RESEARCH PROGRESS IN MECHANICAL AND MANUFACTURING ENGINEERING VOL. 4 NO. 2
(2023) 261-268

RPMME

Homepage: http://penerbit.uthm.edu.my/periodicals/index.php/rpmme
e-ISSN : 2773 - 4765

Fabrication and Physiochemical Properties of Chitosan/Beeswax Biofilm for Wound Healing Applications

Wan Muhammad Faiz Mohd Roslan[1], Maizlinda Izwana Idris[1*]

[1]Faculty of Mechanical and Manufacturing Engineering,
University Tun Hussein Onn Malaysia, Parit Raja, 86400, MALAYSIA

*Corresponding Author Designation

DOI: https://doi.org/10.30880/rpmme.2023.04.02.033
Received 1 Oct 2023; Accepted 5 Dec 2023; Available online 31 Dec 2023

Abstract: Chitosan-based dressings with antimicrobial properties aid wound healing when applied to wounds. Beeswax shows therapeutic potential in wound healing. Combining chitosan and beeswax enhances wound healing capacity. A solution casting method was used to create the chitosan and beeswax biofilm, allowing for varying amounts of beeswax from 1g to 5g. Glutaraldehyde cross-linking formed a firm biofilm. Characterization involved SEM, FTIR, contact angle, and AFM. Beeswax addition resulted in a rough and jagged surface. FTIR confirmed bonding between chitosan and beeswax. Modifications targeted chitosan's hydrophilic properties, increasing biofilm hydrophobicity. AFM demonstrated increased surface roughness with beeswax addition. Overall, the biofilm fabrication was successful for wound healing applications.

Keywords: Chitosan, Beeswax, Glutaraldehyde, Physiochemical, Wound healing

1. Introduction

The principal function of the skin is to act as a barrier between the body's internal organs and external elements such as heat, chemicals, and infectious agents. It is incredible that the healing process happens with such few issues despite how complicated it is. Many circumstances might make recovery challenging, which can lead to slower wound healing, illness or even death, and poor cosmetic results [1]. Natural polymers like chitosan, which are primarily produced by living creatures' cells, are suited for biomedical uses in the early phases of development for wound treatment via biofilm. While chitosan is a derivative of chitin, chitin is derived from the exoskeletons of crustaceans like prawns and crabs. Liquids, membranes, and films can all be employed for medical applications, along with chitosan and its equivalent.

Numerous investigations, both pre-clinical and clinical, are now being carried out in order to gain a better understanding of the behavior and possible toxicity of chitosan and its derivatives. It is utilized widely in the healing of wounds, and the addition of an ingredient such as beeswax may assist in

*Corresponding author: izwana@uthm.edu.my

enhancing the therapeutic characteristics of the product. Because the conditions necessary for wound healing must be maintained, the hydrophobicity of beeswax can assist in maintaining the appropriate level of moisture in the wound. It is important to select wound dressings based on their ability to clear necrotic tissue, keep the skin moisturized, and absorb excess fluid. A wound that loses its moisture might cause the death of surrounding tissue; hence, an absorbent is required for a wound that has a lot of exudates. Even though there was only a small quantity of exudate that was absorbed, the amount of moisture that was trapped inside the dressing would rise due to water seepage, and as a result, the dressing would need to be changed more frequently. As a result of this, a patch that is waterproof is necessary in order to reduce the number of times that dressings need to be changed [2].

Chitosan is utilized for contact disinfectants in numerous biomedical applications, including wound dressing, tissue engineering, and hemodialysis, in various disciplines of hygienic applications and health care for it being a biocompatible substance that degrades slowly into harmless by-products that are totally absorbed by the body. Supplementation of beeswax into a biofilm made of chitosan should induce better wound healing properties. Characterization upon the physiochemical and the fabrication of the chitosan biofilm impregnated with beeswax will be the main focus for this study.

