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Interaction of plum (*prunus salicina lindl.* cv. furong) anthocyanins with *Tremella* polysaccharides and characteristics of their complexes

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Abstract

Polysaccharides and anthocyanins are one of the main components of many foods, and their interaction affects the texture and nutrition of foods. The anthocyanin-polysaccharide complex was prepared from anthocyanin of plum (*Prunus salicina Lindl.* cv. Furong) and polysaccharide of *Tremella fuciformis*. The structure changes of anthocyanin and polysaccharide before and after interaction were investigated by UV-visible spectroscopy, infrared spectroscopy and scanning electron microscopy. The absorption peak at 510 nm of anthocyanin-polysaccharide complex weakened at pH 5.0, and the intensity of absorption spectrum increased with the concentration of polysaccharide. The absorption peaks of anthocyanin-polysaccharide complexes were shifted in the infrared spectral curve of anthocyanin, and the formation of complex was caused by electrostatic interaction and hydrogen bond interaction between anthocyanin and polysaccharide. From microstructural analysis, anthocyanin and polysaccharide were effectively bound together. This can provide theoretical guidance for the structural design of anthocyanin-polysaccharide food and the development and utilization of new food ingredients.

Keywords: anthocyanin; polysaccharide; UV spectrum; infrared spectroscopy; microstructure.

Practical Application: Preparation of polyphenol-polysaccharide binary complex.

1 Introduction

Anthocyanins are a class of water-soluble natural pigments that widely exist in plants in nature. Most of the main coloring substances in fruits, vegetables and flowers are related to anthocyanins (Dangles & Fenger, 2018). Anthocyanins have many beneficial physiological effects, such as reducing the incidence of cardiovascular disease (Kruger et al., 2014), antioxidant and free radical scavenging capacity (Tena et al., 2020), hypouricemic and nephroprotective roles (Qian et al., 2019), and anti-colon cancer and lung cancer (Yudina et al., 2021). The results of a previous study showed that the xanthine oxidase inhibitory activity of Plum *(Prunus salicina Lindl.* cv. Furong*)* anthocyanin in an in *vitro* chemical simulation system had an IC_{50} of 179 μg/mL and had the potential to improve hyperuricemia (Li et al., 2016).

Plum (*Prunus salicina Lindl.* cv. Furong) is the largest variety of plum fruit in southern China and is rich in anthocyanins. Plum anthocyanins has excellent solubility in water, good stability under acidic conditions, and can be extracted under acidic thermohydric conditions (Yu et al., 2021). However, like other anthocyanins, plum anthocyanins are very unstable, is prone to fading and discoloration during storage and processing (Yin et al., 2022), and have low bioavailability in the gastrointestinal digestive system (Yildiz et al., 2020). In recent years, there are many products related to anthocyanins, Cross-linked casein micelles can act as encapsulating agents for anthocyanins under highly acidic conditions (Nogueira et al., 2020). Benchikh et al added anthocyanins to yogurt to replace synthetic antioxidant colorants (Benchikh et al., 2021). Replacing sugar with the natural sugar-free sweetener stevia and adding red beetroot to probiotic yogurt resulted in higher fiber consumption, lower energy intake, and lower body weight (Ozcan et al., 2021).

Extensive literature research proves that the interaction of polysaccharides with anthocyanins improved chemical stability and stability of anthocyanins in the human gastrointestinal tract (Koh et al., 2020; Padayachee et al., 2013). The complexation of anthocyanins with polysaccharides, depending on the type of molecule, pH of the solution and other factors, can have three cases of promotion, reduction and no effect on anthocyanin stability (Renard et al., 2017). For example, anthocyanins can inhibit the degradation of anthocyanins by substances in beverages such as ascorbic acid by forming a complex with glycoproteins on gum Arabic through hydrogen bonding (Chung et al., 2016). And blueberry pectin improves the stability of several anthocyanins in the human gastrointestinal tract (Koh et al., 2020). Studies have shown that when polyphenols are physically mixed with polysaccharides, non-covalent interactions such as hydrogen bonding, hydrophobic interactions, and ionic interactions lead to the formation of reversible or irreversible complexes between polyphenols and polysaccharides (McManus et al., 1985; Dridi & Bordenave, 2021; Soares et al., 2009), and this covalent interaction is mediated by enzymatic or non-enzymatic oxidation of o-quinone mechanisms.

Tremella fucifrmis, belongs to the family Tremellaceae, genus Tremella, and it is a common health food in life (Ma et al., 2021).

