



Preparation and characterisation of Qingzhuan dark tea polysaccharide–zinc

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Abstract

To produce Qingzhuan dark tea polysaccharide–zinc (TPS–Zn), tea polysaccharides (TPS) were isolated from Qingzhuan dark tea and complexed with a zinc salt in the present study. The thermogravimetric analysis, molecular weight analysis, atomic absorption spectrum, Fourier transform infrared spectrum, ultraviolet spectrum, scanning electron microscope, X-ray diffraction, X-ray photoelectron spectroscopy, and atomic mechanical microscope were used for qualitative and quantitative characterisation. The findings demonstrated and verified that complex reactions between zinc ions and TPS had occurred. After the Zn²⁺ complex was modified, the structure of TPS became more stable. The complex was homogeneous in composition and did not comprise any nucleic acid or protein. It is a zinc supplement that may be studied.

Keywords: qingzhuan dark tea; polysaccharides; zinc complex; structure characterisation.

Practical Application: Develop Qingzhuan Dark Tea for pharmaceutical industry.

1 Introduction

The tea resources are abundant in China (Gao et al., 2022; Yao et al., 2021). Qingzhuan dark tea is categorised as dark tea among the six main teas. The tea is produced mainly in Chibi City, Hubei Province, China. It serves as a necessity for ethnic minorities in Northwest China when resources are limited. Qingzhuan dark tea has a wide range of biological characteristics and contains a large number of active compounds (Kayisoglu & Coskun, 2021; Cheng et al., 2021; Liu et al., 2016). Drinking Qingzhuan dark tea has several advantages, as it increases fluid and alleviates thirst, provides refreshment, aids digestion and sterilisation, and prevents diarrhoea. The effects of anti-hypertension, hyperlipidaemia, and hyperglycaemia are more evident (Zhao et al., 2019). At the same time, the content of polysaccharides in Qingzhuan dark tea is higher. TPS is a natural macromolecular active substance that is present in a large amount in tea and has many biological activities. It was reported to exhibit a significant antioxidant capacity and effective scavenging activity against free radicals (Zhou et al., 2022a).

Zinc is a trace element that is essential for the human body because it synthesises numerous enzymes. Inadequate zinc intake, utilisation disorder, and excessive excretion are the common causes of growth retardation, diabetes, coronary heart disease, and other diseases (Boreiko, 2010). In vitro, plant polysaccharides zinc complex has been found to exert improved hypoglycaemic activity and anti-inflammatory effects (Zhang et al., 2019; Xu et al., 2017). Polysaccharides zinc may be produced by combining polysaccharides with zinc ions in a water bath at a constant temperature. Compared with inorganic zinc, the polysaccharide zinc complex has a greater utilisation

rate, less gastrointestinal stimulation, and superior biological activity (Chen et al., 2007; Holen et al., 2020). In the present study, TPS was complexed with zinc ions, and the structure and characterisation of zinc complexes were determined to obtain a zinc supplement with various biological functions.

Zinc is a metal that aids in growth and development. Natural compounds based on plant polysaccharides complexing zinc, on the other hand, have become increasingly popular in recent years. This research, which is rich in the area of further drug development, can be used as a model for future research.

2 Experimental materials

The Qingzhuan dark tea was purchased from Chibi City in the Hubei Province, China. Unless otherwise specified, all reactants were used without any further purification. Deionised water was used, whenever required.

3 Experimental methods

3.1 Preparation of TPS and TPS–Zn

Preparation of TPS

After degreasing and removing small molecules, the tea powder was extracted with water for 4 h, and the supernatant was lyophilised to obtain crude polysaccharides. Next, 50 g of polyamide was soaked in 95% ethanol, 5% NaOH, and 10% hydrochloric acid for 4–5 h, respectively, and the polyamide column was loaded into the column. After natural settlement, the polyamide column was balanced, and TPS was dissolved

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in water, eluted, decolorised, and deproteinised to obtain the eluent. After 48 h of dialysis, the refined polysaccharides were freeze-dried. The sugar content in the refined polysaccharides was determined using the phenol-concentrated sulfuric acid method at 490 nm (Zhou et al., 2022b).