2. Materials and Methods

2.1 Materials

In preparation of this biofilm, material that has been used to fabricate the biofilm is listed in Table 1. The designated composition of the chitosan/beeswax biofilm is shown in Table 2.

Table 1: List of material used

No	Material	Manufacturer
1.	Chitosan (powder)	Sigma-Aldrich
2.	Beeswax (pellet)	Sigma-Aldrich
3.	Acetic Acid (liquid)	Bendosen
4.	Glutaraldehyde (liquid)	Sigma-Aldrich

Table 2: Designation of chitosan/beeswax biofilm composition

No	Composition	Label
1.	1 wt% chitosan + 0g beeswax	CBw0
2.	1 wt% chitosan + 1g beeswax	CBw1
3.	1 wt% chitosan + 2g beeswax	CBw2
4.	1 wt% chitosan + 3g beeswax	CBw3
5.	1 wt% chitosan + 4g beeswax	CBw4
6.	1 wt% chitosan + 5g beeswax	CBw5

2.2 Biofilm preparation

The chitosan/beeswax (CBw) solution was prepared by dissolving 1 wt% of chitosan in acetic acid aqueous solution. Chitosan was added into acetic acid when a vortex was formed. The mixture was stirred on a hot plate magnetic stirrer for 45 minutes at 900 rpm in order to achieve homogeneity. The maximum temperature for this mixing process was 60-70°C. Beeswax was then added gradually into the mixture 15 minutes after chitosan was added. The solution was then rested aside for ½ hour before it was distributed into 3 petri dishes, each with 30ml volume of the solution. Glutaraldehyde was then pipette onto the petri dishes and swirled around with a spatula. The samples were dried at room temperature until a biofilm was formed. CBw0 which is a chitosan/beeswax biofilm without beeswax, was fabricated to compare the differences of the biofilm with and without beeswax. A total of six parameters was set according to the required composition in Table 2.

2.3 Fourier transform infrared (FTIR) spectroscopy

Fourier Transform Infrared (FTIR) data for the films were obtained to identify the functional group present in the biofilm. Chitosan and beeswax each have different levels of transmitted infrared value which can be traced by the unique wavenumbers. The FTIR spectra were performed from wavenumber of 600-4000 cm^{-1}.

2.4 Scanning electron microscope (SEM)

Scanning electron microscope that can produce high resolution images at high magnification level was used to characterize the biofilm. Image produced in grayscale shows the surface morphology of the biofilms. The analytics of the dispersive X-ray spectroscopy was also studied to identify elements composition and crystallographic data. The sample was cut into 10 x 10 mm and coated with. Magnification of 100x, 300x, and 2000x were used.

2.5 Contact angle

Contact angle analysis was used to study the hydrophilicity of the CBw biofilm when beeswax was added to chitosan biofilm. Contact angle lower than 90° characteries as hydrophilic while contact angle higher than 90° as hydrophobic.

2.6 Atomic force microscopy (AFM)

The surface roughness of the CBw biofilm was analyzed by (AFM) based on the data of mean peak value to valley value (Rpv), root mean square of roughness (Rq) and mean roughness (Ra). Non-contact mode is used to scan 10 µm on X and Y-axis.

3. Results and Discussion

3.1 Fabrication of CBw biofilm

Fabrication of the CBw biofilm produced a firm and thin biofilm as in Figure 1 after a modification of the volume of glutaraldehyde and temperature during crosslinking was made. 0.3 ml was the appropriate volume to crosslinked 25 to 30 ml of CBw solution. The temperature when the solution of CBw1, CBw2, CBw3, CBw4, and CBw5 was crosslinked is high to avoid solidification of the beeswax.