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Active ingredients; related studies have shown the anticancer and antitumor, scavenging, oxygen free radical, antioxidant, and anti-aging effects of *Tremella fuciformis* polysaccharides (Chen & Cai, 2008; Zhu & Sun, 2008; Kim et al., 2006; Cheng et al., 2002). In the past processing, *Tremella fuciformis* stems were treated as waste residue. It was found that the content of polysaccharide in stem of *T. fuciformis* was higher than that in fruiting body (Wu et al., 2019).

In this study, plum anthocyanin and *Tremella fuciformis* polysaccharide were selected as the research objects. The interaction between anthocyanin and polysaccharide was analyzed by ultraviolet-visible spectroscopy, infrared spectroscopy and scanning electron microscopy. The effect of the ratio of anthocyanin and polysaccharide on the microstructure and surface characteristics of anthocyanin-polysaccharide complex was discussed. This work will help to understand the physical and chemical properties of the complex in the food system, provide a scientific basis for the optimization of the complex in food formulations, and expand the application prospects of anthocyanins, polysaccharides and their complexes in food and other industrial fields.

2 Materials and methods

2.1 Experimental materials

Plum *(Prunus salicina Lindl.* cv. Furong*)* was originated from Yongtai County, Fujian Province, China. The mature plums were dried by heat pump at 50 °C, then were crushed. Plum powder was frozen for storage at -20 °C.

The stems of fresh *Tremella fuciformis* were obtained from Fujian Xiangyun Biotechnology Co., Ltd. and were stored at -20 °C.

Macroporous resin AB-8 was sourced from Shanghai Yuanye Biotechnology Co., Ltd., China. All other organic solvents used in the study were analytical grade.

2.2 Preparation of plum anthocyanins

Plum anthocyanins were extracted in an ultrasonic bath (KQ-600DV, 40 kHz, 300W, Kunshan Ultrasonic Instrument Co. Jiangsu, China). The extraction parameters were 60% ethanol for extraction solvent, 500 W of ultrasonic power, 25 °C of extraction temperature and 10 min of ultrasonic time. After extraction the mixture was filtered by centrifugation at 3000 rpm for 10 min. The precipitate was extracted again with 60% ethanol according to the above-mentioned extraction method. The supernatant liquid after two centrifugations was combined, then was condensed to 15% solid content with a vacuum rotary evaporator (Senco-GG17, Shanghai Shenke Science and Technology Co., Ltd., China) at 0.08MPa, 40 °C.

The crude anthocyanin extract was purified by macroporous resin AB-8. The specific steps of purification are as follows. The macroporous resin was soaked in ethanol for 24 h, then rinsed with distilled water until no alcohol odor and loaded into the column, the crude extract of polyphenols was adsorbed on the column (the flow rate of the upper sample was 144 mL/h), and after complete adsorption, it was rinsed with distilled water until the outlet was free of viscosity (the flow rate of the water wash was 144 mL/h), and Change the eluent to elute the polyphenols and collect them (the eluent was 80% ethanol, the flow rate of the eluent was 168 mL/h). Then it was concentrated by vacuum rotary evaporator (Senco-GG17, Shanghai Shenke Technology Co., Ltd., China) at 0.08 MPa, 40 °C until no alcoholic taste, and finally freeze-dried to obtain the polyphenol purified material. The anthocyanins of the plum extract was 1.15 g/100 g

2.3 Preparation of polysaccharide from Tremella stem

Tremella polysaccharide was extracted by water extraction. The extraction parameters were liquid to material ratio of 10 mL/g, the extraction temperature was 100 °C, and the temperature was kept at 100 °C for 30 min. After extraction the mixture was passed through a 100 mesh sieve. The precipitate was extracted again according to the above extraction method. The two filtrates were The two filtrates were mixed and concentrated to a concentration of 3% in a vacuum rotary evaporator at 0.08 MPa, 60 °C. The concentrated solution was added with 95% ethanol of three times its volume. The mixture was stirred evenly and placed in a 4 °C refrigerator for 12 h, then centrifuged at 3000 rpm for 10 min, and the precipitate was polysaccharide. The residual alcohol in the polysaccharide was removed by hot air, and then the polysaccharide was freeze-dried, and finally placed in the -40 °C refrigerator for standby.