Preparation of TPS-Zn

The complexation conditions of TPS-Zn were determined in accordance with the preliminary experiment. Schematic illustration for the preparation are shown in Figure 1. UP water was used to prepare 2 mg/mL of the TPS solution and zinc salt solution, respectively, which were then evenly mixed in equal volume. The solution was stored at 30 °C in a constant temperature water bath and continuously stirred for 2 h. The resultant solution was collected, dialysis was performed for 48 h, and the dialysis liquid was freeze-dried to obtain TPS-Zn.

3.2 Structural characterisation of TPS-Zn

Zinc content detection

Atomic absorption spectrometer (Contraa-700 Analytica GMBH Jena, Germany) was used for quantitative analysis of single elements (flame method) at 0.3-MPA Ar pressure. Qualitative and quantitative analyses of zinc were performed in TPS-Zn (Liu et al., 2015).

Purity and homogeneity testing

The upper column was activated with 15 g polyamide, followed by the addition of 1 mL/min physiological saline for balancing for 1.5 h. Normal saline was used to configure 2 mg/mL TPS and TPS-Zn. The saline eluent was collected in 5 mL

tubes. The absorbance was measured at 490 nm by using the phenol-concentrated sulfuric acid method, and the corresponding elution curve was prepared. The relative molecular weights and homogeneity of TPS and TPS-Zn were determined by high-performance gel filtration chromatography. Next, 2 mg of TPS and TPS-Zn were respectively weighed and dissolved in 400 μ L of 0.1 mol/L sodium nitrate solution to determine the homogeneity and molecular weight distribution of TPS and TPS-Zn. The mobile phase was 0.1 mol/L sodium nitrate solution, the flow rate was 0.9 mL/min, and the column temperature was 45 °C for the experiment (Zheng et al., 2014).

Thermal performance analysis

The thermogravimetry analysis (TGA) was conducted using the NETZSCH thermogravimetric analyser (TG 209F3, Germany) under N_2 atmosphere with a heating rate of 10 °C/min from the ambient temperature to 600 °C to detect the relationship between mass and temperature, followed by preparation of the thermogravimetric curve and analysis of the thermal stability of the material.

FT-IR analysis

The organic functional groups in TPS and TPS-Zn complexes were characterised by infrared spectroscopy to detect the structural changes induced by zinc complexation. Specifically, the sample was ground with potassium bromide powder, pressed into 1-mm thin slices, and subjected to FT-IR measurements with the Nicolet 6700 Fourier transformed infrared spectrophotometer in the frequency range of 4000–400 cm^{-1} (Wang & Li, 2019; Iwansyah et al., 2021).



Figure 1. Schematic illustration for the preparation of TPS-Zn.

Raman spectrum analysis

On the basis of infrared analysis, to further obtain the molecular vibration information of TPS and TPS-Zn, the TPS and TPS-Zn were analysed using the RENISHAW Raman microscope.

Ultraviolet spectrum analysis

TPS and TPS-Zn were prepared into 1 mg/mL solution and added into a quartz colorimetric dish, followed by scanning for the UV spectrum with a spectrophotometer in the range of 200–400 nm.

Microstructure analysis (Congo red test)

We mixed equal volumes (1 mg/mL) of TPS and TPS-Zn with 80 $\mu\text{mol/L}$ Congo red solution. To this mixture, 1 mol/L of the NaOH solution was added in different volumes to prepare the final NaOH concentrations of 0–0.5 mol/L, with 0.05 mol/L concentration serving as the gradient. After allowing the mixture to stand for 10 min, the maximum absorption wavelength at 400–700 nm was determined (distilled water was used as the control).

SEM/EDS and XRD analysis

The surface morphology and structure of TPS and TPS-Zn samples were analysed and observed through field emission scanning electron microscopy (Zeiss Ultra Plus) with 5-kV acceleration voltage. Meanwhile, the surface element composition between TPS and TPS-Zn was compared by EDS. The crystal structures of TPS and TPS-Zn were characterised using an Empyrean X-ray diffractometer (Panaco, The Netherlands).