Figure 1: Fabricated CBw0 biofilm

3.2 Interaction of chitosan and beeswax

FTIR experiments were performed in order to study the interaction when a chitosan film was integrated with beeswax ranging from 1 gram to 5 grams. Figure 2 displays FTIR spectroscopy of CBw0 biofilm and beeswax. The measurements for the functional group of the CBw were recorded in the range of 4000-600 cm^{-1} . The broad peak over the region of 3000-3500 cm^{-1} refers to the stretching of hydrogen bonding (OH) of chitosan [3]. Strong sharp signal at 2916.056 cm^{-1} and 2849 cm^{-1} indicates the adjoining of beeswax represent by aliphatic $-CH_2$ asymmetric and asymmetric stretching. The specific spectral range at 1063 cm^{-1} and 1027 cm^{-1} further confirms chitosan's C-O stretching vibrations.

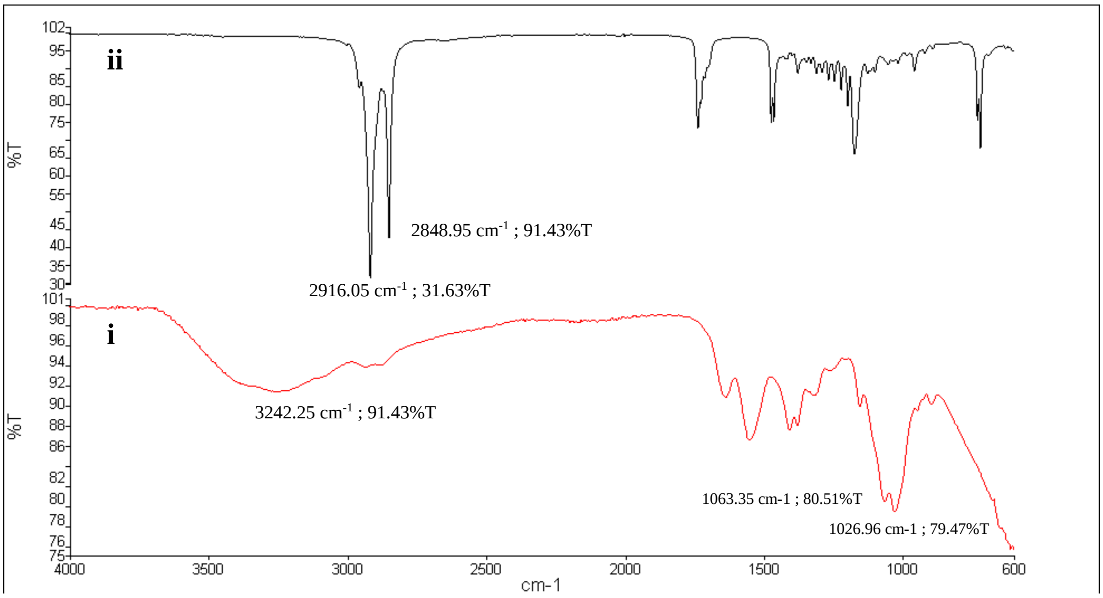

Figure 2: FTIR spectroscopy of chitosan (i) and beeswax (i)

Figure 3 shows the absorption peaks 1639 cm^{-1} and 1555 cm^{-1} are due to -C-O stretching and -OH deformation vibrations with decreasing trends when higher beeswax content was added.

