RASĀYAN *J. Chem.*
110-117| Special Issue | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

EVALUATION OF CROSS-LINKED CASSAVA STARCH MICROSPHERES FOR DRUG DELIVERY MATRICES APPLICATION

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ABSTRACT

The preparation of cross-linked cassava starch microspheres (CCSM) using catalyst sodium chloride and cross-linker sodium trimetaphosphate as drug delivery matrices has been studied. The CCSM's functional groups, freeze-thaw stability, swelling power, solubility, and gelatinization capabilities have been evaluated. The morphological structure, drug loading, and particle size of the AA encapsulation were also investigated. The water solubility and swelling degree increased after the modification via cross-linking, and then the morphology exhibited bell-shaped granules and a smooth, rounded surface. The drug delivery was analyzed using three dissolution mediums: 0.1 M of HCl, buffered saline phosphate (PBS pH of 7.4), and NaCl solution (9%). The PBS solvent resulted in a much better performance in drug delivery. The loading capacity, encapsulation efficiency, and release rate after 150 min of the AA were 31.490.20%, 89.840.23%, and 81.580.10%, respectively.

Keywords: Cassava Starch, Drug Delivery, Encapsulation, Ascorbic Acid, Cross-Linked.

RASĀYAN *J. Chem.*, Special Issue, 2022

This manuscript is focusing **SDG-7:** Affordable and Clean Energy

INTRODUCTION

Starch is a polysaccharide with numerous applications in the food, pharmaceutical, and environmental industries, including food coating, bioremediation, packaging, edible film, and drug delivery.[1-4] Recently, the research topic focused on the drug delivered in the human body has been increasing growth as well as the demand and efforts to produce safer drugs. Drug-carrying matrix materials currently being developed are biopolymers, such as starch.[5] Research about drug delivery matrix from water-soluble starch has been reported by Fang *et al.* using methylene blue as a drug model, which shows the amount of drug quantity attained its highest in sodium chloride (0.9%) dissolving media at ambient temperature and drug release amount rose prominently in the first 24 hours of release.[6] Previously, we successfully modified the process of sago starch using the catalyst sodium chloride and the cross-linker STMP. It has also been reported how the cross-linker and catalyst affect the product's FTIR, swelling index, and solubilization index characterization.[7] However, the application of a drug delivery matrix has yet to be thoroughly investigated. Due to this, this research presents the findings of a thorough investigation into cross-linked cassava starch microspheres (CCSM) as the drug delivery matrix. The research objective is to evaluate the CSSM chemical and physical characteristics and their application as drug delivery systems using ascorbic acid (AA) as a drug model.

http://doi.org/10.31788/RJC.2022.1558139

EXPERIMENTAL

Material and Methods

Cassava starch was obtained from a local market (Bogor, Indonesia). Analytical grade chemicals were used in this research and had obtained from Merck KGaA (Darmstadt, Germany), except for the sodium trimeta phosphate (STMP), which was obtained from Honeywell (North Carolina, United States). A technical grade of the white oil was obtained from a local chemical manufacturer, Bratachem (Bogor, Indonesia).

General Procedure

The CCSM was modified following Fang *et al.*[6] with modification. Firstly, the water phase was made by suspension of 20 g of cross-linked cassava starch into 100 ml distilled water in a 300 ml beaker glass. Then, it was mixed continuously at 300 rpm for 30 min. Next, the oil phase was created by heating the surfactants with HLB 5.5 (Span 80: Tween 80 = 33.64:6.35, w/w) on a hotplate (Thermo Scientific Cimarec, USA) at 60°C, with constant stirring at 700 rpm. As much as 200 ml of white oil was combined progressively until the mixture became homogeneous. Following that, 10 ml of ethanol was poured gradually and then agitated for 2 h. Subsequently, the water phase solution was put into the oil phase solution dropwise so that the water phase was utterly dispersed in the oil phase. Finally, the emulsion was homogenized with a magnetic stirrer at 50 °C and 1200 rpm for 3 h.

