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THE EFFECT OF VARIOUS ORGANIC ACIDS AND CONCENTRATIONS ON THE MECHANICAL AND PHYSICAL CHARACTERISTICS OF HYDROGELS MADE FROM FRUIT FLOUR OF MANGROVE *Bruguiera gymnorrhiza*

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ABSTRACT

This research utilized organic acids in different concentrations as crosslinking agents with polyvinyl alcohol (PVA) to induce an esterification reaction for *lindur* (*Bruguiera gymnorrhiza*) fruit flour-based hydrogels. The aim was to discover the impact of different organic acids, namely lactic and acetic acids, on physical, mechanical, and antibacterial activity against *Staphylococcus aureus* on hydrogels. The concentration variations of lactic acid and acetic acid used were 6%, 8%, and 10% (w/v). The characteristics of the physical properties test (swelling index, gel fraction, pH, thickness) indicate its classification as a superabsorbent material. The characteristics of the mechanical properties test (tensile strength, elongation, foldability) indicate that hydrogels meet mechanical standards for human skin. Adding lactic acid demonstrated the most significant bacterial inhibition against *S. aureus* among the analyzed hydrogels. Hydrogel, with the addition of organic acids, especially lactic and acetic acids, holds the promise of enhancing the inherent qualities of hydrogels for potential application as a wound dressing.

Keywords: Acetic Acid, Elongation, Gel Fraction, Lactic Acid, Swelling Index, Tensile Strength, Thickness
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INTRODUCTION

The mangrove forests along the coastal areas of East Java Province span 27,221 hectares, which accounts for approximately 48% of the total mangrove forest area on Java Island.[1] One promising species for development is *Bruguiera gymnorrhiza*, commonly known as *lindur*. Research has shown that starch from *lindur* fruit flour can be utilized in hydrogel production. Large, highly magnified polymeric networks that are hydrophilic and can hold a significant amount of water inside their pores are known as hydrogels.[2] Hydrogel synthesis is a three-step process that includes hybrid, chemical, and physical bonds. Important characteristics of the synthesized hydrogels include their sensitivity to stimuli, swellability, biocompatibility, and biodegradability.[3] The starch from *B. gymnorrhiza* mangrove fruit, being a natural polymer, is well-suited for use as a hydrogel precursor due to its large hydroxyl group content, which results in significant water-absorbing capabilities.[4] However, hydrogels made from starch tend to be less flexible, rigid, and prone to brittleness because increased starch concentration can raise amylose content, leading to more numerous and stronger hydrogen bonds.[5] Cross-linking in starch can enhance physical properties but often falls short of meeting the mechanical strength required for hydrogel products. Hence, hydrogels need modification with other materials to improve their mechanical properties, one of which is the addition of polyvinyl alcohol (PVA), a substance widely used in bioplastic blends for its ability to enhance elasticity and tensile strength.[6] Research has shown that incorporating organic acids like citric acid into PVA during hydrogel production can induce esterification reactions, facilitate cross-linking, and impart plasticization effects, thereby enhancing composite film properties and resulting in hydrogels with improved mechanical characteristics.[7] In addition to enhancing physical and mechanical properties, organic acids can also play a role in antibacterial inhibition by controlling bacterial growth through low pH levels. Acetic and lactic acids have been deemed effective in inhibiting the growth of *S.* aureus.[8,9,10] Adding organic acids, specifically lactic acid and acetic acid, to PVA in the formulation of hydrogels from *B. gymnorrhiza* mangrove fruit

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flour is expected to induce esterification reactions that improve the mechanical properties of the hydrogel. Thus, this study aims to ascertain how different kinds of organic acids—namely, lactic and acetic acids—affect *S. aureus*'s mechanical, physical, and anti-bacterial characteristics about the hydrogel products produced at varied concentrations.

EXPERIMENTAL

Material and Methods

Bruguiera gymnorrhiza fruit flour, polyvinyl alcohol (88% hydrolyzed), lactic acid (LA), acetic acid (AA), and distilled water. The research method employed in this study is an experimental approach utilizing a Full Factorial Randomized Complete Block Design (RCBD) with two factors. The first factor represents the variation in acid type (A1 = lactic acid; A2 = acetic acid). The second factor represents the variation in acid concentration (B1 = 6%; B2 = 8%; B3 = 10%). The experiments were conducted with three replications. Data obtained from the research were analyzed using Minitab 21 software.