2.4 Preparation of anthocyanin tremella polysaccharide complex

In the preparation of anthocyanin-polysaccharide complex, the anthocyanin concentration was 0.5 mg/mL, and the anthocyanin and *T. fuciformis* polysaccharide were mixed according to different polysaccharide ratios to have different polysaccharide concentrations. The mass ratios of anthocyanin and polysaccharide were 4 : 1, 3 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3 and 1 : 4, respectively. The anthocyanin and polysaccharide were dispersed and stirred at high speed (8000 rpm) for 10 min at 25 °C to make a full compound. The compound was freeze-dried for later use. Figure 1 is Anthocyanin-polysaccharide complex preparation process flow diagram

2.5 UV-Vis spectral analysis

The prepared complex samples were scanned by UV-Vis spectrophotometer (METTLER TOLDEO; TU-1810 UV spectrophotometer, Beijing Pu-Analysis General Instrument Co., Ltd., China) and the spectral changes of four groups of samples were detected in the wavelength range of 200-600 nm at pH 2.0, 3.5, 5.0 and 7.3.

2.6 Fourier infrared spectroscopy

The lyophilized powder was placed in a desiccator (room temperature) to be measured. The sample and KBr were prepared in the ratio of 1 : 20 and then ground well and put into a mold, and the sample was pressed into a thin film with an oil press and scanned 32 times with infrared spectroscopy (iCAN 9 Fourier infrared spectroscopy, Tianjin Energy Spectrum Technology Co., Ltd., Tianjin, China) at a resolution of 32 cm^{-1} with a wave number range condition of 400~4000 cm^{-1}.

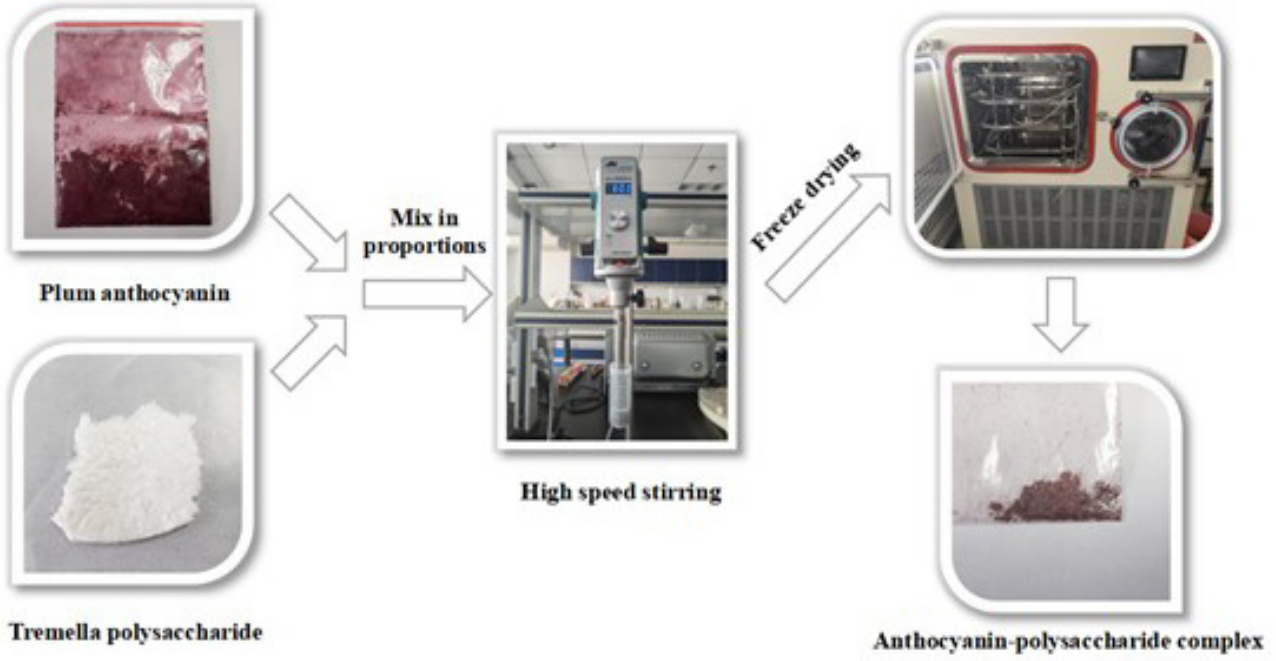

Figure 1. Anthocyanin-polysaccharide complex preparation process.

2.7 Scanning electron microscope observation

A certain amount of plum anthocyanin, *Tremella fuciformis* polysaccharide and anthocyanin-polysaccharide complex powder was prepared and put into an ion sputtering apparatus to coat the surface with a metal film. Then the samples were put into a scanning electron microscope (SOP-A406 scanning electron microscope, Nippon Electron) for observation at a voltage of 15 kV.

3 Results and discussion

3.1 UV-vis spectroscopy analysis of anthocyanin and polysaccharide complex

Determination of the maximum absorption wavelength

25 mg of plum anthocyanin was configured as 0.5 mg/L solution and scanned at full wavelength with UV-vis spectrophotometer in the wavelength range of 200~600 nm.