X-ray photoelectron spectroscopy

An X-ray photoelectron spectroscopy (Escalab 250XI, USA) was used for determination of the zinc valence states in the TPS-Zn complex and the binding energy before and after the reaction.

Atomic force microscopy

The morphology of TPS and TPS-Zn complexes was studied using an atomic force microscope (AFM) Nanoscope IV (Veeco, USA). Then, 10 $\mu\text{g/mL}$ of TPS and TPS-Zn solutions were precipitated on newly cut surfaces and allowed to dry in the air for approximately 10 min. Then, 1- μm images were processed and analysed using Gwyddion-SPM data analysis software.

4 Statistical analysis

All results are expressed as mean \pm standard deviation of six repetitions. Statistical analysis was performed using Origin 2019 software (Origin Lab Corporation, Northampton, MA, USA). One-way analysis of variance (ANOVA) was performed using SPSS (version 18.0, IBM, Armonk, New York, USA). A P value < 0.05 was considered to be statistically significant.

5 Experimental result

5.1 Structural characterisation of TPS-Zn

Determination of sugar content and zinc content of TPS-Zn

The sugar content of TPS-Zn was measured using the phenol-concentrated sulfuric acid technique. The sugar content of TPS is 69.4%. The zinc content of TPS-Zn was determined using the atomic absorption method. The zinc content of TPS-Zn is 4.43%.

Determination of purity and homogeneity

Plant polysaccharides are polymerised systems with variable degrees of polymerisation. It is defined as a homogenous polysaccharide, which refers to polysaccharides in a certain molecular weight range (Han et al., 2015). The detection results of the polyamide chromatography column are shown in Figures 2A and 2B. Because there was just one elution peak for TPS and TPS-Zn, suggesting that each had only one component, polysaccharides and zinc polysaccharides may be considered homogeneous based on the existing separation methods. Meanwhile, as illustrated in Figures 2C and 2D, the molecular weight distribution of TPS has only a symmetric peak, indicating that TPS is a homogenous polysaccharide with a relative molecular weight of 21839; the molecular weight distribution of TPS-Zn has only a symmetric peak, indicating that TPS-Zn composition is uniform and its relative molecular weight is 33722. It indicated that the TPS and TPS-Zn products obtained in this experiment were homogeneous in composition, with TPS-Zn having a higher molecular weight.

Thermal stability analysis

As shown in Figure 3A, the high mass fraction of TPS-Zn suggested lesser weight loss and improved thermal stability compared with TPS with an increase in temperature. Probably, the sugar chain's -COO-, -CH₃, -OSO₃- atoms can establish a coordination link with zinc ions, making the structure more stable and decreasing TPS-Zn weight loss when heated (Zhang et al., 2020).

Infrared spectroscopic analysis

The functional groups of polysaccharide zinc were identified through infrared analysis. Figure 3B depicts that the absorption peak near 3406 cm^{-1} is generated by the stretching vibration of -OH. The absorption peak at 2936 cm^{-1} is generated by the stretching vibration of sugar C-H. It demonstrates that TPS and TPS-Zn both have polysaccharide IR signature peaks. The absorption peaks of TPS-Zn are weaker than those of TPS. TPS's C=O stretching vibration peak is stronger than TPS-absorption Zn's peak. These findings suggest that a coordination bond can form between Zn²⁺ and a carboxylic acid, which alters the stretching vibration of C=O and increases the contact between molecules (Rupérez et al., 2002).