Preparation of *B. gymnorrhiza* Fruit Flour

Preparing *B. gymnorrhiza* (*lindur* fruit) flour begins with boiling the *lindur* fruit, which has been washed, in boiling water at 100°C for 20 minutes. Subsequently, the boiled *lindur* fruit is peeled to separate the skin from the flesh. Next, the peeled *lindur* fruit is boiled again for 15 minutes. The *lindur* fruit is cut into smaller pieces and dried in an oven at 60°C for 24 hours. Finally, the dried *lindur* fruit is ground using a disk mill and sieved through a 100-mesh sieve.[5,11]

Hydrogel Preparation

The hydrogel preparation process begins with dissolving 3.25% PVA in 50 mL of distilled water. This PVA solution is then heated at 95°C and stirred at 300 rpm for 1 hour. Afterward, the PVA solution is left overnight to remove bubbles formed during stirring. Following an overnight rest, PVA is combined with 6%, 8%, and 10% lactic acid and acetic acid, respectively, as per the treatment, and heated at 80°C for 15-20 minutes. The next step involves dissolving *B. gymnorrhiza* (*lindur* fruit) flour at a concentration of 6% in 40 mL of aquades in a glass beaker, heated on a hotplate at 90°C for 45 minutes. During heating, the solution is stirred at 300 rpm to gelatinize the *lindur* fruit starch. The subsequent process entails mixing both solutions, i.e., the *lendur* fruit starch solution and the PVA solution, with 10 mL of aquades for homogenization. This mixture is heated on a hotplate at 80°C for 15-20 minutes. The mixture is poured into a disposable petri dish until the surface is evenly covered. The final step is oven-dry at 60°C for 8 hours.[7,11]

FTIR Analysis

FT-IR testing of the hydrogel is conducted using the FTIR Spectrometers Shimadzu IRSpirit-T.

pH Analysis

pH testing is performed using a digital pH meter. Before use, the pH meter is calibrated using standard solutions. The pH testing procedure on the hydrogel involves weighing 1 gram of the hydrogel sample. Subsequently, 10 mL of distilled water is added, and the pH meter electrode is immersed in the hydrogel solution container. Observations are made on the pH meter until a constant reading is achieved, representing the hydrogel's pH value.[12]

Swelling Capacity (SC) Analysis

Swelling capacity is conducted by cutting the hydrogel into 2x2 cm pieces. Afterward, the hydrogel is dried at 60°C for 24 hours. Following this, the hydrogel is immersed in distilled water for 24 hours. The water on the surface of the hydrogel is removed with tissue and weighed (Wb). Then, the previously soaked hydrogel is dried again at 60°C for 24 hours. Subsequently, the dry hydrogel is weighed once more (Wk). The swelling capacity of the hydrogel is calculated using the following formula:

$$\%\text{Swelling Capacity} = \frac{\text{Wb}-\text{Wk}}{\text{Wk}} \times 100\%$$

Wb represents the weight of hydrogel after swelling (g), and Wk is the dry hydrogel's (g) weight.[13]

Gel Fraction (GF) Analysis

Gel fraction testing is conducted by weighing 0.25 grams of the hydrogel (W0). Afterward, the hydrogel is wrapped in gauze and soaked for 24 hours in 20 mL of distilled water. After 24 hours, the hydrogel is

removed from the soaking container. Subsequently, the hydrogel is dried at 60°C for 4 hours. Then, the dry hydrogel is weighed again (W1). The gel fraction of the hydrogel is calculated using the following formula:

$$\%\text{Gel Fraction} = \frac{W1}{W0} \times 100\%$$

W0 represents the initial weight of hydrogel (0.25 g), and W1 represents the weight of hydrogel after redrying (g).[3]

Thickness (Thi) Analysis

Thickness testing of the hydrogel is performed using a thickness gauge at three different points, and the measurements are averaged.

Foldability (Fol) Analysis

Foldability testing involves folding the hydrogel into half circles, then quarter circles, until it reaches the point of damage or tearing. The number of folds the hydrogel can endure before damage occurs is recorded as the foldability test result.[3,14]

Tensile Strength (TS) Analysis

Tensile strength measurement of the hydrogel is conducted using an Imada Tension Testing instrument. The testing operates at a speed of 1 mm/s.[15]

Elongation (Elo) Analysis

Elongation measurement of the hydrogel is carried out using an Imada Tension Testing instrument. The testing operates at a speed of 1 mm/s.[15]

