Dual-shell microencapsulation enhances nucleopolyhedrovirus stability and insecticidal activity

B. H. Zhang [b], Y. Y. Zhan [a], M. H. Gao [a], Z. H. Jia [a], C. Y. Li [a], D. Chu [a], Q. Li [b], G. L. Zheng [a,*]

[a] *College of Plant Health and Medicine，Qingdao Agricultural University, Qingdao 266109, China*

[b] *College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, China*

Insect viruses are a type of environmentally friendly and safe microbial insecticide. However, they are prone to deactivation in the environment due to exposure to light. In this study, to enhance the insecticidal activity and UV resistance of the *Spodoptera exigua nucleopolyhedrovirus* (SeNPV), chitosan and polydopamine were selected as embedding materials, A chitosan-dopamine SeNPV (CS / PDA-NPV) microcapsule was successfully developed, and characterized using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and Zeta potential analysis. The results show that CS / PDA-NPV microcapsule exhibit a well spherical morphology with a particle size range of 3-5μm. Compared to free SeNPV viruses, CS / PDA-NPV microcapsule exhibit enhanced thermal stability. After UV treatment, the insecticidal activity of the virus microcapsule was 1.5 times higher than that of the non-encapsulated viruses, demonstrating that the chitosan-polydopamine bilayer structure provides effective UV shielding for SeNPV.

(Received April 3, 2025; Accepted July 17, 2025)

Keywords: SeNPV, Polydopamine, Chitosan, Ultraviolet resistance

1. Introduction

Currently, excessive use of chemical pesticides in agriculture is causing environmental pollution and ecological imbalance, such as the energence of resistance, resurgence, and residue issues of pest [1]. Increasing global attention is being paid to biopesticides because of their eco-friendliness, effectiveness, and minimal toxicity [2]. Insect viruses constitute a remarkable category of biological insecticides possessing numerous advantages such as remarkable specificity, outstanding pest control effectiveness, safety towards plants and vertebrates, and the capability to trigger disease outbreaks. Nevertheless, insect viruses inadequate UV tolerance and delayed action have restricted their use in agriculture [3-4]. Microencapsulation emerges as a viable approach to offer ultraviolet shielding for viruses agents. The microencapsulation technique employs a core-shell architecture to safeguard the active ingredients inside the capsule, thus improving the stability and prolonged effectiveness of the core substances. Consequently, microencapsulation

* Corresponding author: glzheng@qau.edu.cn
https://doi.org/10.15251/DJNB.2025.203.823

technology is widely applied, particularly in biopesticide formulation studies, where it is frequently employed to encapsulate bacterial, fungal, and viral agents, thus improving the formulations' storage stability and extending their shelf life [5-7].

Polydopamine (PDA) is a polymeric compound synthesized via dopamine self-polymerization under weakly alkaline conditions [8-9]. The presence of catechol and amino groups in dopamine allows for self-oxidative polymerization on diverse solid surfaces, forming polydopamine coatings or microcapsular architectures [10-11]. These coatings, rich in phenolic hydroxyl and amino/imino functional groups, remarkably improve surface adhesion characteristics and ultraviolet protection capabilities [12-13]. Chitosan arises from the removal of the N-acetyl group from chitin through treatment with concentrated alkali [14]. It stands as the second polysaccharide after cellulose among natural organic polymers, underscores the widespread applications of chitosan. As a cationic polysaccharide macromolecule, chitosan can be crosslinked with sodium tripolyphosphate to form a gel, making it an effective drug carrier [15].

Spodoptera exigua nucleopolyhedrovirus (SeNPV) is a highly pathogenic, host-specific alpha baculovirus that has been utilized as a biological insecticide to manage the population of S. exigua (*Lepidoptera: Noctuidae*) [16-18]. The preparation of SeNPV effectively suppresses the population of S. exigua, playing a pivotal role in biological control. It holds significant economic, ecological, and environmental value, presenting a vast application prospect. Nevertheless, SeNPV is susceptible to light and temperature, potentially losing its biological activity [19].

In this study, in order to improve the anti UV performance of the virus, chitosan and polydopamine were selected as the encapsulation materials, and the SeNPV microcapsules were prepared by electrostatic adsorption assembly method. The morphology and structure of SeNPV microcapsules were characterized, the insecticial activity and anti-UV performance were evaluated. Results from this study provide reference for developing novel stable virus formulation to ensure sustainable agriculture.