Ultraviolet spectrum analysis

As demonstrated in Figure 3C, TPS and TPS-Zn have no unique absorption peaks at 260 nm and 280 nm, indicating

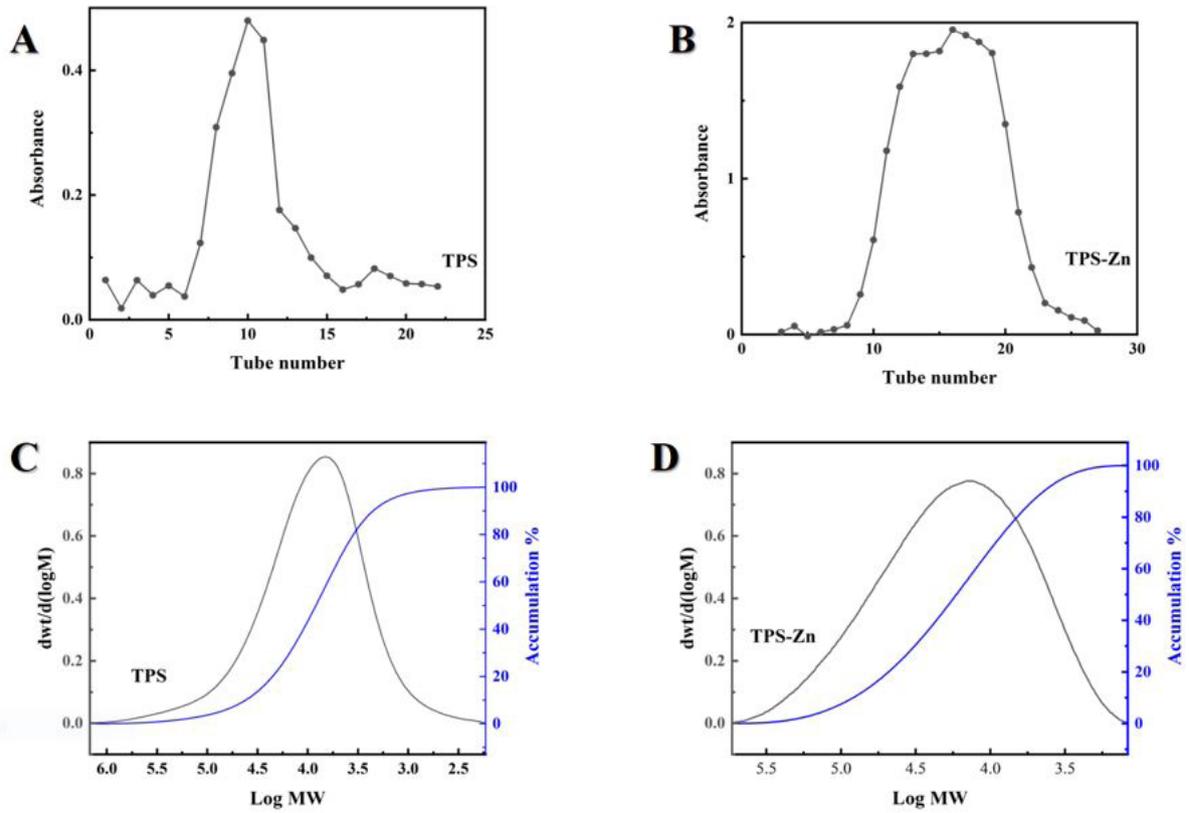


Figure 2. Elution curves and relative molecular weights of TPS and TPS-Zn.

