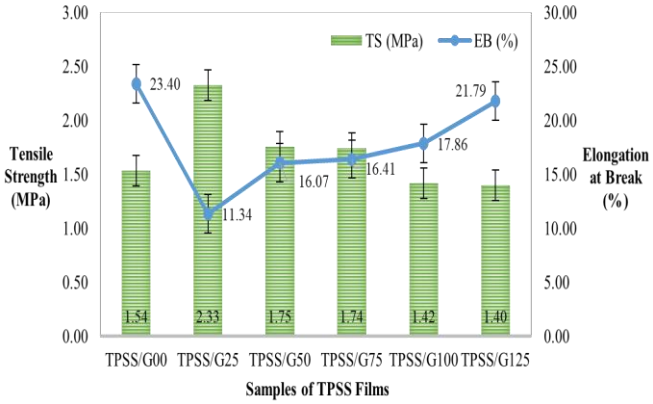

Fig. 1. Graphs of tensile strength (TS) and elongation at break (EB) of fabricated TPSS films

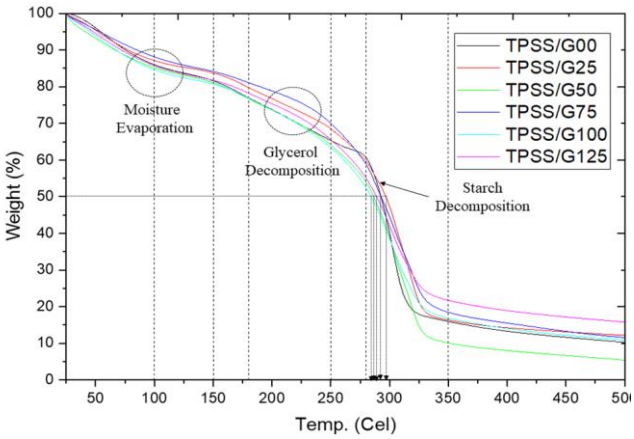

Fig. 2. TG curves for fabricated TPSS films of six different compositions

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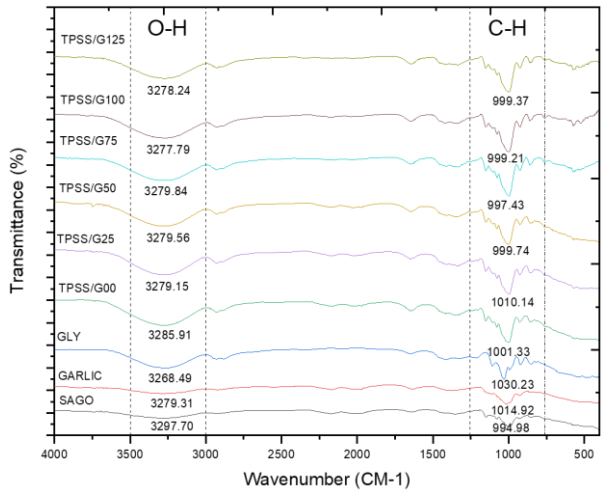

Fig. 3. FTIR spectra of glycerol, sago starch, garlic and fabricated TPSS films

Garlic shows weak O-H peak at 3279.31 cm^{-1}. After blending, the O-H peaks were observed in TPSS/G25, TPSS/G50, TPSS/G75, TPSS/G100 and TPSS/G125 at 3279.15 cm^{-1}, 3279.56 cm^{-1}, 3279.84 cm^{-1}, 3277.79 cm^{-1} and 3278.24 cm^{-1}, respectively. Shifting of the O-H peaks is also observed in the mixture. Furthermore, after sago, glycerol and garlic were blended together, shifting of C-H peaks could be observed from the peaks of TPSS/G25, TPSS/G50, TPSS/G75, TPSS/G100 and TPSS/G125 at 1001.33 cm^{-1}, 1010.14 cm^{-1}, 999.74 cm^{-1}, 997.43 cm^{-1}, 999.21 cm^{-1} and 999.37 cm^{-1}, respectively. It is also noted that no thiol groups were observed, whereby the typical peaks of thiol, S-H group are within 2550 – 2620 cm^{-1}[17]; sulfoxide, S=O group stretches approximately 1050 cm^{-1}; and sulfones, S=O group stretches within 1300 – 1150 cm^{-1} or S-O group stretches within 1000 – 750 cm^{-1}. This explains the absence of allicin since the functional groups of organosulfur compounds were not observed.