Antibacterial Activity against *S. aureus*

Antibacterial testing against *S. aureus* is conducted using the disc diffusion method. The antibacterial testing involves introducing one colony of pure *S. aureus* bacteria into a TSB solution and homogenizing it using a vortex mixer. Then, the bacterial suspension is incubated for 2 - 6 hours until the turbidity level matches McFarland standard 0.5. A liquid culture with a turbidity equivalent to McFarland 0.5 contains a bacterial population of $1.5x10^8$ CFU/mL.[16] Once the same turbidity level is reached, the *S. aureus* bacterial suspension from the TSB medium is inoculated onto MHA media using a sterile cotton swab by the streaking method and left for 3 - 5 minutes. Afterward, sterile blank paper discs measuring 6 mm in diameter are used as negative controls, paper discs previously dipped in gentamicin sulfate (10µL/L) serve as positive controls, and hydrogel measuring 5 mm is placed on the MHA media using sterile forceps. Subsequently, the petri dishes are sealed with plastic wrap and incubated at 37°C for 16 - 18 hours. The diameter of the inhibition zone formed around the hydrogel is measured using calipers with the following formula:[17,18]

$$\text{The diameter of the inhibition zone} = \frac{d1+d2}{2} - x$$

Where d1 is the vertical diameter of the clear zone (mm), d2 represents the horizontal diameter of the clear zone (mm), and x is the diameter of the paper disc (mm).

RESULTS AND DISCUSSION

Functional Group Analysis of Hydrogel using FTIR

FTIR analysis was performed to confirm the occurrence of esterification reactions in the acid-PVA mixture, which can lead to cross-linking and plasticization effects, resulting in hydrogels with improved mechanical properties. The functional groups in the hydrogel with lactic and acetic acids can be seen in Fig.-1 and Fig.-2.

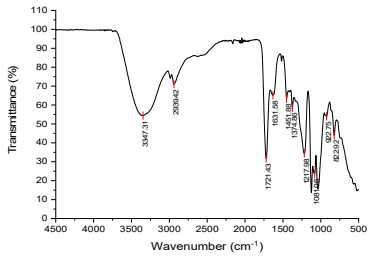

Fig.-1: Functional Number of Spectra Hydrogel-LA using FTIR

Previous studies by[19] have shown that the formation of C-O-C and C=O groups characterizes the esterification reactions between PVA and organic acids such as lactic acid. This study confirms the esterification reactions between the lactic acid-PVA mixture by absorption peaks at about 1721.43 cm^{-1} (C=O, ester) and 1217.98 cm^{-1} (C-O-C).

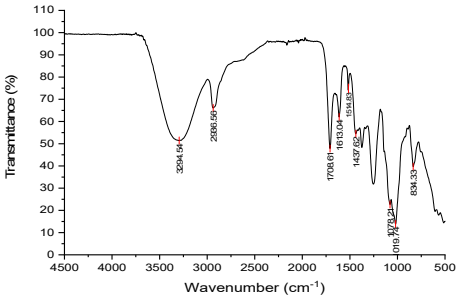

Fig.-1: Functional Number of Spectra Hydrogel-AA using FTIR

The acetic acid-PVA mixture showed absorption peaks at 1708.61 cm^{-1} (C=O, ester), which is consistent with the research results.

Effect of Treatments on Hydrogel Characteristics

As a film, each sample was produced. Figure-4 shows that the hydrogel films containing LA are darker than those containing AA. The kind and concentration of the acid had an impact on the properties of the resultant hydrogel, according to the analysis of variance. The effects of treatments on hydrogel characteristics are summarized in Table-1.