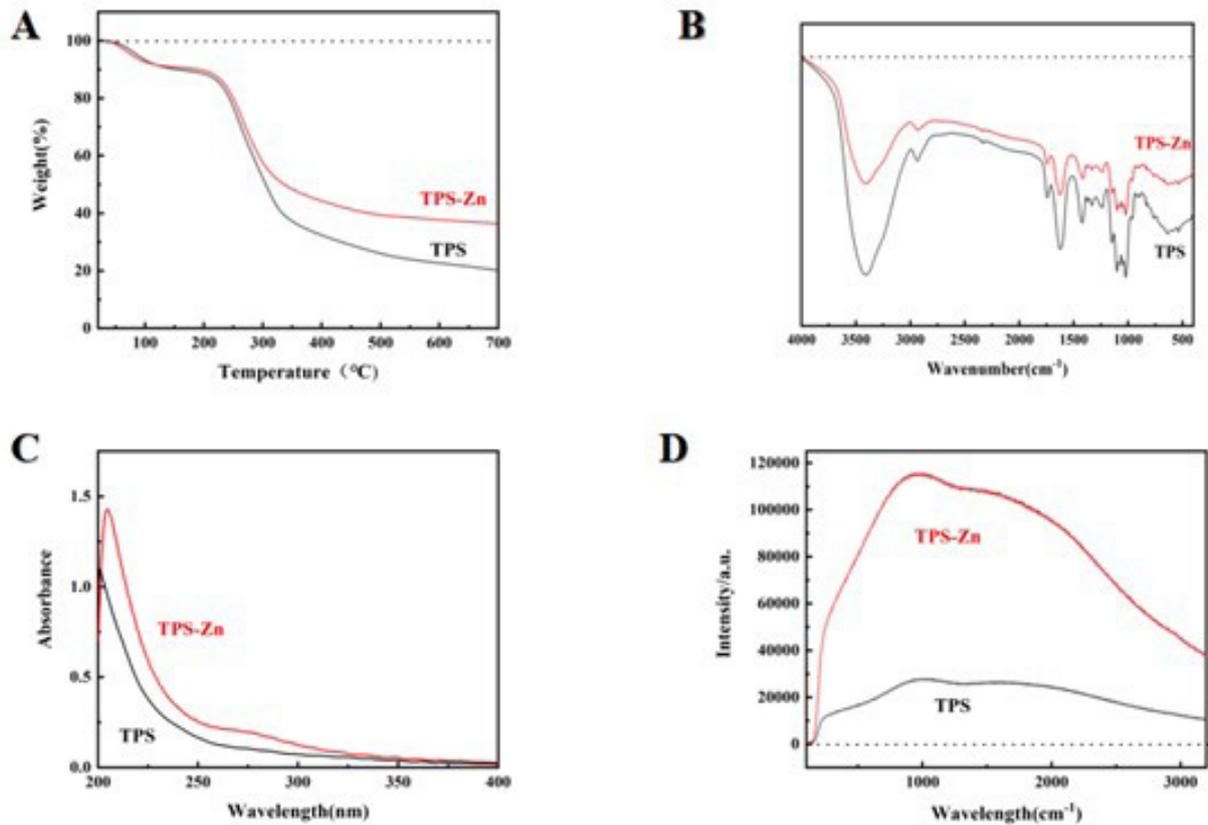


Figure 3. TGA of TPS and TPS-Zn(A); Infrared spectra of TPS and TPS-Zn(B); UV spectra of TPS and TPS-Zn(C); Raman spectra of TPS and TPS-Zn(D).

that they do not contain nucleic acid or protein. The absorption strength of TPS-Zn is smaller than that of TPS. It might be caused by the complex interaction of auxochrome or chromophore in TPS with Zn^{2+} (Zheng et al., 2015).

Raman spectrum analysis

Both TPS and TPS-Zn have absorption maxima of approximately 900 cm^{-1} , as shown in Figure 3D. As illustrated in the figure, these are β -glycosidic bonds with the pyran ring as their sugar ring. The results showed that the main structure of polysaccharides did not change after zinc complexation, which was compatible with the results of IR spectra.

X-ray photoelectron spectroscopy

Figure 4A depicts the full XPS scanning spectrum of TPS and TPS-Zn of Qingzhu dark tea, whereas Figure 4B depicts the TPS and TPS-Zn Zn2p scanning spectrum. There were no characteristic peaks of zinc in the full scan spectra of polysaccharide samples or the XPS spectra of Zn2p, indicating that the samples did not contain zinc. The characteristic electron binding energy peak of zinc was found in the XPS scanning spectrum of zinc polysaccharides and Zn2p. Following the reaction, it is reasonable to conclude that Zn successfully altered the polysaccharide (Guo et al., 2014).