Detection Method

Solubility in water was examined via the gravimetric technique.[8] The water solubility and swelling degree were calculated according to Equation 1 and Equation 2, respectively:

$$Water\ solubility\ (\%) = \frac{m_2 - m_4}{m_4} \quad (1)$$

$$Degree\ of\ swelling\ (\%) = \frac{m_3 - m_2}{m_2} \quad (2)$$

Freeze-thaw stability analysis following procedures in this work.[9] The particle size of starch was determined by Particle Size Analyzer (Horiba, Nano Partica SZ-100, Japan). The functional group was analyzed by Fourier transform infrared (FTIR) ATR spectrometer (Bruker, Tensor II, USA). Gelatinization analysis of microspheres starch samples was determined following Thirathumthavorn and Trisuth.[10] A scanning electron microscope (SEM JEOL, JSM-IT30, Japan) was used to examine the morphology of the starches. The method described in the works was used to determine drug loading and drug release analysis samples.[6,11]

RESULTS AND DISCUSSION

The Water Solubility, Swelling Degree, Freeze-Thaw Stability, and Particle Size

The modified cassava starch captured through this cross-linking technique transpired into starch phosphate. Table-1 shows the influence of the cross-linking preparation on the Physico-chemical characteristics of starches. Cross-linked starch had a superior water solubility and swelling degree than the native starch. Because of the covalent connections created for both the starch and the STMP, the existence of phosphate in the starch gives the cross-linked starch a significantly greater affinity for water molecules. The STMP molecules are polar covalent connections that have different electronegativity. Negatively charged cross-linked starch has a higher solubility due to the increased affinity of starch molecules toward the water molecules. Therefore, the molecules have no association or union due to the electrostatic effect that results from the charge itself.[12,13] The CCSM had more excellent freeze-thaw stability compared with the native starch due to the addition of the phosphate group, which could blend with water and create hydrogen bonding with the amylase's hydroxyl groups, prohibiting them from reforming and re-assembly.[14] The previous results found that retrogradation of non-waxy cross-linked from corn starch inhibited.[15] Cross-linking is one method of reducing starch syneresis so that the starch does not lead to retrogradation. By obstructing the starch chain with a recently added functional group, the cross-linkers prevent it from reassembling. Starch must have a high degree of freeze-thaw stability to be an effective matrix for drug delivery.[9] The water loss rates in native and cross-linked starches were 59.91% and 17.29%, respectively, after only one freeze-thaw cycle. The stability of the emulsion is significantly impacted by its production using HLB calculations. The result of HLB values for the Span 80 is only 4.3, indicating poor adherence to the water phase but strong coherence to the oil phase. The HLB value of Tween 80 was 15, which marked

an opposite trend. When the HLB values of the mixed emulsifiers reached 5.5, its adhesion in both phases attained an equilibrium. It decreased the interface stress tension between the aqueous phase and the oil phase so that the microspheres were revealed to be small particles, only 20.83769 ± 0.02 µM. Other than the HLB value, proper stirring speeds and emulsification temperature also influenced the preparation of the starch microparticles.[16]

Table-1: Physico-Chemical Characteristics of Starches

No	Sample	Solubility (%)	Swelling power (%)	Freeze-thaw stability (%)	Particle size (µm)
1	Native starch	15.65±0.28	7.99±0.08	40.09±0.01	149.15 ± 0.03
2	Cross-linked starch	26.37±0.01	19.10±0.06	82.71±0.02	20.83 ± 0.02

Functional Groups of CCSM

The binding sites attributed to the effectiveness of ionic attachment of phosphate groups on the surface of the cassava starch can be further investigated using FTIR. Several new peaks at 1260 cm^{-1} from P=O bonding and 998 cm^{-1} from P-O-C stretching were inspected in the spectrum of the treatment starch and could not be recognized in the untreated starch spectrum. It is noticed that the phosphate groups have been successfully attached to the cassava starch structure. Heo *et al.*[17] and Liu *et al.*[9] reported similar outcomes as well, who researched the modification of potato and maize starch using cross-linking techniques with sodium Tri metaphosphate and sodium tripolyphosphate as cross-linking agents, as well as with the catalysts of sodium sulfate and sodium hydroxide. The assignments of the other peaks in microspheres starch samples spectra are seen in Fig.-1. A wave number in the 3300- 3400 cm^{-1} region was associated with the hydroxyl bonds presence. The peak at 2925 cm^{-1} showed C-H bands stretching of the glucose, and the peaks at 1336–1413 cm^{-1} were defined for the C-H bending vibrations. The peak at band 1636 cm^{-1} was typical of starch spectra, as were its derivatives, defined as an H_2O bending vibration That was a result of the intramolecular hydrogen bond's existence. The highest peak at 1167 cm^{-1} corresponds to C-O stretching, while band 756 cm^{-1} belongs to the C-C stretch. The C-OH and CH_2 deformation characteristics in starch molecules were assigned to the peak of 991 and 925 cm^{-1}, respectively.[18-20]