Table-1: Effect of Treatments on Hydrogel Characteristics

Treatment	SC (%)	GF (%)	Thi (mm)	Fol (times)	TS (N/mm^2)	Elo (%)	pH	*S. aureus* Antibacterial Activity (mm)
A1B1	557.13± 0.50^{c}	59.54±0. 97^{c}	0.30±0.01^{c}	138.33± 1.53^{cd}	4.29±0.3 9^{c}	79.06±0. 43^{c}	1.90± 0.00^{d}	14.91±0.99^{c}
A1B2	604.48± 0.49^{b}	55.82±0. 27^{d}	0.32±0.00^{b}	147.00± 1.00^{bc}	4.25±0.8 1^{c}	101.66±0 .57^{b}	2.26± 0.00^{e}	19.46±0.06^{b}
A1B3	675.15± 0.54^{a}	52.60±0. 53^{e}	0.34±0.00^{a}	158.67± 0.58^{a}	2.78±0.0 5^{d}	124.26±0 .70^{a}	2.63± 0.00^{f}	22.21±0.10^{a}
A2B1	456.33± 0.35^{f}	77.43±0. 35^{a}	0.18±0.01^{f}	114.67± 7.02^{e}	11.72±0. 20^{a}	11.27±0. 03^{f}	3.15± 0.05^{a}	3.97±0.07^{f}
A2B2	490.93± 0.85^{e}	79.50±0. 72^{a}	0.24±0.01^{e}	136.00± 3.46^{d}	9.77±0.6 4^{ab}	33.87±0. 17^{e}	3.35± 0.00^{b}	5.49±0.15^{e}
A2B3	548.08± 0.31^{d}	73.29±1. 25^{b}	0.26±0.00^{d}	148.67± 4.16^{ab}	9.32±0.2 6^{b}	46.46±0. 30^{d}	2.71± 0.01^{c}	9.53±0.33^{d}

Note: Mean±St. Dev. Different notations in the same column indicate statistically significant differences (P<0.05)

Fig.-2: Hydrogel Films in Different Treatments

Swelling Capacity

The lowest swelling capacity was observed in treatment A2B1 (AA 6%), while the highest swelling capacity was observed in treatment A1B3 (LA 10%). Esterification reactions with polyvinyl alcohol (PVA) leave behind free lactic acid molecules that provide -OH groups[19], which are hydrophilic and can increase absorption capacity with increasing acid concentration. Similarly, acetic acid, which is a carboxylic acid,

will leave a hydrophilic group in the esterification reaction with PVA.[20] So that with the presence of the hydrophilic group, water absorption will be increased according to[7], hydrogels with absorption capacities more than 260% are categorized as superabsorbents, making them suitable for wound dressings. The hydrogel in this study exhibited an absorption capacity of more than 260%, indicating its suitability as a wound dressing material.

Gel Fraction

The lowest gel fraction was obtained in treatment A1B3 (AL 10%), while the highest gel fraction was obtained in treatment A2B2 (AA 8%). The gel fraction values are influenced by the presence of -OH groups in the material. Hydroxyl groups can inhibit cross-linking, and the more hydroxyl groups react, the more cross-linking is inhibited, resulting in lower gel fraction values.[21] Therefore, the -OH group contained in acetic acid can also be considered to contribute to inhibiting the crosslinking which has an impact on decreasing the value of the gel fraction. Furthermore, lactic acid, acting as a plasticizer, can weaken the hydrogel's strength and increase its flexibility.[7,22]

Thickness

The highest thickness was observed in treatment A1B3 (AL 10%), while the lowest thickness was observed in treatment A2B1 (AA 6%). The thickness of the hydrogel is influenced by the differences in the constituent materials' concentrations, which affect its viscosity. Increased viscosity can result in thicker films.[8] The dissociation constant (pKa) of lactic acid (3.85) is lower than that of acetic acid (4.75), which can lead to a higher degree of dissociation and a larger volume of acid solution in the polymer matrix.[23]

Foldability

The highest foldability was observed in treatment A1B3 (AL 10%), while the lowest foldability was observed in treatment A2B1 (AA 6%). Hydrogel foldability is influenced by its elasticity and thickness. Thin and overly elastic hydrogels can tear easily.[18] The use of lactic acid causes the intermolecular space between the polymer chains to increase, so it's resulting in great material flexibility and increasingly pliable film.[23,24] Other than that, the use of acetic acid also affects the folding power characteristics where[25], suggested that the addition of acetic acid will produce a flexible film so that when folded, it is not easily torn. Additional -OH groups in the structure of hydrogels made of lactic acid material can enhance the intermolecular space between polymer chains, increasing the material's flexibility.[23] This better flexibility properties of lactic acid leads to a better value of lactic acid folding ability compared to acetic acid.

Tensile Strength

Treatment A2B1 (AA 6%) exhibited the maximum tensile strength, whereas treatment A3B1 (AL 10%) displayed the lowest. Acid, acting as a plasticizer, can reduce the energy required for molecular movement, thus decreasing its rigidity and resulting in low tensile strength.[26] It's related to the presence of -OH groups that form spaces in the polymer chain. Other than that, the addition of acetic acid concentration also tends to increase the tensile strength value. It's in accordance with the research of[27], which states that the higher concentration of acetic acid added, leads to the increase of tensile strength value. Compared with acetic acid, a hydrogel with lactic acid mixture produces material with a better flexibility value.[23] Therefore, films with lactic acid mixtures do not require great tensile strength because they are more flexible than acetic acid. According to the standards, the tensile strength of wound dressing hydrogels should fall within the range of 5-15 N/mm^2 for tendons and 0.3-25 N/mm^2 for heels.[21] Based on these standards, this hydrogel meets the tensile strength requirements for wound dressing hydrogels.