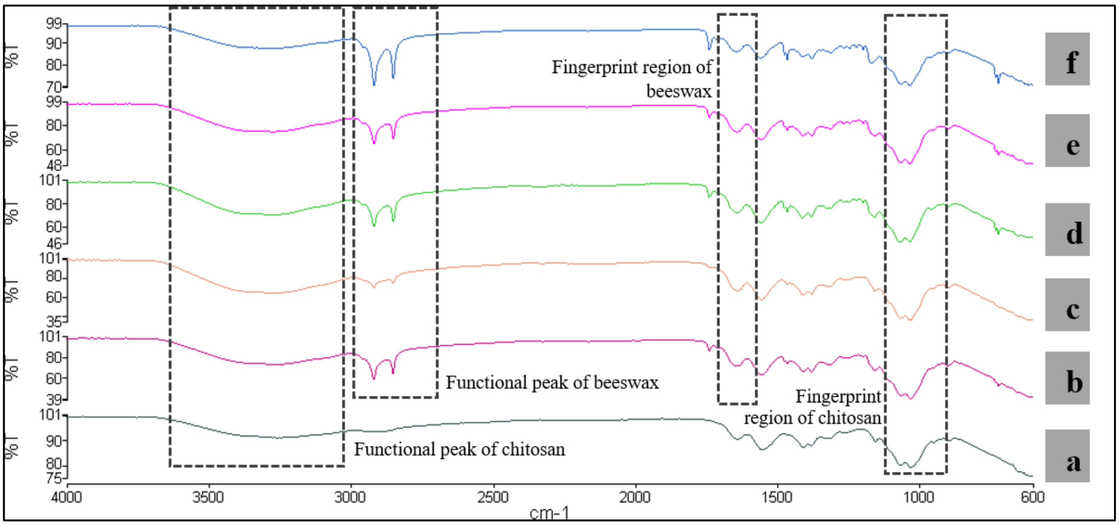

Figure 3: FTIR spectra of CBw0 (a), CBw1 (b), CBw2 (c), CBw3 (d), CBw4 (e) and CBw5 (f)

3.3 Microstructure of the composite biofilm

SEM analysis on the CBw biofilms was able to reveal its microstructure. Figure 4 shows the topography of the CBw0 under 2000x magnification and CBw1, CBw2, CBw3, CBw4, CBw5 under 300x magnification. It was found that chitosan biofilm (a) without beeswax has a smooth surface since chitosan on its on [4]. Image of CBw1 (b) showed the integrity of the biofilm was still higher when compared to CBw5 (f) where higher beeswax content showed the compact arrangement of beeswax structure on the biofilm's surface. Comparison of the element composition in the CBw biofilm that was observed through the SEM imaging is in Table 3 and Table 4 shows the increment of weight percentages of carbon within the biofilm.

Table 3: Elements composition of CBw3

Element	Weight %	Atomic %
C K	74.75	79.77
O K	25.25	20.23
Total	100	

Table 4: Elements composition of CBw5

Element	Weight %	Atomic %
C K	85.31	88.55
O K	14.69	11.45
Total	100	

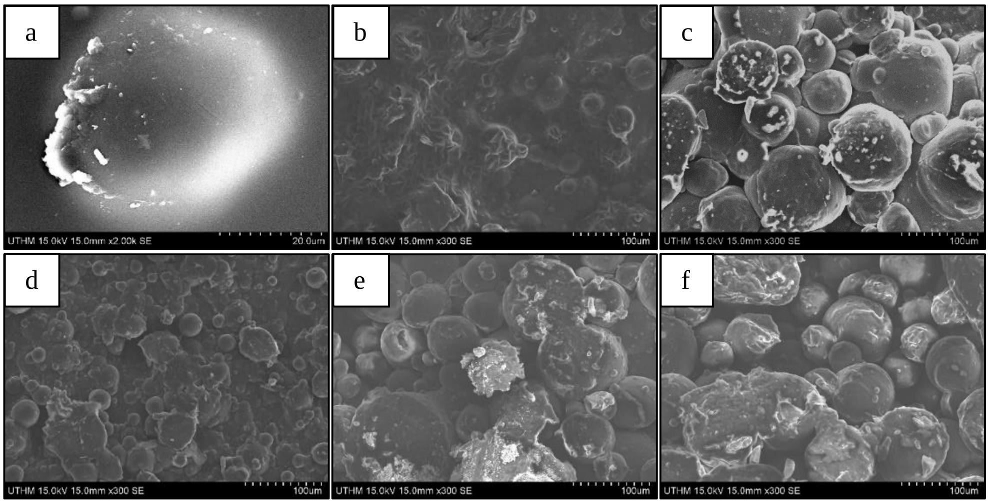

Figure 4: SEM images of CBw0 (a) at 2000x magnification and CBw1 (b), CBw2 (c), CBw3 (d), CBw4 (e) and CBw5 (f) at 300x magnification

3.4 Contact angle

The hydrophilicity of the CBw biofilm was analyzed with contact angle. Contact angle lower than 90° indicates a hydrophilic surface while higher angle means hydrophobicity. Table 5 summarized the contact angle values obtained for CBw film parameters.