As shown in Figure 2, there are two maximum absorption peaks of anthocyanin extract from Furong plum, which are located in the visible region near 510 nm and the ultraviolet absorption peak near 290 nm The characteristic absorption peak of anthocyanins was near 510 nm and the characteristic absorption peak of polyphenols was near 290 nm. Among them, the maximum absorbance of geranium pigments was around 510 nm, cornflower pigments and peony pigments were around 530 nm, and delphinium pigments, petunia pigments and mallow pigments were around 540 nm (Liu et al., 2020). In the UV-Vis absorption spectrum, the maximum absorption wavelengths of anthocyanins of plum were found at two locations, one at 510 nm in the visible region and the other at 292 nm in the UV region, suggesting that they may originate from individual pigments.

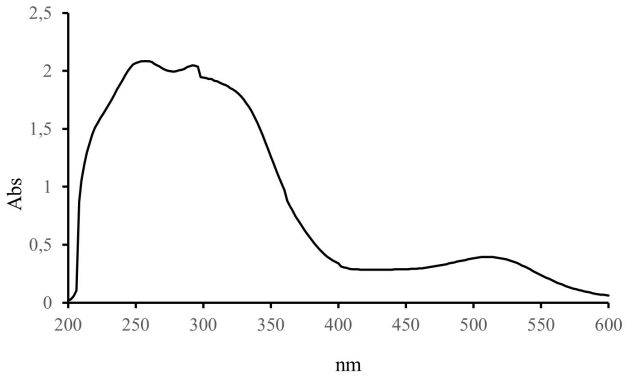

Figure 2. UV-Vis spectrum scanning of anthocyanin extract from plum (200-600 nm).

The presence or absence of absorption peaks of anthocyanins in the range of 300~330 nm was used as an indicator to determine whether there were acyl groups in the pigment molecules. It was found that there were no maximum absorption peaks in the range of 300~330 nm for anthocyanins on the UV spectrum, indicating that there were no acyl groups in the pigment molecules.

Ultraviolet-visible spectroscopy analysis of anthocyanin and polysaccharide complex

It was shown that in acidic aqueous solutions, anthocyanins exhibited three chemical equilibria: acid-base equilibrium,

hydration equilibrium and ring-chain isomerization, and anthocyanins showed four structures in this equilibrium reaction and caused a change in anthocyanin color. Acid-base equilibrium is the main reaction when pH is greater than 7, which makes the anthocyanin structure less stable and more easily decomposed to produce other products. When pH is less than 7, 3 chemical equilibria exist simultaneously, which equilibrium is dominated by the structure of anthocyanins, and the higher the pH the faster the decomposition of anthocyanins under the same external conditions (Castañeda-Ovando et al., 2009).

As can be seen in Figures 3-6, under pH 2.5 conditions plum (*Prunus salicina Lindl.* cv. Furong) anthocyanins have maximum absorption wavelengths at 510 nm and 290 nm, while the addition of *T. fuciformis* polysaccharides had no significant effect on anthocyanins from 350 to 600 nm. However, the absorbance at 290 nm increased gradually with the increase in the concentration of *T. fuciformis* polysaccharide, and the absorption peak showed a red-shift phenomenon here. This may be a direct interaction between anthocyanin and *T. fuciformis* polysaccharide. The red-shift phenomenon of the anthocyanin tremella polysaccharide complex disappeared at pH3.5, and there was no significant change at 510 nm. The results showed that at pH 5.0, the anthocyanin tremella polysaccharide complex had a weak absorption peak at 510 nm, and the intensity of the absorption spectrum increased with the increase of the concentration of *T. fuciformis* polysaccharides. At pH 7.3, the intensity of the absorption peak of anthocyanin extract increased significantly, which may be due to the fact that the structural stability of anthocyanin decreases when the pH is greater than 7 and decomposes to produce other products, making the absorption peak of anthocyanin extract at 200-350 nm significantly larger than that of the complex, indicating that the stability of anthocyanin tremella polysaccharide complex of plum (*Prunus salicina Lindl.* cv. Furong) is significantly improved compared with that of anthocyanin, and the anthocyanin extract and its complex at The absorption peaks of anthocyanin extract and its complex were enhanced at 510 nm, but the UV spectrum