D. Scanning Electron Microscopy (SEM)

The SEM images show the morphology of the thermoplastic thin films, which is a smooth surface with irregular particles scattered throughout. This suggests that the fabricated thermoplastic thin films had solidified with no formation of observable grains. It can be observed that as the garlic loading increased, the agglomerated particles enlarged. Moreover, the smooth surface of the thermoplastic appears to become rougher with the increase in garlic loading. It can also be noted that some striation marks are visible on the surface of thermoplastic thin films such as depicted in Figure 4 (d, f). The agglomeration and irregular marks were attributed to processing defects. Longer mixing time may be needed to encourage a more homogenous distribution of garlic particles. From the tensile strength obtained, four samples (TPSS/G50, TPSSS/G75, TPSS/G100 and TPSS/G125) have lower values with increased garlic loading. Meanwhile, the SEM images of all four samples show agglomerated particles. This may indicate a weaker bonding of alkane groups that made the film less rigid with poor tensile strength. In comparison, TPSS/G25 has a higher tensile strength due to more uniformly distributed garlic particles.

E. Antimicrobial Properties

The inhibition of *E. coli* strains by the thin films was studied after 48 h of incubation. From the test conducted, it can be observed in Figure 5 that the *E. coli* culture growth was not inhibited by the addition of garlic into the thermoplastic thin film composition. This indicates that the garlic addition did not introduce any antimicrobial properties to the thermoplastic thin films. This may be due to the absence of allicin in the garlic powder. As seen in the FTIR analysis of the garlic powder in Figure 3, no thiols or sulfur functional groups were observed, thus suggesting that allicin compound was not present in the garlic powder. The antimicrobial properties of garlic are largely derived from organosulfur compounds such as allicin [19], [20]. With the absence of these compounds, the thermoplastic thin films are unable to inhibit the growth of bacterial strains such as the *E. coli* culture. The absence of allicin in garlic powder may be due to fabrication processes that involve heating. It was found in a study by Prati et al. that almost 99% of allicin is lost during processing by heating [10]. This explains the absence of allicin in the garlic powder. In short, it can be deduced that due to the absence of allicin in the garlic powder, the fabricated thermoplastic thin film samples were unable to inhibit bacterial growth.

IV. CONCLUSION

Thermoplastic sago starch thin film samples were successfully fabricated. The samples were studied

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under SEM, which revealed a smooth morphology contributed by glycerol with agglomerated particles of garlic powder due to processing defects. FTIR spectra analysis revealed good blending between all constituents of the samples with shifting of hydroxyl and alkane groups observed. The thermal study showed that thermal stability of the samples decreased with the increase in garlic loading. Thickness and bulk density studies revealed that the garlic loading does not affect the measurements since no clear trends can be observed. The mechanical properties of the samples showed low tensile strength but higher flexibility. Lastly, the antimicrobial evaluation reported that the samples were unable to inhibit bacterial culture growth due to the absence of antimicrobial compounds in the garlic powder used. The missing allicin was a ramification of the processing method for the garlic. It can be deduced that the organic sulfur compound, specifically allicin, is a crucial compound in garlic for antibacterial properties.

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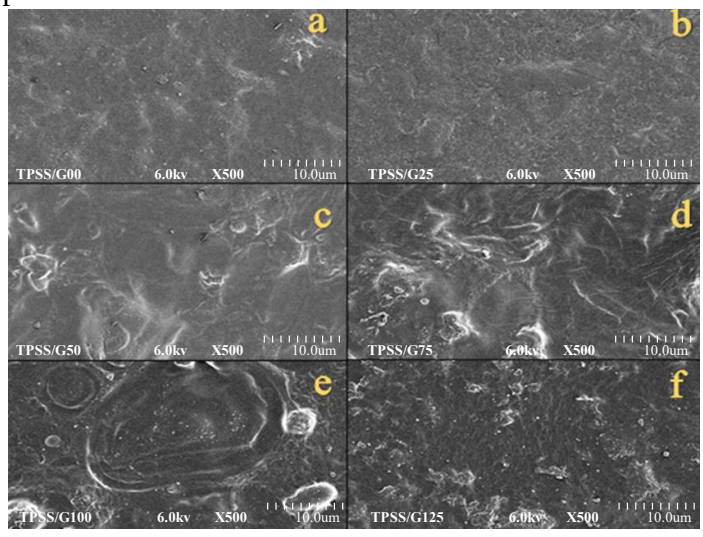

Fig. 4. SEM micrographs for fabricated TPSS films for samples of a) TPSS/G00, b) TPSS/G25, c) TPSS/G50, d) TPSS/G75, e) TPSS/G100 and f) TPSS/G125

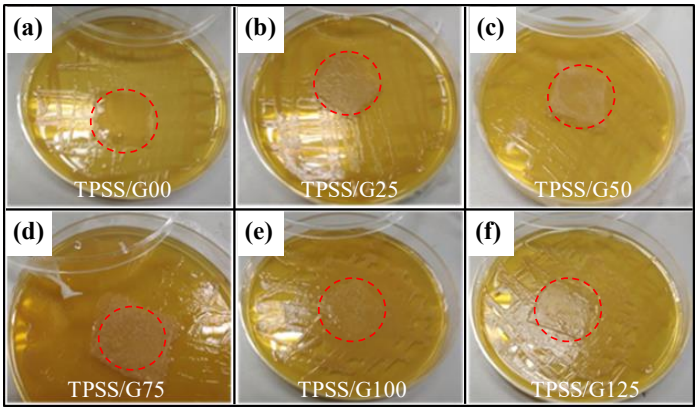

Fig. 5. Antimicrobial evaluation on the fabricated TPSS films (a) TPSS/G00, (b) TPSS/G25, (c) TPSS/G50, (d)TPSS/G75, (e) TPSS/G100 and (f) TPSS/125, in red-dotted lines.