2. Experimental

2.1. Experimental reagents and instruments

The third instar larvae of *Spodoptera exigua* and *Spodoptera exigua Nuclear Polyhedrosis Virus* (SeNPV) were provided by the College of Plant Medicine at Qingdao Agricultural University; Dopamine hydrochloride, with a mass fraction of 98%, was sourced from Shanghai Aladdin Biochemical Technology Co., Ltd; Chitosan (CS, deacylation degree 80%-90%, MW≥100,000 g·mol-1) were purchased from Sinopharm Chemical, Reagent Co. Ltd. (Beijing, China); Other reagents, of AR grade, were commercially available domestically.

2.2 Method

2.2.1. Virus amplification and extraction

The 3rd instar *Spodoptera exigua* larvae were fed with the SeNPV using the feed mixed virus method. Five days later, the infected larvae were ground and extracted with 1% sodium dodecyl sulfonate (SDS), followed by centrifugation at 8000 rpm for 10 minutes to obtain the virus.

2.2.2. Preparation of polydopamine virus microcapsule (P-N)

P-N microcapsule were prepared by referencing our previous works[5,20].briefly, the SeNPV suspension with concentration of 1×10^6 PIB/mL were added into Tris-HCl buffer (10 mmol/L, pH=8.5).Then, 0.2g dopamine (DA) was added into above mixture suspension and stirred in the dark for 12 h and centrifuged at 11,000 rpm for 10 min, wash three times with sterile water, and freeze-dried to obtain polydopamine virus particles SeNPV@PDA (P-N). Polydopamine (PDA) was prepared by the same method without adding virus.

2.2.3. Preparation of chitosan-polydopamine virus microcapsule (C/P-N)

First, 5 mL of chitosan (CS) solution (prepared in 1% acetic acid with a mass concentration of 2 mg/mL) was added into 5 mL of SeNPV@PDA (P-N) suspension and stirred continuously for 6 h. Subsequently, 2.5 mL of 0.5% sodium tripolyphosphate (TPP) was slowly introduced to crosslink the chitosan under constant stirring for 30 minutes. The resulting mixture was centrifuged at 11,000 rpm for 15 min and washed three times with distilled water to obtain the final product, designated as chitosan-polydopamine encapsulated viral particles SeNPV@CS-P (C/P-N).

2.2.4. Characterization

The morphology and structure of samples were observed by FT-IR (IR200 Fourier Transform Infrared Spectrometer, Nicolet, USA), Zeta Sizer Nano instrument (ZEN3700, Malvern Panalytical Ltd), scanning electron microscope (JEOL7500F, JEOL), differential scanning calorimetry analyzer (DSC-60, Shimadzu (Shanghai) Experimental Equipment Co., Ltd.), and thermogravimetric analyzer (TGADiscoverySDT650, TA Instruments Menu, USA). The contact angle measured by using contact angle tester.

2.2.5. Activity and anti-UV evaluation

The insecticidal activity of virus microcapsules was determined according to our previously established methodology [5]. Briefly, suspensions of free virus and virus microcapsules (C/P-N) at identical concentrations were evenly coated onto feed surfaces. Ten third-instar larvae of *Spodoptera exigua* (beet armyworm) were introduced to each treated feed sample, and their mortality rates of 24 h were recorded to evaluate bioactivity. To assess the UV resistance of the microcapsules, both free virus and virus microcapsule (C/P-N) suspensions were exposed to ultraviolet irradiation (3W, 254 nm) for 2 hours. Following this treatment, the residual insecticidal activity of the irradiated samples was analyzed using the aforementioned bioassay method. Three replicates were used for each treament concentration to ensure the accuracy of the experiments.

3. Results and discussion

3.1. Characterization

3.1.1. Particle and zeta potential of microcapsule

It can be seen from Fig.1 that the particle size of microcapsule increases gradually after embedding of polydopamine and chitosan in turn. However, the particle size is still below 5 μ m, which is within the particle size requirements of microcapsule suspension agent, and can be processed into microcapsule suspension agent later. The zeta potential analysis demonstrated that the surface charge of native virus shifted from -36.6 mV to +18.2 mV after polydopamine coating, and subsequently to +45.75 mV following chitosan encapsulation. This stepwise charge reversal

provides conclusive evidence for the successful sequential incorporation of polydopamine and chitosan into the microcapsules.