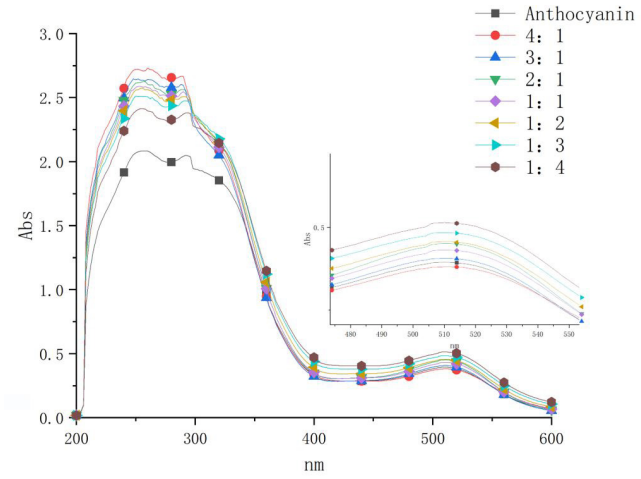

Figure 3. UV spectrum of the complex of anthocyanin and *T. fuciformis* polysaccharide at pH = 2.5.

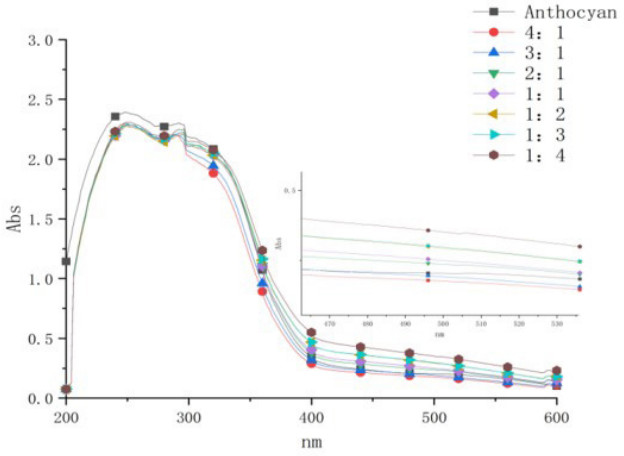

Figure 5. UV spectrum of the complex of anthocyanin and *T. fuciformis* polysaccharide at pH = 5.

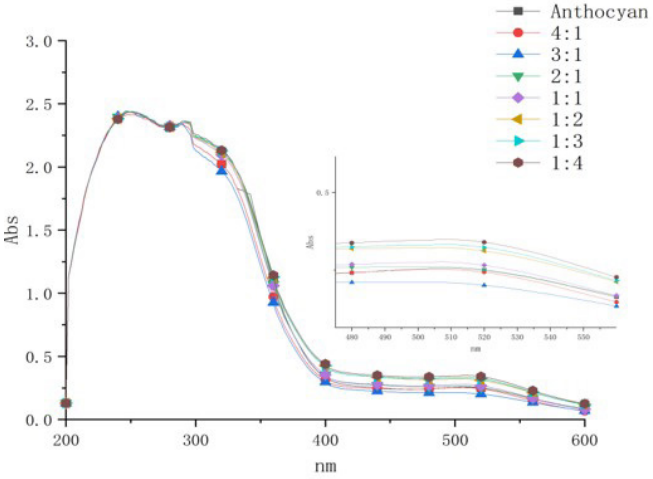

Figure 4. UV spectrum of the complex of anthocyanin and *T. fuciformis* polysaccharide at pH = 3.5.

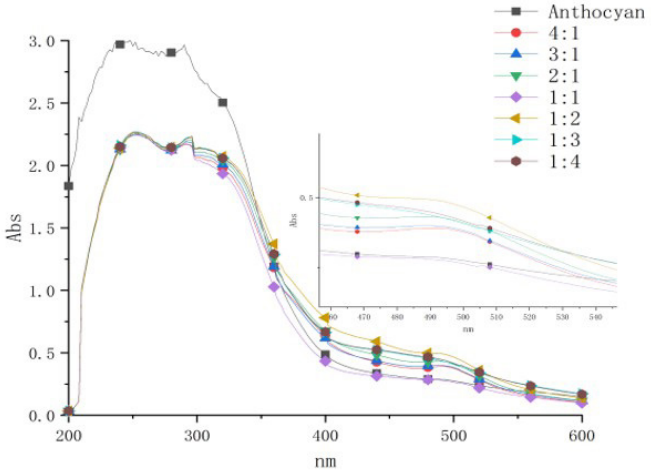

Figure 6. UV spectrum of the complex of anthocyanin and *T. fuciformis* polysaccharide at pH = 7.

of anthocyanin tremella polysaccharide complex was not significantly different compared with that of pH = 5 except for the enhancement at 510 nm.