XRD crystal structure

Figure 4C shows an X-ray diffraction pattern. XRD analysis is a method for studying the structural distribution of atoms. Because the atoms or ions within the crystal are organised in a regular pattern, X-rays of a certain wavelength can be distributed across a crystalline material. The phase of scattered X-rays is enhanced in various orientations. As a result, the crystal structure is related to certain diffraction occurrences. The crystallinity of both complexes is low, with no obvious crystallisation peaks. The crystal structure changes as a result of a coordination reaction. TPS-Zn XRD results revealed a variety of peak positions, indicating that polysaccharide crystallinity changed following zinc addition (Wang & Li, 2019). According to XRD analysis, zinc was effectively injected into TPS, which resulted in the formation of a TPS-Zn complex.

Microstructure analysis (Congo red test)

When compared with Congo red solution, the maximum absorption wavelength of the product formed by a complex reaction between the substance with triple helix structure and Congo red will be red-shifted. To put it another way, the position of the absorption peak change with time, but within a certain range of NaOH concentration, the position of the absorption peak will basically remain unchanged, showing metastability

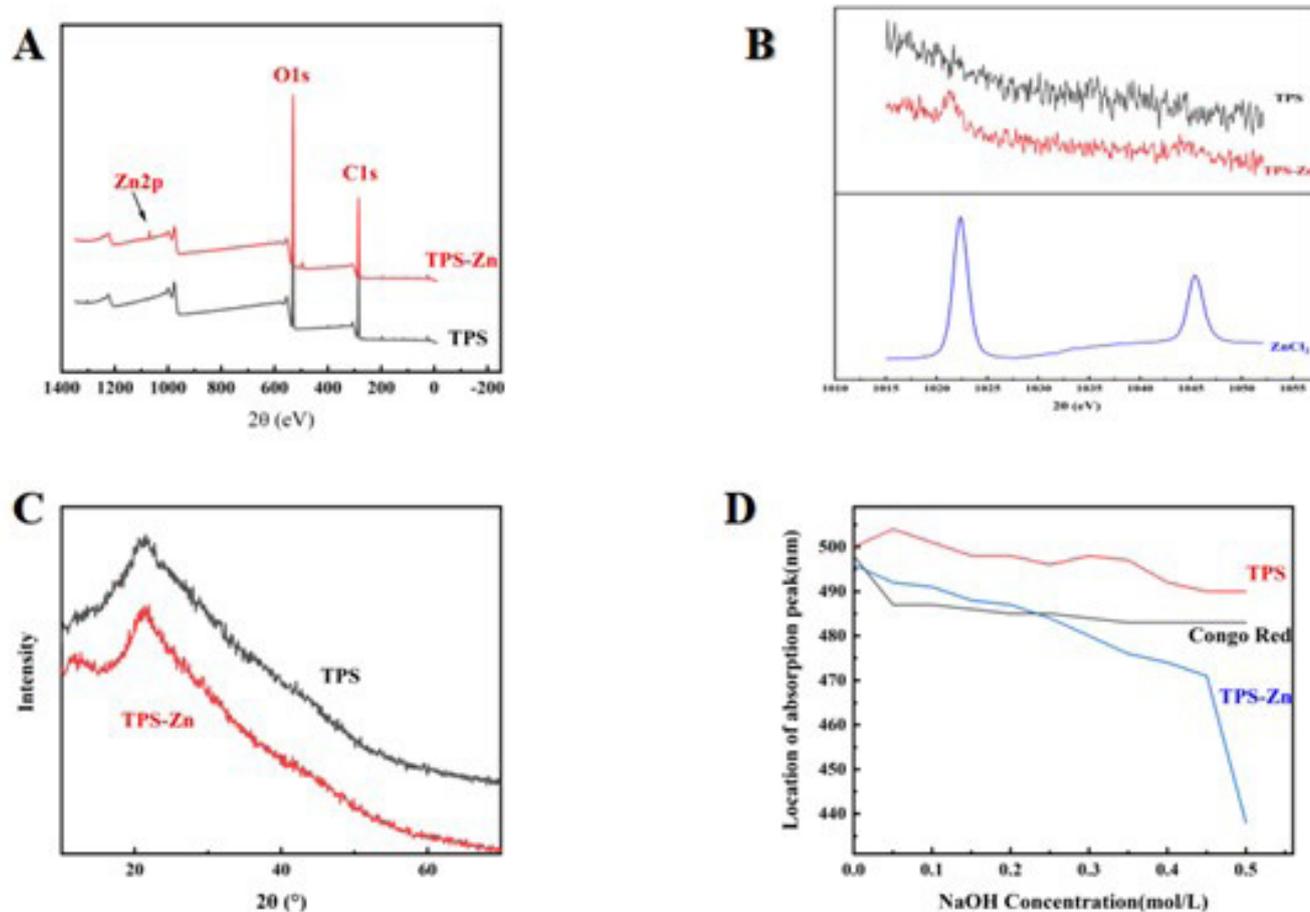


Figure 4. XPS full scan atlas of TPS and TPS-Zn (A); XPS Zn2p spectra of TPS and TPS-Zn (B); Crystal structure of TPS and TPS-Zn(C); Triple helix structure of TPS and TPS-Zn(D).