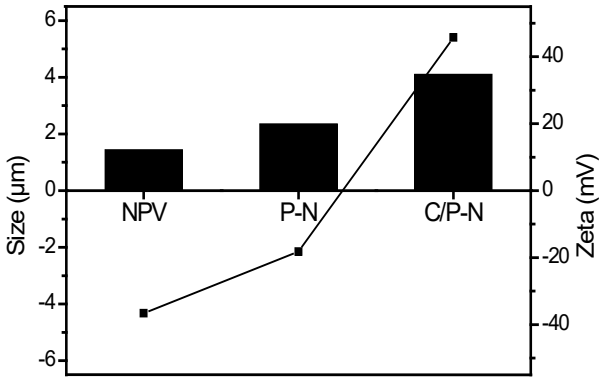

Fig. 1. Particle size and potential of SeNPV, P-N and C/P-N.

3.1.2. SEM analysis

Fig.2 is the SEM images of free virus, P-N and C/P-N microcapsules. The SEM of the virus reveals that its surface is smooth and exhibited a regular polyhedral shape. The SEM images of P-N indicated that polydopamine polymerization formed an adherent coating on the viral surface, resulting in significant surface roughening (fig.2 b). Subsequent chitosan crosslinking of the P-N composite (C/P-N) produced a comparatively smoother surface morphology (fig.2 c), though particle aggregation became evident due to polysaccharide chain interactions.

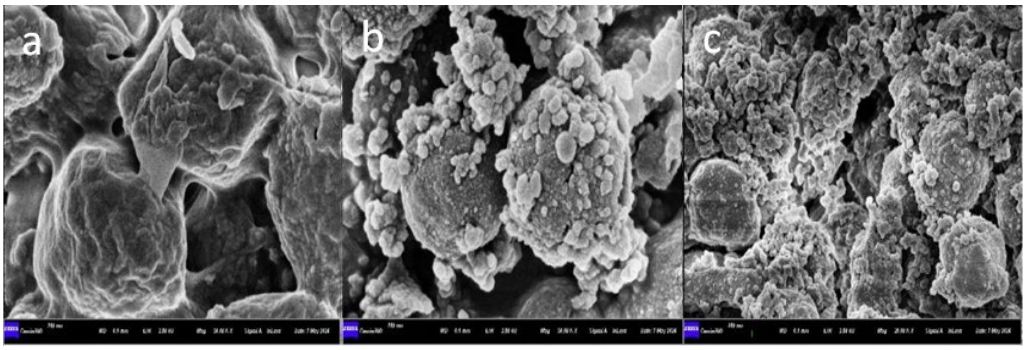

Fig. 2. SEM images of SeNPV, P-N and C/P-N.

3.1.3. FTIR analysis

The FTIR spectra of CS, PDA, C/P-N, and P-N are presented in Figure 3. Since chitosan (CS) and polydopamine (PDA) share structural similarities with polysaccharides and proteins, their FTIR spectra exhibits multiple characteristic peaks corresponding to similar functional groups. The abundance of hydroxyl and amino groups of CS and PDA results in broad, overlapping peaks in the 3000–3500 cm^{-1} region. Compared with P-N, C/P-N shows a broader absorption peak, suggesting the formation of additional hydrogen bonds. In both the CS and C/P-N spectra, the peaks at 1651 cm^{-1} and 1600 cm^{-1} are attributed to the C=O stretching vibration of amides and the N–H bending vibration, respectively. The absorption peak at 883 cm^{-1} is attributed

to the β-configuration glycosidic bond in chitosan (CS). These findings confirm the presence of chitosan in the C/P-N microcapsules, supporting the conclusion that chitosan constitutes the shell structure of the microcapsules.

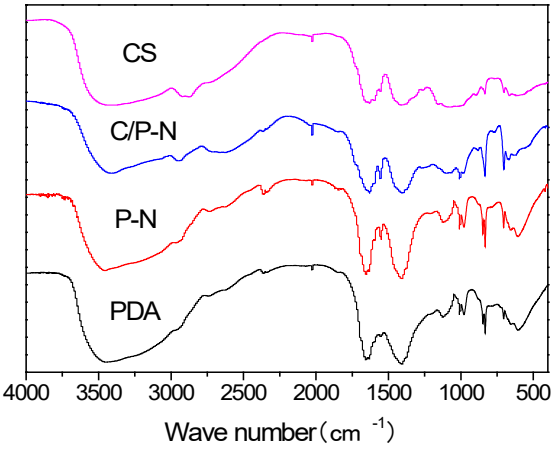

Fig. 3. Infrared image of PDA, P-N, C/P-Nand CS.