3.2 Infrared spectroscopy analysis of plum anthocyanin and polysaccharide complex

The functional groups contained in the chemical substances can be specifically analyzed by infrared spectroscopy, and the interaction between various substances constituting the complex can be investigated. The infrared spectra of plum anthocyanin, *T. fuciformis* polysaccharide and anthocyanin-polysaccharide complex are shown in Figure 7 and Figure 8. In the infrared spectra of plum anthocyanin, the main infrared absorption peaks of anthocyanin all contain 3388.316, 2911.985, 1677.766, 1444.422, 1207.384, 1060.657 cm^{-1}.According to the analysis in the literature (Amanah et al., 2020), 3388.316 cm^{-1} was the stretching vibration absorption peak of hydroxyl (–OH), 2911.985 cm^{-1} was the stretching vibration absorption peak of methyl (C–H), 1677.766 cm^{-1} was the coupling stretching vibration absorption peak of (C=O), and 1444.422 cm^{-1} might be the stretching vibration absorption peak of aromatic skeleton (C=C) bond. From the IR spectra of *T. fuciformis* polysaccharide, the broad peaks appearing at 3600~3200 cm^{-1} were (O-H) stretching vibrations, 2918.985 cm^{-1} was the absorption peak of (C=O) coupling and 1060.657 cm^{-1} was the absorption peak of aromatic skeleton (C=C) bond., 2918.2985 cm^{-1} was the (C-H) stretching vibration, 1400~1200 cm^{-1} was the variable angle vibration of (C-H), and these three characteristic peaks proved that the compound was a sugar substance (Ge et al., 2020). The absorption peak of the stretching vibration of -OH group near 3600~3200 cm^{-1} was the characteristic peak of the polysaccharide (Coates, 2006). It can be found from Figures 7, Figure 8 and Figure 9 that the infrared spectra of anthocyanin and polysaccharide have typical characteristic peaks. These peaks also exist in the spectra of anthocyanin-polysaccharide complexes, but their positions have changed, indicating that the electrostatic driving complex formation.

In Figure 9, the spectral lines of all anthocyanin-polysaccharide complexes are basically similar, and the position of -OH absorption peak in anthocyanin deviates from that in the complex. Compared with anthocyanin and polysaccharide, the absorption peak intensity of the complex is weaker and wider, and red shift occurs with the increase of polysaccharide ratio. It can be seen that after the interaction between anthocyanins and polysaccharides, there is a stretching vibration of (-OH) group between the molecules, which results in a decrease of the absorption peak intensity. The absorption peak of the infrared spectral curve of anthocyanins has stretching oscillation of (C-H) bond in the range of 3000 to 2800 cm^{-1}, the anthocyanin-polysaccharide complex deviates from its absorption peak. It indicates that there are (C-H) bond stretching vibrations between the molecules of anthocyanin and polysaccharide.

The FTIR spectra of anthocyanin-polysaccharide complexes prepared with different mass ratios were not significantly different, except for a slight change in the intensity of the peaks. Compared with anthocyanin, the IR spectra of the complex only increased the peak intensity. To sum up, comparing the infrared spectrum curves of anthocyanin, polysaccharide and anthocyanin-polysaccharide complex, it can be concluded that the formation of complex is caused by electrostatic interaction and hydrogen bond interaction between anthocyanin and polysaccharide.

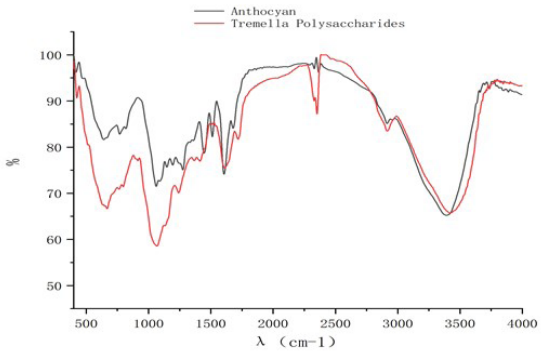

Figure 7. Infrared spectra of plum anthocyanins and *T. fuciformis* polysaccharides.

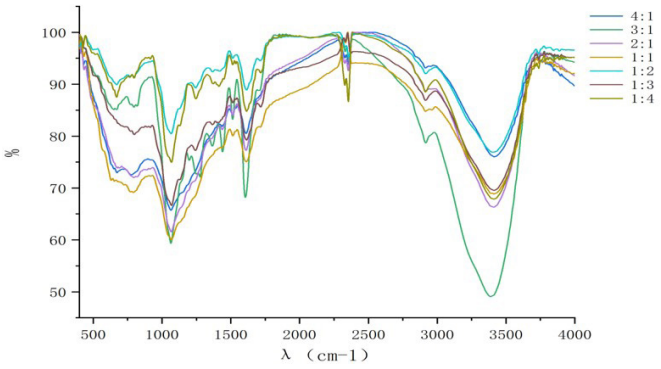

Figure 8. Infrared spectrum of the complex of plum anthocyanin and *T. fuciformis* polysaccharide.