Table 5: Contact angle values for CBw film parameters

Sample	Contact Angle (°)				Observation
	1st	2nd	3rd	Average	
CBw0	85.50	87.70	80.40	84.53	Hydrophilic
CBw1	115.60	117.40	121.00	118.00	Hydrophobic
CBw2	133.20	134.90	128.40	132.17	
CBw3	138.10	134.10	138.60	136.93	
CBw4	119.70	113.70	113.80	115.73	
CBw5	135.60	126.90	126.50	129.67	

Increased amount of beeswax in chitosan biofilm resulted in higher contact angle with relation to fatty acids compounds provided by the beeswax bond. But data from Table 5 showed the contact angle value for CBw4 and CBw5 had decreased fairly due to reduction of NH_3^+ group lacking bonding availability with fatty acids [5]. Figure 5 shows the contact angle measurement obtained from the CBw biofilm where all except the chitosan biofilm without beeswax (a) had lower contact angle.

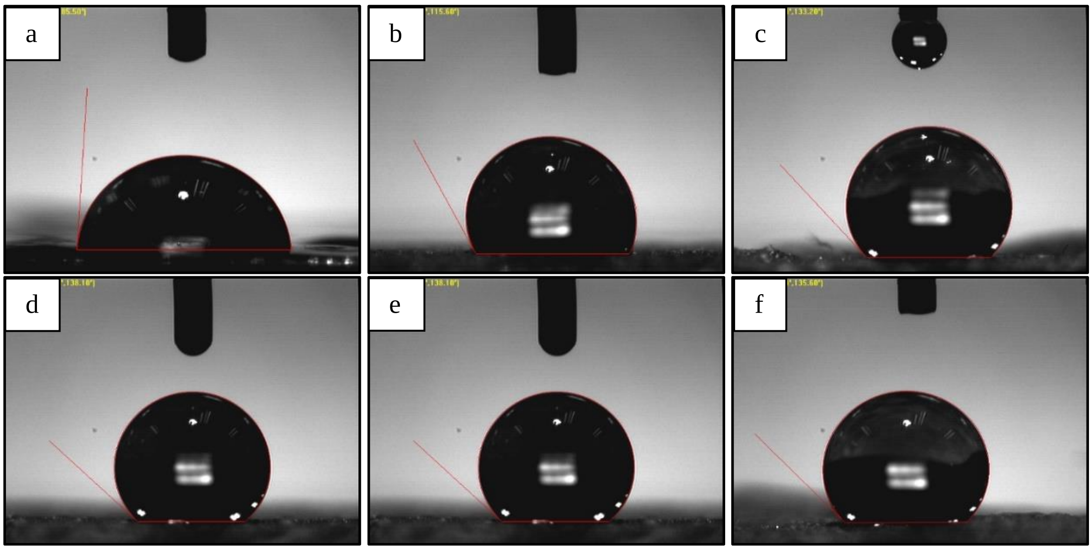

Figure 5: Contact angle measurement of CBw0 (a), CBw1 (b), CBw2 (c), CBw3 (d), CBw4 (e) and CBw5 (f)

3.4 Surface roughness

Table 6 shows the surface roughness parameters of CBw with different concentrations of beeswax. Surface roughness was studied through the comparison of the mean peak value to valley (Rpv), root mean square of roughness (Rq) and mean roughness (Ra) [6](Liu et al). The 3D

Table 6: Surface roughness parameter for CBw biofilm

Sample	Surface Roughness		
	Rpv (nm)	**Rq (nm)**	**Ra (nm)**
CBw0	105.181	5.993	3.838
CBw1	1843.182	273.052	205.463
CBw2	583.483	76.832	59.335
CBw3	1591.389	194.420	154.515
CBw4	472.488	72.240	56.586
CBw5	1197.404	137.512	102.127