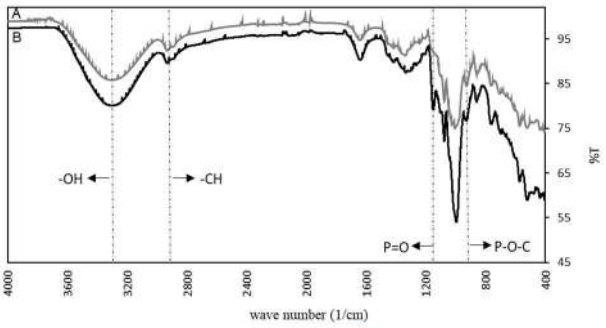

Fig.-1: The Spectra of Untreated Starch (A) and Treated Starch (B)

Gelatinization of CCSM

The parameters of gelatinization of samples produced with STMP are seen in Table-2. The transition temperatures during the gelatinization of cross-linked starch were higher than those of untreated starch. However, its enthalpy was vice versa. This suggested that the development of covalent connections in cross-linked starch tightens the molecular structure of starch molecules, hence enhancing their structural integrity. Therefore, the cross-linked starch was gelatinized at higher temperatures, requiring higher energy for its gelatinization than native starch. This was in line with Liu *et al.*, who observed the physicochemical, functional, and structural aspects of cross-linking, oxidizing, and cross-linking-oxidizing of maize starch.[9] The enthalpy value of modified maize starch with STMP increased across the three transition temperatures, but the enthalpy value decreased slightly. Carmona-Garcia *et al.* investigated banana starch's physicochemical and functional properties using several cross-linked reagent types.[21] They discovered that modification of starch-containing STMP/STPP and EPI (epoxy chloropropane) increased enthalpy value but dropped in temperature across the three transition temperatures. Chatakanonda *et al.* discovered that when the cross-linking degree with STMP/STPP grew, so did the gelatinization temperatures.[14] However,

it was found that cross-linking did not affect the gelatinization enthalpy, indicating the melting process from the cross-linked starch in the crystalline regions.

Table-2: The Thermal Characteristics of Starch Microspheres

Sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Native starch	48.18±0.1	57.77±0.2	72.99±0.0	98.62±0.2
Cross-linked starch	70.09±0.1	77.24±0.1	82.74±0.1	57.76±0.2

T_o: the onset temperatures; T_p: the peak temperatures; T_c: the conclusion gelatinization temperatures ΔH is the gelatinization enthalpy.

Surface Morphology of Native, Cross-Linked Starches, and Microsphere Formation

The surface morphology analysis of the starches using SEM illustrated the alteration of CCSM granules. The CCSM granules showed a slightly rough surface, good consistency with tight surfaces, and good dispersibility. However, certain particles congregated because of a significant van der Waals force and electrostatic attraction, while the native starch granules were polygonal with defined edges. Microscopic observations of samples are shown in Fig.-2, furthermore, Fig.-3 shows the microsphere cassava starch at a magnification of x100 and x2000. The surface morphology of the starch microsphere was detected at x100 and ×2000 magnification. According to Fig.-3, microsphere starch has a sharp edge, large starch particles, an apparent crack, a slightly blurry structure, and aberrations on the surface.[22]

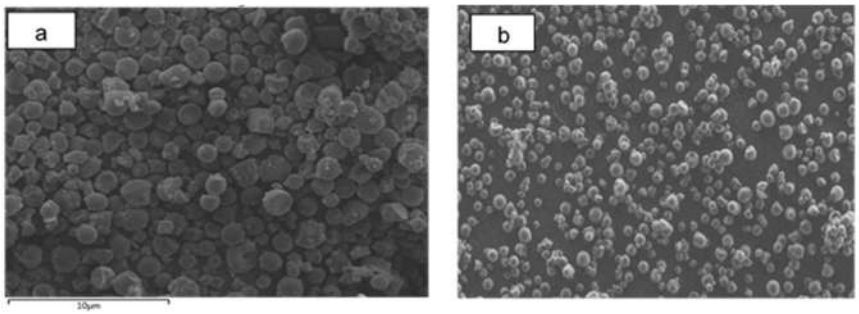

Fig.-2**:** Morphology Structure from Cross-Linked Starch (a) and Native Starch (b)