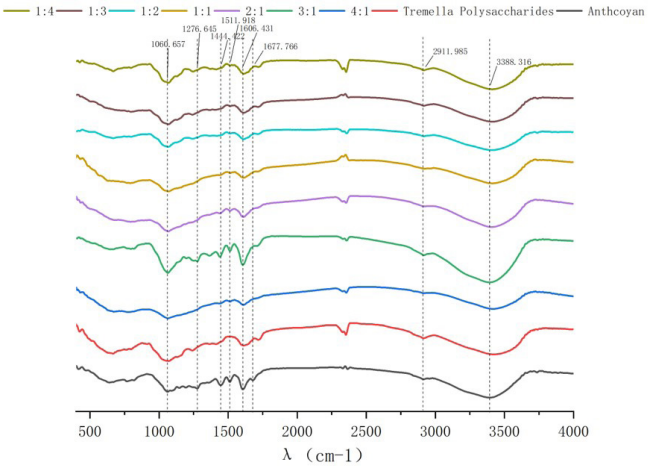

Figure 9. Y-axis shift of infrared spectra of plum (*Prunus salicina Lindl.*cv. Furong) anthocyanins, *T. fuciformis* polysaccharides and their complexes.

3.3 Microstructure observation

Figure 10 showed that the anthocyanins of plum were flakes with spherical particles on the surface. It is speculated that the pectin was not completely removed during the extraction process, and the anthocyanins combined with the pectin on the cell wall, which may form spherical structures during the drying process (Bourvellec et al., 2009). In Figure 11, the polysaccharides showed irregular lamellar structures with smooth surfaces and dense structures with different diameters, similar to the results of Chen et al. (2022) The complexes in Figure 12 showed irregular

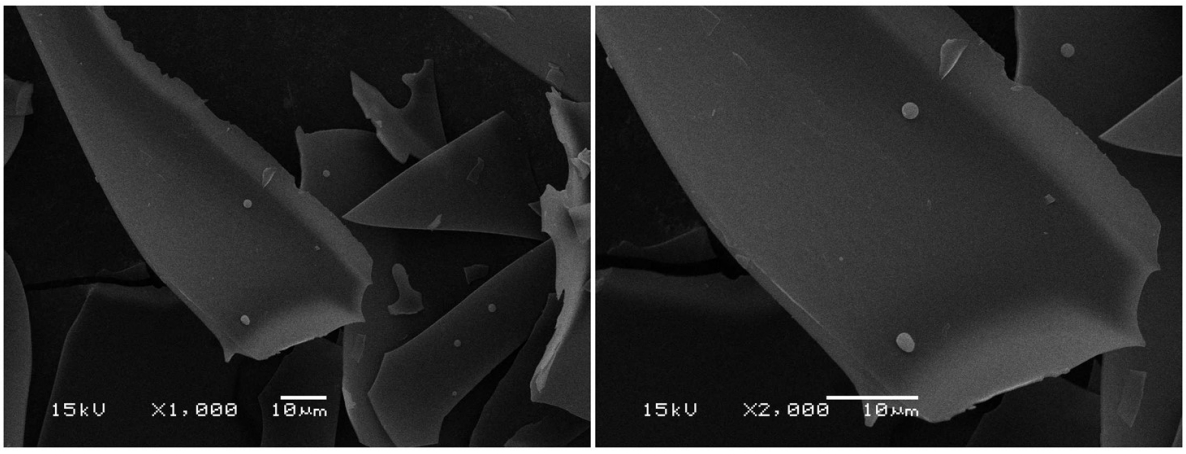

Figure 10. Microstructure of plum anthocyanins.