Table 6 highlighted that CBw2 and CBw4 had lower surface roughness than CBw1, Cbw3, and CBw5 in that sequence. The value fluctuation for the surface roughness result can be influenced by the selection of testing area for the AFM characterization. Additional homogenization strength produced a more robust film-forming emulsion with reduced beeswax particle sizes that was less affected by destabilization phenomena during drying process, which could be the cause in the different levels of roughness of the surface [7](Zhang et al). Figure 6 showed that CBw0 biofilm has the lowest mean roughness compared to other CBw biofilm with beeswax where hills and valleys were present. This outcome was seen as a result of beeswax particles migrated to the biofilms surface after drying, which increased the surface roughness [8].

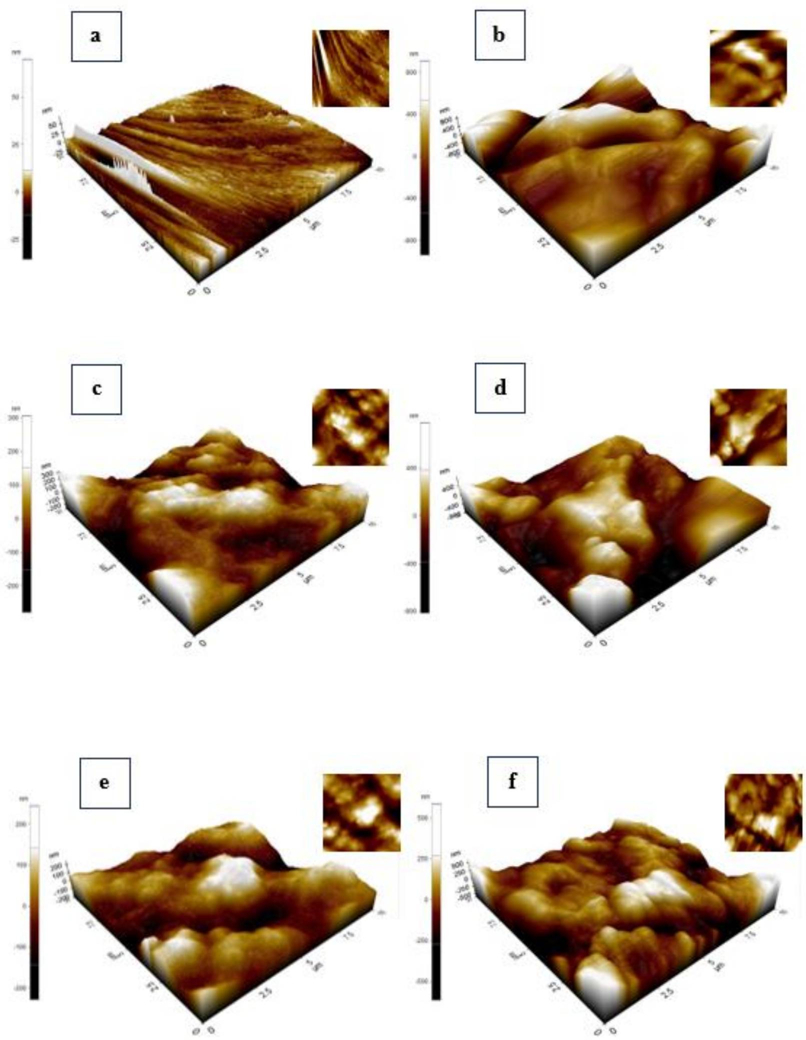

Figure 6: 3D and 2D AFM images of CBw0 (a), CBw1 (b), CBw2 (c), CBw3 (d), CBw4 (e), and CBw5 (f)