3.1.4. DSC and TGA analysis

Through DSC testing, the change in enthalpy with temperature can be obtained. Due to the different structures of substances, the change in enthalpy also varies to some extent. Therefore, by comparing the DSC curves of different samples, we can qualitatively analyze whether there is interaction between sample components and the strength of the interaction [21]. From Figure 4a, it can be seen that compared with the CS, NPV, and P-N curves, C/P-N has an endothermic peak at around 232 ℃, which is due to the melting of the capsule wall material and the removal of bound water in the microcapsules.

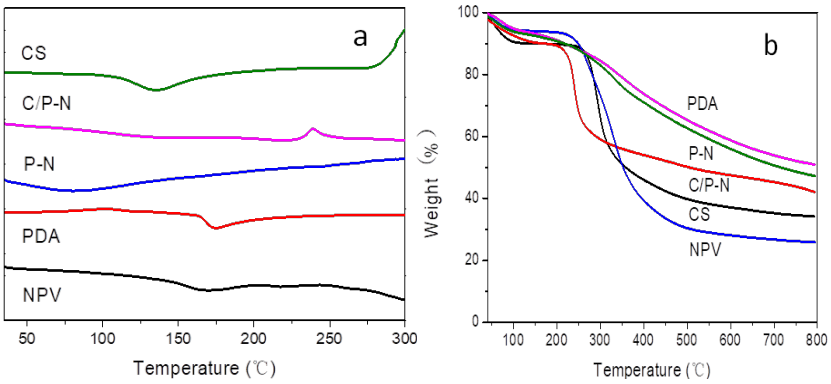

Fig. 4. Cs, C/P-N, P-N, PDA, NPV: DSC(a), TGA(b).

From the TGA curves, SeNPV underwent a drastic thermal decomposition in the range 200- 400 ℃ under air flow (fig.4 b), and the mass change was 73.67%. While the weight loss rate of P-N and C/P-N microcapsule were only 56.47% and 42.24%, respectively. Although the thermal stability of Microcapsule C/P-N was not as good as Microcapsule P-N, the weight loss rate of Microcapsule C/P-N was still higher than that of chitosan and virus. This indicates that insect viruses have improved thermal stability after embedding with polydopamine and chitosan.

3.2. Contact angle and retention analysis

The wetting performance and retention of pesticide solution on crop leaves can affect the utilization rate and control effect [22]. The contact angle of pesticide droplets was obtained by dropping viruses and virus microcapsule suspension with the same virus concentration onto the surface of cabbage mesophyll (fig.5a). The contact angle of the SeNPV suspension was higher than that of pure water, while the contact angle of the P-N suspension was significantly lower than that of the uncoated viral suspension. This indicates that the polydopamine coating enhanced the hydrophilicity of the virus and reduced the surface tension of the suspension. The contact angle of viruses co-embedded with polydopamine and chitosan (C/P-N) increased but remained lower than that of the viral suspension. Combined with the retention volume of different formulations on cabbage leaves (fig.5b), C/P-N exhibited the highest retention, suggesting that the adhesive properties of chitosan improved the persistence of the virus on the leaf surface.

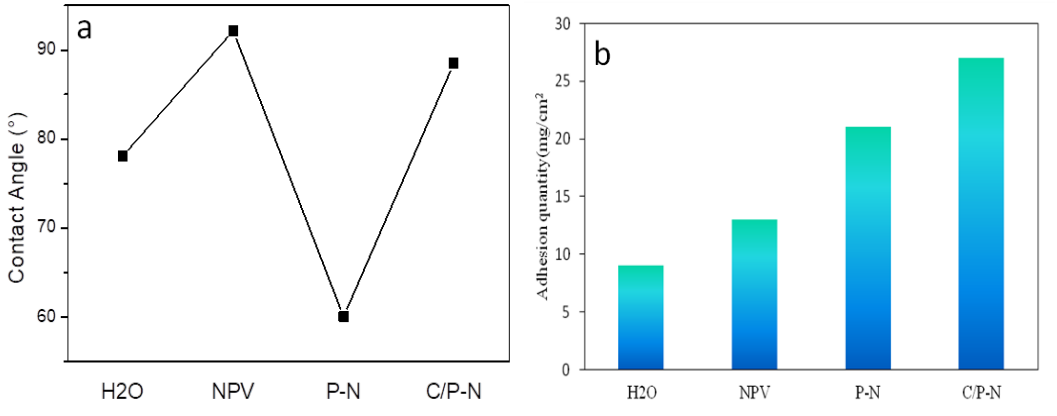

Fig. 5. Contact angle and retention of H2O, NPV, P-N and C/P-N on leaf surfaces.