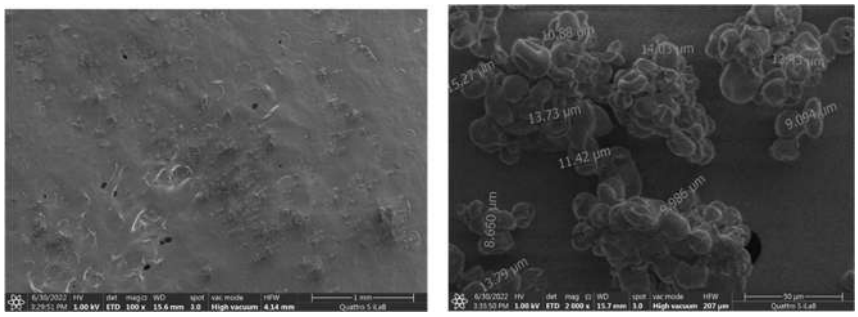

Fig.-3: Morphology Structure of Microsphere Cassava Starch at a Magnification of x100 and x2000

Drug Loading Analysis

The result of wavelengths of HCl, PBS, and NaCl were 280, 278, and 280 nm, respectively. The AA standard curves absorbance concentrations (0.002- 0.01 mg/ml) across these three solutions were, y = 0.0274x - 0.0508 (R^2 = 0.09988), y = 0.0365x - 0.0134 (R^2 = 0.9987), y = 0.0306x - 0.0212 (R^2 = 0.9957), respectively. The different dissolution mediums affected by the drug loading are shown in Table-3. Evidently, the AA's drug loading ratios and encapsulation efficiencies differed significantly depending on the dissolving media. Under the acidic solution, the microspheres were only loaded to 9.120±0.20 %, lower than that in the alkaline solution, and under neutral conditions. It is possible that the starch microspheres were more susceptible to decomposition in acidic and neutral conditions, hence decreasing their drug-carrying ability. The AA encapsulation efficiency displayed a similar trend. The encapsulation efficiency of the AA in the alkali solution (89.84±0.23%) was significantly higher than those in the acidic solution (67.94±0.24%) and the neutral solution (69.89±0.32 %). This likely occurred because the starch

microspheres tended to degrade under acidic conditions, and consequently, the drug loading capacity decreased. The lower release of AA in the neutral solution is probably caused by salt ions present in the release media. Salt ions reduce the system's osmotic pressure, which makes microspheres less likely to swell. The results of our study were supported by that research previously by Bajpai, and Bhanu [23] investigated the heparin excretion from cross-linked starch microspheres. They found that the release of heparin gradually rose in the PBS medium compared to the other dissolution mediums.

Table-3: Influence of Solvent Type on AA Loading and Efficiency of Encapsulation

Dissolution medium	AA loading ratio (%)	Encapsulation efficiency (%)
PBS	31.49±0.20	89.84±0.23
HCl	9.12±0.20	67.94±0.24
NaCl	14.53±0.16	69.89±0.32

Figure-4 showed the effect of drug loading temperature, indicating that temperature fluctuations influenced the drug loading of AA substantially for all mediums (0.1M HCl, 9% NaCl, and Phosphate Buffered Saline (pH 7.4) showed that microspheres loaded with more AA at a temperature of 25°C than at higher temperatures. The explanation was that AA's sorption was primarily due to opposing charges with a high affinity. As a result of a large amount of heat created during this process, which high temperatures will hamper, the drug loading ratio at 50°C decreased from 36% to that at 25°C. The affinity between microspheres and AA was decreased further at low temperatures, the drug loading ratio at 6°C was only 10% of that at 25°C, and encapsulation efficiency decreased from 89.84% to 19,83% for PBS medium.