(Bao et al., 2001). Figure 4D shows that when Congo red is mixed with TPS, the wavelength of its maximum absorption wavelength increases, causing a red shift to occur. Furthermore, the metastable zone appeared in the NaOH concentration range of 0.15–0.35 mol/L, whereas TPS-Zn did not appear. TPS appears to contain a triple helix structure, whereas TPS-Zn does not. The trihelix structure of TPS may be disrupted during the complexation process, suggesting that the microstructure of TPS-Zn is closer.

Morphology and elemental distribution of TPS-Zn

Figure 5 shows that the microscopic morphology of TPS and TPS-Zn was uneven, which may be attributed to the cross-linking aggregation of TPS and TPS-Zn due to their strong

contact. Furthermore, TPS was lumpy, and the TPS-Zn structure was more uniform, which might be due to an increase in the intermolecular force after TPS complexation with zinc ions, which alters the structure distribution (Dong et al., 2018). Zinc ions accounted for 3.98% of the total, according to EDS analysis.

AFM analysis

The morphology of TPS was studied using an atomic mechanics microscope. Polysaccharide chemical modification can change their spatial structure and hence their structure-activity relationship. The spatial structure or conformation of polysaccharides is important in biological activity (Wang et al., 2014). Figure 6 depicts TPS and TPS-Zn 1- μm AFM images. The polysaccharide distribution is uniform, as depicted in the

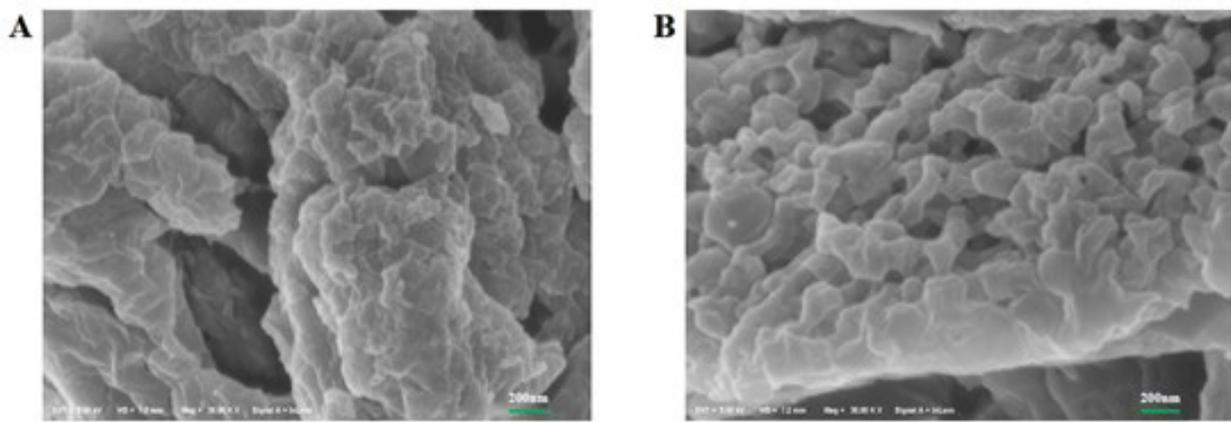


Figure 5. SEM images for TPS (A) and TPS-Zn (B).