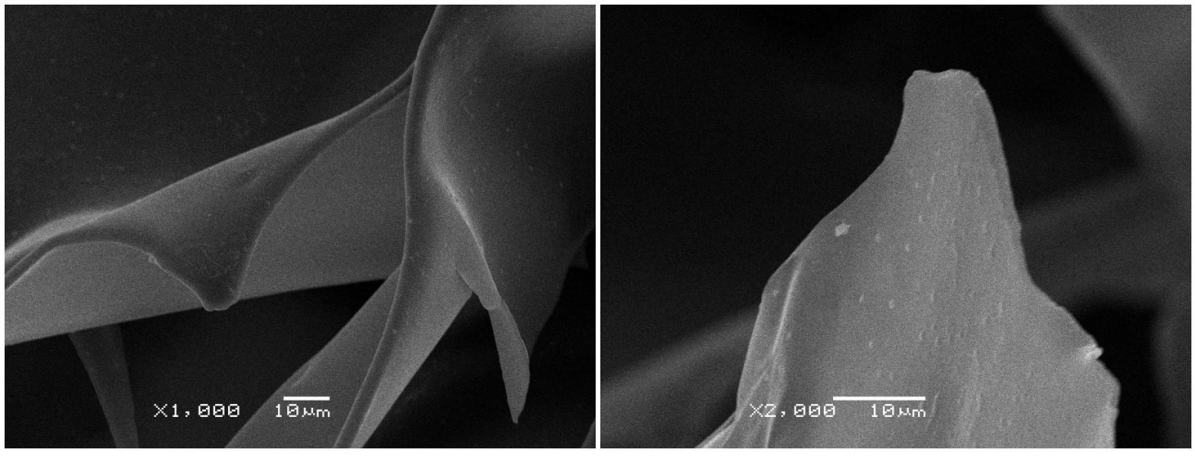

Figure 11. Microstructure of *T. fuciformis* polysaccharide.

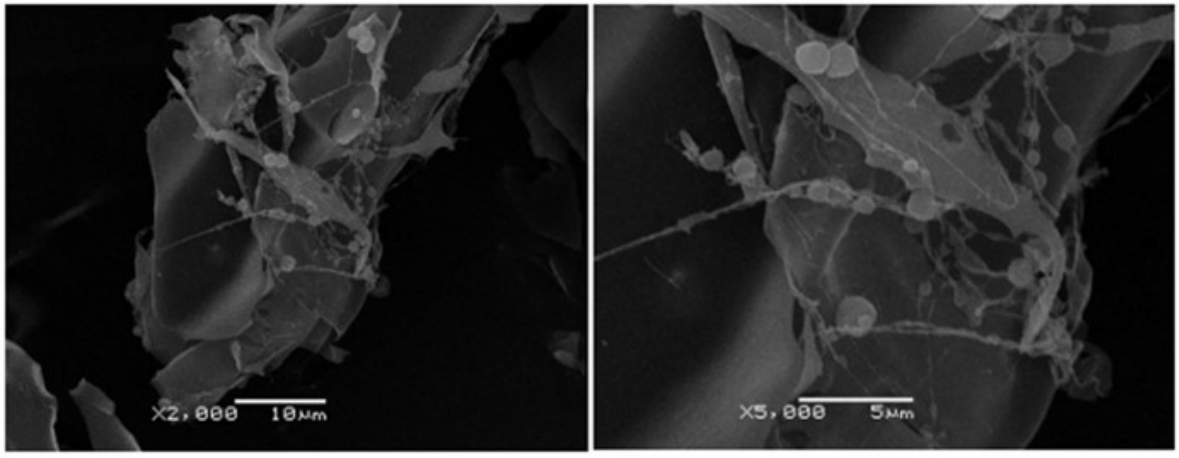

Figure 12. Microstructure of anthocyanin-polysaccharide complex.

morphology, and the polysaccharide and anthocyanin molecules were adsorbed together with each other, which indicated that the combination of anthocyanin and polysaccharide to form oligomers was a viscous, mutually repulsive physical mixing caused purely by non-covalent interactions, and no chemical reaction occurred.

4 Conclusions

In this study, anthocyanin-polysaccharide complex was prepared by combining plum anthocyanin with *T. fuciformis* polysaccharide by physical means.

The absorption peak at 510 nm of anthocyanin-polysaccharide complex weakened at pH 5.0, and the intensity of absorption spectrum increased with the increase of polysaccharide concentration. The FTIR spectra of anthocyanin-polysaccharide complexes prepared with different mass ratios did not differ significantly, but the peak intensities changed slightly. Compared with anthocyanins, the FTIR spectra of the complexes only increased the peak intensities. From microstructural analysis, anthocyanin and polysaccharide were effectively bound together. Comparing the experimental results of anthocyanins, polysaccharides and anthocyanin-polysaccharide complexes, it can be concluded that the formation of complexes was caused by non-covalent interactions such as electrostatic interactions and hydrogen bonding interactions between anthocyanins and polysaccharides. The above results indicate that the anthocyanin-polysaccharide complex has good prospects for development and application in food production, and it is worthy of further study of its properties. The in *vivo* stability between *T. fuciformis* polysaccharide and plum anthocyanin should be explored in future studies.

Acknowledgements

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