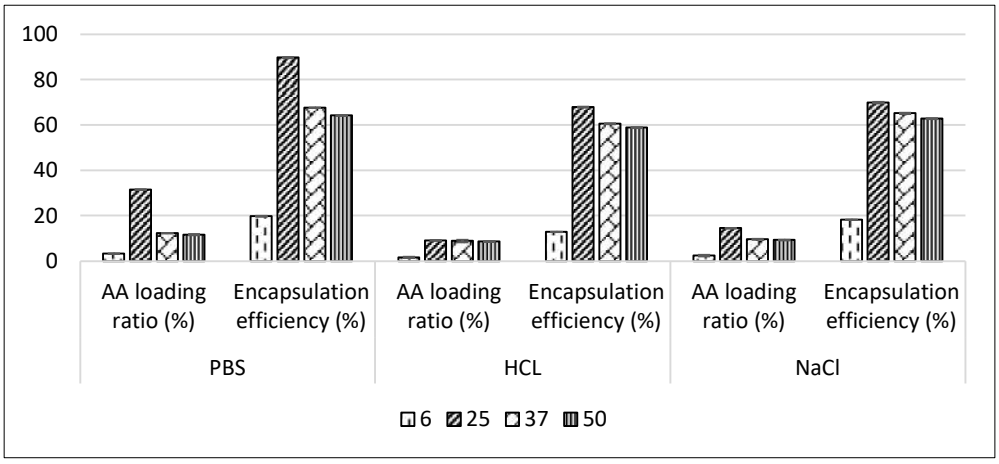

Fig.-4: Effect of Loading Temperature on AA Loading at PBS, HCl, and NaCl Mediums

Figure-5 illustrates how the duration of loading affects the ratio of drug loading and efficiency of encapsulation in HCl, NaCl, and PBS media. It demonstrates that as the loading duration increased, the drug loading ratio was boosted and AA encapsulation efficiency considerably increased. The time variation (1.0h-1.5h) and the drug loading ratio climbed progressively from 1.71±0.10% to 12.29±0.14%, with AA encapsulation efficiency increasing from 11.66±0.15 to 68.54±0.15% for PBS medium. The maximum AA loading ratio of 8.85±0.10% was achieved for the HCl medium with an encapsulation efficiency of 60.64±0.22% at 1.5 h. Also, in the present investigation, the maximum AA loading encapsulation efficiency for the cassava cross-linked starch at the same optimal condition was obtained as 65.22±0.28% in the NaCl medium. However, at 2 and 2.5 h, there was a decrease in drug loading and efficiency of encapsulation. This occurs in all dissolution mediums. The research showed that drug loading and encapsulation efficiency are optimal at 1.5 h and decrease again after 1.5 h. The AA encapsulation process probably influences this by starch microspheres. The interaction between the drug and starch microparticles is optimal at 1.5 h. The functional group in the starch phosphate will present more van der Waals bonds and hydrogen bonding so that the drug will be easily encapsulated into starch microparticles, causing the value of drug loading and efficiency encapsulation will increase. According to previous researchers, the factors that affect drug

loading and encapsulation efficiency are the interaction between the drug and starch microparticles, starch microparticle concentration, and surfactant concentration.[16]