Elongation

The highest elongation was observed in treatment A1B3 (AL 10%), while the lowest elongation was observed in treatment A2B1 (AA 6%). The elongation value correlates with the tensile strength. The esterification reaction between PVA and lactic acid, which provides plasticization effects[7], increases elongation with increasing concentration. Besides that, the addition of acetic acid concentration also affects the elongation value, as explained in the study of[28], that the addition of acetic acid results in changes in the elongation value. Hydrogel with a lactic acid mixture has a higher elongation value. The use of lactic acid can produce materials with better flexibility values compared to acetic acid.[23] Therefore, a hydrogel with a

lactic acid mixture has a greater elongation value because it's more flexible with a good elasticity ability. The standard for elongation in wound dressing hydrogels is typically within the range of 1-25%.[21] Therefore, this hydrogel is considered to meet the standard for elongation in wound dressing applications.

pH

Treatment A2B1 (AA 6%) had the highest pH value, while treatment A1B3 (AL 10%) had the lowest pH value. pH decreases with increasing acid concentration due to the dissociation of acid molecules in solution, releasing free protons.[29] Lactic acid has a lower pH value compared to acetic acid due to its lower dissociation constant (pKa) of 3.85, whereas acetic acid has a higher pKa of 4.75.

Antibacterial Activity against *S. aureus*

Treatment A1B3 (AL 10%) exhibited the maximum antibacterial activity against *S. aureus*, whereas treatment A2B1 (AA 6%) showed the lowest activity. Lactic acid has more excellent antibacterial action than other organic acids like acetic, citric, and propionic acids. The acid's molecular weight may impact how well it inhibits bacteria. Compared to citric acid (192.13 Da) and tartaric acid (150.09 Da), lactic acid has a lesser molecular weight (90.08 Da), which makes it easier to enter bacterial cells and change their internal pH, which increases its antibacterial activity.[30]

CONCLUSION

The use of various acid kinds and concentrations dramatically influences the properties of the hydrogel. Because organic acids like lactic and acetic acids contain functional OH groups, using them to produce hydrogels impacts the hydrogel's quality, particularly its water absorbency and gel fraction features.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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REFERENCES

1. Z. Hidayah, H. A. Rachman, and A. R. As-Syakur, *Majalah Geografi Indonesia*, **37(1),** 84(2023), https://doi.org/10.22146/mgi.78136
2. B. S. Kaith, A. Singh, A. K. Sharma, and D. Sud, *Journal of Polymers and Environment*, **29,** 3827(2021), https://doi.org/10.1007/s10924-021-02184-5
3. S. Bashir, M. Hina, J. Iqbal, A. H. Rajpar, M. A. Mujtaba, N. A. Alghamdi, S. Wageh, K. Ramesh, and S. Ramesh, *Polymers,* **12(11),** 2702(2020), https://doi.org/10.3390/polym12112702
4. D. H. Kusumawati and W. D. R. Putri, *Jurnal Pangan dan Agroindustri*, **1(1),** 90(2013), http://jpa.ub.ac.id/index.php/jpa/article/view/9
5. J. Budiman, R. Nopianti, and S. D. Lestari, *Jurnal FishtecH*, **7(1),** 49(2018),