REFERENCES

[1] K. F. Cutting, S. Dowd, S. L. Percival, and R. D. Wolcott, "Types of Wounds and Infections," in Microbiology of Wounds, no. July 2014, 2010, pp. 219–234.

[2] E. Cevher and A. D. Sezer, "Biopolymers as Wound Healing Materials: Challenges and New Strategies," 1992.

[3] A. Asthana and G. S. Asthana, "Polymeric Sustained Biodegradable Patch Formulation for Wound Healing," vol. 9, no. 7, 2015, pp. 577–580.

[4] A. Asthana, G. Shilakari, and S. Toshkhani, "Advancements in wound healing biodegradable dermal patch formulation designing," Inven. Rapid Pharm Tech., 2013.

[5] L. Da, Y. Huang, and H. Xie, "Progress in development of bioderived materials for dermal wound healing," Regen. Biomater., 2017, pp. 325–334.

[6] M. R. Ishak, M. Jawaid, J. Sahari, M. L. Sanyang, and S. M. Sapuan, "Effect of Plasticizer Type and Concentration on Tensile, Thermal and Barrier Properties of Biodegradable Films Based on Sugar Palm (Arenga pinnata) Starch," Polymers (Basel)., vol. 7, 2015, pp. 1106–1124.

[7] S. Ad and K. Chantawee, "Effect of Glycerol on the Physical Properties of Carboxylated Styrene-Butadiene Rubber / Cassava Starch Blend Films," J. Polym. Environ., vol. 0, no. 0, p. 0, 2018.

[8] T. Mekonnen, P. Mussone, and D. Bressler, "Progress in bio-based plastics and plasticizing modifications," J. Mater. Chem. A, vol. 1, 2013, pp. 13379–13398.

[9] S. W. Bigger, M. J. Cran, K. K. Kuorwel, J. Miltz, and K. Sonneveld, "Antimicrobial Activity of

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ISSN: 0193-4120 Page No. 1014 - 1019

Biodegradable Polysaccharide and Protein-Based Films Containing Active Agents," vol. 76, no. 3, 2011.
[10] C. M. Henrique, M. T. B. Pacheco, P. Prati, V. S. N. da Silva, and A. S. de Souza, "Evaluation of allicin stability in processed garlic of different cultivars," Food Sci. Technol., vol. 34, no. 3, 2018, pp. 623–628.
[11] L. L. M. Lagunas, "Effect of temperature cycling on allinase activity in garlic," vol. 111, 2008, pp. 56–60.
[12] H. Y. Hermain, "Preparation And Characterisation of Halloysite/Sago Starch Nanocomposites.," 2014.
[13] ASTM International, "ASTM D882 - 18 : Standard Test Method for Tensile Properties of Thin Plastic Sheeting," ASTM, 2018. [Online]. Available: https://www.astm.org/Standards/D882. [Accessed: 05-Dec-2018].
[14] L. Guz, O. Ochoa-yepes, and S. Goyanes, "Influence of extrusion process conditions on starch film morphology," LWT - Food Sci. Technol., no. June, 2017.
[15] Y. Dai, P. Guan, L. Hao, L. Zhang, and Z. Zhang, "Physicochemical characteristics of complexes between amylose and garlic bioactive components generated by milling activating method," Food Res. Int., vol. 105, no. July 2017, 2018, pp. 499–506.
[16] Y. Jiugao and W. Ning, "The Effects of Citric Acid on the Properties of Thermoplastic Starch Plasticized by Glycerol," Starch/Starke, vol. 57, pp. 494–504, 2005.
[17] I. Koca and H. Kutuk, "Determination of alliin and allicin in the plant of Allium scorodoprasum L . subsp . rotundum by using the infrared spectroscopy technique," no. November, 2016.
[18] O. Kang, "Evaluation of Melanoidins Formed from Black Garlic after Different Thermal Processing Steps," vol. 21, no. December, pp. 398–405, 2016.
[19] T. Ariga et al., "Antibacterial Potential of Garlic-Derived Allicin and Its Cancellation by Sulfhydryl Compounds Antibacterial Potential of Garlic-Derived Allicin and Its Cancellation by Sulfhydryl Compounds," Biosci. Biotechnol. Biochem., vol. 73, no. 9, pp. 1948–1955, 2014.
[20] R. Mardomi, "Determining the Chemical Compositions of Garlic Plant and its Existing Active Element," J. Appl. Chem., vol. 10, no. 1, pp. 63–66, 2017.

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