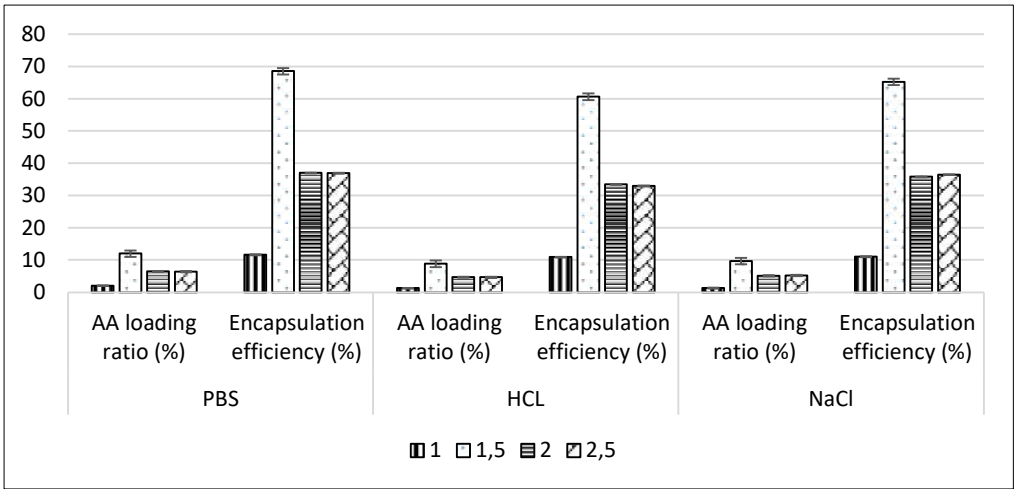

Fig.-5: Effect of Loading Time on AA Loading at PBS, HCl, and NaCl Mediums

Drug Release Study

In order to evaluate the state of the medium during the release kinetics of the AA, release experiments used several physiological fluids. The release profile of the CCSM is presented in Fig.-6. The cross-linked starch microspheres in drug release exhibited a gradual plateauing during the release. It was clear that the medication release amount rose dramatically throughout 150 min. Following the starch microspheres' immersion in the release media, a steady release was initially noticed in the first 60 minutes. The fast dispersal of the AA, which was positioned close to the surface of the starch microsphere, was related to a high release rate of 18.73±0.10%in in the alkaline medium. The cross-linked starch microspheres formed the mechanism in swelling properties and continued release after 120 minutes. During this time, the rate of the release tailed off and 60.890.14% of AA composed in the starch microspheres was disclosed. As the swelling progressed, several AA molecules were distributed and released into the media via channels and pores of the microspheres of starch. Nevertheless, a gel diffusion layer formed as the microspheres grew in the physiological fluid, blocking AA release and causing a persistent slowdown in AA release.[23] As the gel diffusion layer thickened, the microsphere pores and channels for water transport shrunk significantly, causing less AA to be pushed out of the microspheres. The starch particles degraded with time but at a slow rate. Bajpai and Bhanu[23] discovered that the drug's percent loading significantly impacted the medication's release profile. The drug's release rate will increase as the drug loading ratio rises. The cumulative drug release in the alkaline solution (81.58±0.10%) was the highest at 79.02±0.08% and 74.02±0.10%, respectively, achieved in the acidic and neutral solutions.[24]

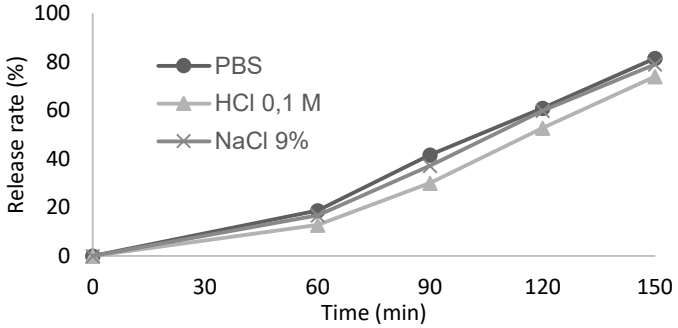

Fig.-6: Drug Release Profile of AA From Cross-Linked Starch Microspheres in Dissolution Medium

CONCLUSION

The CCSM was successfully prepared from the cassava starch using sodium trimethaphosphateas, a cross-linking agent, through an emulsification cross-linking reaction. This is evident from the observed FTIR

absorption. In addition, it was discovered that the dissolution media had a substantial impact on the loading ratio of the AA from the perspective of the starch microspheres' ability to hold drugs. In this study, the PBS medium had a maximum loading ratio of 31.49±0.20%, with an encapsulation efficiency of 89.84±0.53%.

ACKNOWLEDGMENTS

The authors thanked The Ministry of Research, Technology and Higher Education/ BRIN and LPDP grant for research support via the National Research Program in 2020. The authors also acknowledge BRIN's Integrated Laboratory of Bioproducts (iLaB) for the research facilities.

CONFLICT OF INTERESTS

The authors declare that no conflicts of interest exist.

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. S. Fauzia, H. Aziz, D. Dahlan and R. Zein, *Rasayan Journal of Chemistry*, **12(4)**, 1889(2019), http://dx.doi.org/10.31788/RJC.2019.1245444
2. Chairul Amni, Ismet, Sri Aprilia, Mariana and Said Ali Akbar, *Rasayan Journal of Chemistry*, **13(1)**, 275(2020), http://dx.doi.org/10.31788/RJC.2020.1315492
3. D.A. Tanjung, N. Jamarun, S. Arief, H. Aziz, A.H. Ritonga and B. Isfa, *Rasayan Journal of Chemistry*, **14(4),** 24519(2021), http://doi.org/10.31788/RJC.2021.1446563
4. M. Hasan, R.F.I. Rahmayani, W.R. Hazi and Rusman, *Rasayan Journal of Chemistry*, **15(4)**, 2534(2021), http://doi.org/10.31788/RJC.2022.1547001
5. A.D.N. Lestari, D. Siswanta, and R. M. Mudasir, *Rasayan Journal of Chemistry*, **14(3)**, 1729(2021), http://doi.org/10.31788/RJC.2021.1436217
6. Y.Y. Fang, L.J. Wang, D. Li, BZ Li, B. Bhandari and X. D. Chen, *Carbohydrate Polymers,* **74**, 379(2008), http://doi.org/10.1016/j.carbopol.2008.03.005
7. D. Sondari, W.K. Restu, A.A. Septevani, R. Suryaningrum, D. Burhani, B.A. Widyaningrum, and R. Putri, *Starch – Stärke,* **2000266**, 1(2022), http://doi.org/10.1002/star.202000266
8. S. Senanayake, A. Gunaratne, K. Ranaweera and A. Bamunuarachchi, *ISRN Agronomy*, 1(2013), http://doi.org/10.1155/2013/502457
9. J. Liu, B. Wang, L. Lin, J. Zhang, W. Liu, J. Xie and Y. Ding, *Food Hydrocolloids*, **36**, 45(2014), https://doi.org/10.1016/j.foodhyd.2013.08.013