3.3. Bioactivity and UV resistance properties

The bioactivity and UV resistance of SeNPV and C/P-N microcapsule were showed in the Fig. 6. It can be seen from Fig. 6a that the insecticidal activity of SeNPV and C/P-N microcapsule against Spodoptera exigua larvae in the previous two days was not significantly different. From the third day, the survival rate of SeNPV and C/P-N microcapsule decreased. On the fourth day, the survival rate caused by the C/P-N microcapsule changed the most, was about 1.25 times that of the virus, because chitosan and PDA improved the insecticidal activity of the virus.

The residual biological activities of both native SeNPV and C/P-N formulations after 2 h of UV-C irradiation (254 nm, 5W) were compared in Figure 7b. The biological activity of each treatment group against Spodoptera exigua larvae demonstrated differential temporal variations

following UV irradiation. By day 4 post-treatment, the C/P-N microcapsule exhibited significantly higher insecticidal activity compared to the non-encapsulated viral treatment, with a 1.5-fold difference in efficacy. These results confirmed that the microencapsulation process effectively protects virus activity from UV degradation, thereby maintaining the insecticidal potency of the formulation.

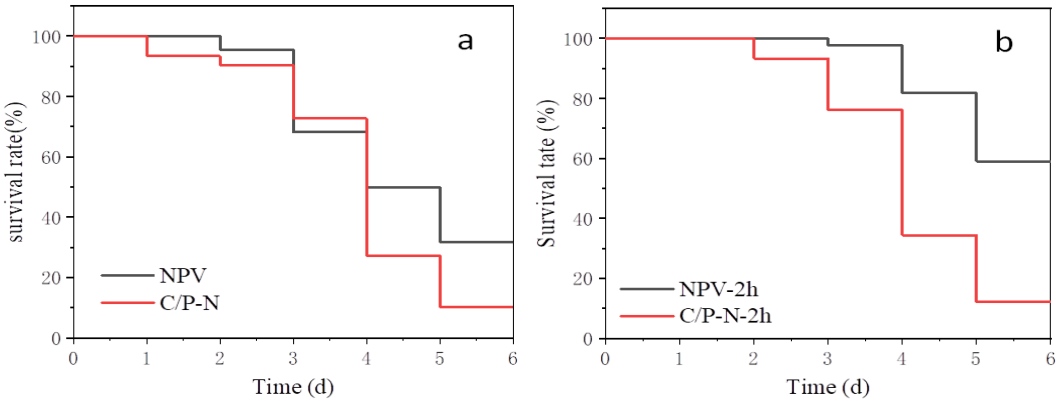

Fig. 6. Activity evaluation of SeNPV and C/P-N.

4. Conclusion

In this study, we successfully developed a dual-shell viral microcapsule system using polydopamine and chitosan. Compared to non-encapsulated viruses, the C/P-N microcapsule exhibited superior thermal stability and UV resistance, along with significantly improved retention on plant leaves, thereby ensuring high insecticidal activity. These findings provide valuable insights for developing highly efficient and stable insect-virus-based formulations.

Acknowledgments

This work was supported by the Shandong Province Science and technology-based maximum innovation capacity improvement project (2022TSGC1311), the Key R&D Plan of Shandong Province (2022CXGC020709), the Science and Technology Benefiting the People Demonstration Project of Qingdao (24-1-8-xdny-10- nsh), and the National Key R&D program of China (2022YFD2300100).