4. Conclusion

Both the fabrication of the chitosan/beeswax biofilm and the analysis of its physiochemical properties were successful. To make the solution, it was determined that 0.3 ml of glutaraldehyde was the appropriate quantity for 25 to 30 ml of CBw solution. Characterization using SEM revealed an increase in the number of crevasses, separations, and fractures. The weight percentages of the materials found in the films were analyzed, and any abnormalities that may have been caused by handling or contamination were identified. The films have rather high carbon and oxygen concentrations. The use of FTIR spectroscopy made it easier to identify the functional groups that were present in the biofilms. The samples that contained beeswax did not have any peaks at 2917 cm-1 or 2849 cm-1. The hydrophilicity of chitosan was changed as a result of the addition of beeswax to the mixture. The composition of CBw had the no obvious trends on hydrophilic properties from the amount of beeswax present in the biofilm, and this contributed to improved water retention on the surface of the biofilm. According to the findings of the AFM research, the addition of beeswax resulted to an increase in surface roughness. The CBw0 biofilm had the smoothest surface, which was comparable to chitosan.

It is recommended to use less involvement of heat when fabricating the CBw biofilm since this can affect the success of fabrication and the biofilm volume. Continuously stir the solution with a spatula during crosslinking to allow it homogenously mixed. Crosslinking involving beeswax should be done in closer time frame to avoid clumping from solidified beeswax.

Acknowledgement

The authors would also like to thank the Faculty of Mechanical and Manufacturing Engineering, Universiti Tun Hussein Onn Malaysia for its support in making this research successfully conducted.

References

[1] Singh, S., Young, A., & Mcnaught, C.-E. (n.d.). The physiology of wound healing

[2] Maharsih, I. K., Tarmidzi, F. M., Alviany, R., Aurelia, M., & Putri, S. A. (2020a). The Effect of Beeswax and Chitosan Concentrations as Superhydrophobic Coating on Wound Dressing. 58–63. https://doi.org/10.5220/0009405300580063

[3] Hromiš, N. M., Lazić, V. L., Markov, S. L., Vaštag, Ž. G., Popović, S. Z., Šuput, D. Z., Džinić, N. R., Velićanski, A. S., & Popović, L. M. (2015). Optimization of chitosan biofilm properties by addition of caraway essential oil and beeswax. Journal of Food Engineering, 158, 86–93. https://doi.org/10.1016/j.jfoodeng.2015.01.001

[4] Yu, Z., Rao, G., Wei, Y., Yu, J., Wu, S., & Fang, Y. (2019). Preparation, characterization, and antibacterial properties of biofilms comprising chitosan and ε-polylysine. International Journal of Biological Macromolecules, 141, 545–552. https://doi.org/10.1016/j.ijbiomac.2019.09.035

[5] Maharsih, I. K., Tarmidzi, F. M., Alviany, R., Aurelia, M., & Putri, S. A. (2020a). The Effect of Beeswax and Chitosan Concentrations as Superhydrophobic Coating on Wound Dressing. 58–63. https://doi.org/10.5220/0009405300580063

[6] Liu, R., Zhang, R., Zhai, X., Li, C., Hou, H., & Wang, W. (2022). Effects of beeswax emulsified by octenyl succinate starch on the structure and physicochemical properties of acid-modified starchfilms. International Journal of Biological Macromolecules, 219, 262–272. https://doi.org/10.1016/j.ijbiomac.2022.07.235

[7] Zhang, R., Wang, W., Zhang, H., Dai, Y., Dong, H., Kong, L., & Hou, H. (2020). Effects of preparation conditions on the properties of agar/maltodextrin-beeswax pseudo-bilayer films. Carbohydrate Polymers, 236. https://doi.org/10.1016/j.carbpol.2020.116029

[8] Liu, R., Zhang, R., Zhai, X., Li, C., Hou, H., & Wang, W. (2022). Effects of beeswax emulsified by octenyl succinate starch on the structure and physicochemical properties of acid-modified starchfilms. International Journal of Biological Macromolecules, 219, 262–272. https://doi.org/10.1016/j.ijbiomac.2022.07.235