10. D. Thirathumthavorn and T. Truth, *International Journal of Food Properties,* **11(4)**, 858(2008), https://doi.org/10.1080/10942910701659567
11. M. Salkić and A. Selimović, *Croatica Chemica Acta,* **88(1)**, 73(2015), https://doi.org/10.5562/cca2551
12. M. Q. Guo, X. Hu, C. Wang and L. Ai, 2017, Polysaccharides: Structure and Solubility. Solubility of Polysaccharides, https://doi.org/10.5772/intechopen.71570
13. R. Wongsagonsup, T. Pujchakarn, S. Jitrakbumrung, W. Chaiwat, Fuongfuchat, S. Asira Varavinit and S. Dangtip, *Carbohydrate Polymers,* **101**, 656(2014), https://doi.org/10.1016/j.carbopol.2013.09.100
14. P. Chatakanonda, S. Varavinit and P. Chinachoti, *Food Science and Technology,* **33**, 276(2000), https://doi.org/10.1006/fstl.2000.0662
15. F. Han, M. Liu, H. Gong, S. Lü, B. Ni and B. Zhang, *International Journal of Biological Macromolecules*, ***50*(4)**, 1026(2012) https://doi.org/10.1016/j.ijbiomac.2012.02.030.
16. G. Subedi, A. K. Shrestha and S. Shakya, O*pen Pharmaceutical Sciences Journal*, **3(1)**, (2006), https://doi.org/10.2174/1874844901603010182
17. H. Heo, Y. Lee and Y. H. Chang, *Emirates Journal of Food and Agriculture*, **29(6)**, 463(2017), https://doi.org/10.9755/ejfa.2017-01-237
18. B. Zhang, D. Cui, M. Liu, H. Gong, Y. Huang and F. Han, *International Journal of Biological Macromolecule,* **50(1)**, 250(2012), http://dx.doi.org/10.1016/j.ijbiomac. 2011.11.002.
19. F. Gao, D. Li, C. Bi, Z. Mao and B. Adhikari, *Carbohydrate Polymers,* **103**, 310(2014). http://dx.doi.org/10.1016/j.carbpol.2013.12.028
20. D. M. Suflet, A. Nicolescu, I. Popescu and G. C. Chitanu, *Carbohydrate Polymers,* **84(3),** 1176 (2011), http://dx.doi.org/10.1016/j.carbpol. 2011.01.010
21. R. Carmona-Garcia, M. M. Sanchez-Rivera, G. Méndez-Montealvo, B. Garza-Montoya and L. A. Bello-Pérez, *Carbohydrate Polymers,* **76(1)**, 117(2009), http://dx.doi.org/10.1016/j.carbpol.2008.09.029.
22. N. L. Garcia, L. Ribba, A. Dufresne, M. I. Aranguren and S. Goyanes, *Macromolecule Material Engineering,* **294(3)**, 169(2009), https://doi.org/10.1002/mame.200800271
23. A. K. Bajpai and S. Bhanu, *Journal of Material Science: Material in Medicine,* **18(8),** 161(2007), https://doi.org/10.1007/s10856-007-3020-y
24. R. C. Mundargi, N.B. Shelke, A. P. Rokhade, S.A. Patil, T.M. Aminabhavi, *Carbohydrate Polymers*, **71,** 42(2008), https://doi.org/10.1016/j.carbopol.2007.05.013

[RJC-8139/2022]