References

[1] C. Zeng, L. Wu, Y. Zhao, Y. Yun, Y Peng, PeerJ. 6, No. e4534(2018); https://doi.org/10.7717/peerj.4534

[2] A. Abbas, F. Ullah, M. Hafeez, X. Han, M.Z. Dara, H Gul, C.R. Zhao, Agron J. 12(11):2704 (2022); https://doi.org/10.3390/agronomy12112704

[3] L. A. Lacey, S. P. Arthurs, Journal of Invertebrate Pathology, 90(2): 85 (2005); https://doi.org/10.1016/j.jip.2005.07.002

[4] A. Gifani, R.Marzban, A.Safekordi, M. Ardjmand, A.Dezianian, Biocontrol Science and

Technology 25(7), 8147(2015); https://doi.org/10.1080/09583157.2015.1018814

[5] G.Y. Yan, H.M.Wang, J.J. Lv, C.Y. Li, B.H. Zhang, Pest Management Science. 78: 456(2021); https://doi.org/10.1002/ps.6640

[6] X.H. Pan, F. Cao, X.P. Guo, Y.L. Wang, Z.Q. Cui, T.P. Huang, Y.M. Hou, X. Guan,J. Agric. Food Chem. 72, 7807(2024); https://doi.org/10.1021/acs.jafc.4c00833

[7] Y. F. Liu, M. H. Gao, J. X. Liu, Y. Y. Zhan, Z. W. Gu, C. Y. Li, H. Y. Zhuc, F.Y. Du a, B. H. Zhang, Digest Journal of Nanomaterials and Biostructures 19(1), 53(2024); https://doi.org/10.15251/DJNB.2024.191.53

[8] M.Kohri, K. Yanagimoto, A. Kawamura, H.Kosuke, I.Yoshihiko, W.Takaichi, O.Tsutomu, T.Tatsuo , K.Keiki, ACS Applied Materials & Interfaces 7b-3453b (2017)

[9] A.H.Zou, Y. Yang, J.G.Cheng, V. M. Garamus, N. Li, Journal of Agricultural and Food Chemistry 66,6262 (2018); https://doi.org/10.1021/acs.jafc.8b00877

[10] B. Zhu, J.D.Liu, J.T. Wang, M. Liu, Z.K. Yan, F.Shi, J.H. Li, Y.F. Li, Journal of Membrane Science 13,9 (2019)

[11] C.X. Tang, Y.Z. Li, J. Pun, A.S.M.Osman , K. C. Tam, Colloids and Surfaces a: Physicochemical and Engineering Aspects 570,403 (2019); https://doi.org/10.1016/j.colsurfa.2019.03.049

[12] H.X.Zhang, Q.T.Hu, X.W.Zheng, Y.h.Yin, H.Wu, Z.y.Jiang, Journal of Membrane Science 33,18 (2019)

[13] Y. Wei, N.Wang, K.Hu, L.C.Zhang, A.H.Liu, Ch.J. Pan, T. Gong, T. Liu, H.Y. Ding, Rsc Advances 8(48), 27253 (2018); https://doi.org/10.1039/C8RA04273J

[14] C.Lin, F. Gong, Z. Yang, Zh.J. Yang , L.P. Pan , S.J. Liu , J. Li , S.Y. Guo, Polymer Testing 68, 126 (2018); https://doi.org/10.1016/j.polymertesting.2018.03.046

[15] H.M. Zhao, X. He, Ch.J.Tan, A.M. Jakhar, F.Y. He, J.H. Ma, Carbohydrate Polymers 122886 (2024); https://doi.org/10.1016/j.carbpol.2024.122886

[16] D. Ma, Z.C.Tu, H. Wang, Z.P. Zhang, D. J. McClements, Food Hydrocolloids 71,149 (2017); https://doi.org/10.1016/j.foodhyd.2017.05.015

[17] B.A.Muhammad, N.Ahmad, J.A.Muhammad , A.Luqman, Egyptian Journal of Biological Pest Control 67 (2019)

[18] X.L. Sun, Viruses 7(1),306 (2015); https://doi.org/10.3390/v7010306

[19] Y.Liang, Y.H.Duan, C.Fan, H.Q. Dong, J.L. Yang, J.Y. Tang, G.Tang,W.C.Wang, N.Jiang,Y.S. Cao, Chemical Engineering Journal 361,671 (2019); https://doi.org/10.1016/j.cej.2018.12.129

[20] G.Y.1Yan, C.Y.Li, J.Sun, Q.Z.Yang, X.L.Pang, B.h.Zhang, Fine Chemical 37(08),1684 (2020)

[21] H.X.Wang, K.Y. Ding, J.Liu, Journal of food science and biotechnology 34(04),407 (2015).

[22] T.Huhtamaki, X.Tian, J. T.Korhonen, R. H. A.Ras, Nat.Protoc. 13, 1521(2018) https://doi.org/10.1038/s41596-018-0003-z