https://doi.org/10.36706/fishtech.v7i1.5980
6. N. Sari, M. Mairisya, R. Kurniasari, and S. Purnavita, *Metana*, **15(2),** 71(2019).
7. A. Das, R. Uppaluri, and C. Das, *International Journal of Biological Macromolecules*, **131,** 998(2019), https://doi.org/10.1016/j.ijbiomac.2019.03.160
8. M. Ewadh, H. Hasan, I. Bnyan, and F. Mousa, *Advances in Life Science and Technology*, **7,** 15(2013), http://iiste.org/Journals/index.php/ALST/article/view/5447
9. Å. Rosengren, M. Lindblad, and R. Lindqvist, *International Journal of Food Microbiology*, **162(2),** 159(2013), https://doi.org/10.1016/j.ijfoodmicro.2013.01.006
10. E. E. Safani, W. A. C. Kunharjito, A. Lestari, and E. R. Purnama, *Biotropic*, **3(1),** 68(2019), https://doi.org/10.29080/biotropic.2019.3.1.68-78
11. D. Setijawati, Yahya, H. Kartikaningsih, Sukoso, A. L. Fitri, and I. Hidayat, *Prosiding Seminar Nasional Perikanan dan Kelautan*, **9(1),** 95(2022).
12. D. Nofiandi, W. Ningsih, and A. S. L. Putri, *Jurnal Katalisator*, **1(2),** 1(2016), https://doi.org/10.22216/jk.v1i2.1113
13. B. S. B. Utomo, D. Fransiska, and M. Darmawan, *Jurnal Pascapanen Bioteknologi Kelautan dan Perikanan*, **11(1),** 55(2016), https://doi.org/10.15578/jpbkp.v11i1.258
14. M. A. Rahussidi, Sumardianto, and I Wijayanti, *Jurnal Pengolahan dan Bioteknologi Hasil Perikanan*, **5(2),** 17(2016), https://ejournal3.undip.ac.id/index.php/jpbhp/article/view/16011
15. S. Alvarado, G. Sandoval, I. Palos, S. Tellez, Y. Aguirre-Loredo, and G. Velazquez, *Food Science and Technology*, **35(4),** 690(2015), https://doi.org/10.1590/1678-457X.6797
16. R. Triwibowo, N. Rachmawati, and I Hermana, *Jurnal Pascapanen Bioteknologi Kelautan dan Perikanan*, **8(2),** 151(2014), https://doi.org/10.15578/jpbkp.v8i2.59
17. S. A. Estikomah, A. S. S. Amal, and S. F. Safaatsih, *Journal of Islamic Pharmacy*, **5(1),** 36(2021), https://doi.org/10.21111/pharmasipha.v5i1.5705
18. E. N. Wiratno, D. Aliviyanti, H. Djamaludin, and M. Dailami, Mikrobiologi Perairan, *UB Press*, (2023).
19. V. Sedlařík, N. Saha, I. Kuřitka, and P. Sáha, *International Journal of Polymer Analysis and Characterization*, **11(4),** 253(2006), https://doi.org/10.1080/10236660600750190
20. E. Rynkowska, K. Fatyeyeva, S. Marais, J. Kujawa, and W Kujawski, *Polymers*, **11(11),** 7(2019), https://doi.org/10.3390/polym11111799
21. U. L. Fadiana and Haryanto, *Technology*, **22(2),** 177(2021), https://doi.org/10.30595/techno.v22i2.11593
22. J. M. Onyari and S. J. Huang, *Journal of Applied Polymer Science*, **113,** 2053(2009), https://doi.org/10.1002/app
23. J. M. F. Pavoni, C. L. Luchese, and I. C. Tessaro, *International Journal of Biological Macromolecules*, **138,** 693(2019), https://doi.org/10.1016/j.ijbiomac.2019.07.089
24. P. Kaur, T. Alam, H. Singh, J. Jain, G. Singh, and A. A. Broadway, *Journal of Pure and Applied Microbiology*, **17(1),** 241(2023), https://doi.org/10.22207/JPAM.17.1.14
25. A. Santoso, W. Ambalinggi, and H. Niawanti, *Jurnal Chemurgy*, **3(2),** 8(2019), https://doi.org/10.30872/cmg.v3i2.3577
26. Sismaini, I. S. Nasution, and B. S. Putra, *Jurnal Ilmiah Mahasiswa Pertanian*, **7(2),** 464(2022), https://doi.org/10.17969/jimfp.v7i2.19599
27. R. Apriani and N. N. M. Susantini, *Jurnal Vokasi Teknologi Industri*, **1(1),** 14(2019), https://doi.org/10.36870/jvti.v1i1.41
28. W. Saptahadi, V. Anggraeni, P. S. Nazmi, and Rahmayetty, *Jurnal Integrasi Proses*, **10(1),** 37(2021), http://dx.doi.org/10.36055/jip.v10i1.11399
29. G. Sengupta, M Zheng, and N. L. Prisle, *Atmospheric Chemistry and Physics*, **24,** 1467(2024), https://doi.org/10.5194/acp-24-1467-2024
30. S. Stanojević-Nikolić, G. Dimić, L. Mojović, J. Pejin, A. Djukić-Vuković, and S. Kocić-Tanackov, *Journal of Food Processing and Preservation*, **40(5),** 990(2016), https://doi.org/10.1111/jfpp.12679

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