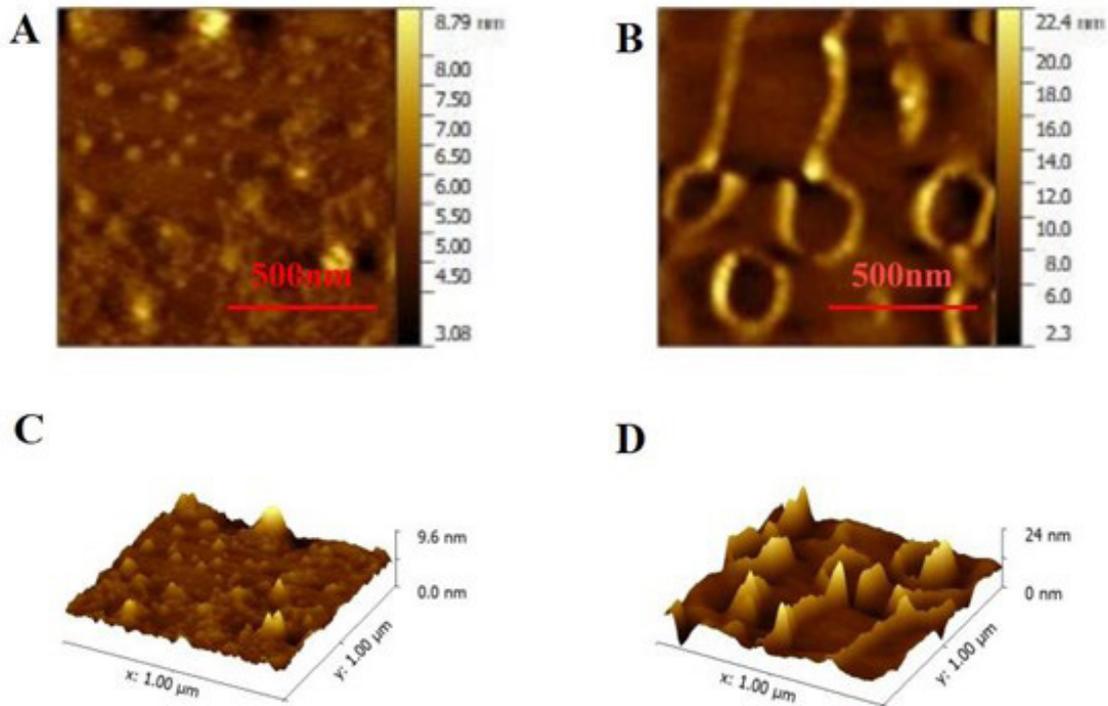


Figure 6. AFM topography images in tapping mode. (A) Topography image of TPS; (B) Topography image of TPS-Zn; (C) Height distribution of TPS; (D) Height distribution of TPS-Zn.

images, although aggregation is lower than that in TPS–Zn, and a particle size shift is apparent. TPS–Zn has a larger volume and a more concentrated distribution, suggesting that it has more molecular aggregation, which may be related to its stronger intermolecular interaction force. This may be due to alteration of the spatial structure of polysaccharides by zinc, causing sugar chains to cluster together.

6 Discussion

Inorganic zinc, on the other hand, stimulates the gastrointestinal system and has lesser biological activity than organic zinc. Zinc ions may easily complex with various groups in plant polysaccharides, including –CH₃, –COO–, and other groups. TPS and zinc salt were generated in this experiment and used to create TPS–Zn. TPS–Zn has higher thermal stability than TPS and comprises 4.43% zinc. TPS has a triple helix structure, whereas TPS–Zn does not, and both have pyran rings as sugar rings. TPS displays unique UV–Vis, FT-IR, and XRD spectra from TPS–Zn, indicating that TPS has a different structure than TPS–Zn. According to structural studies, zinc has been successfully complexed into TPS.

This experiment generated a high zinc TPS–Zn complex with good thermal stability. The complex was homogeneous in composition and did not comprise any nucleic acid or protein. It is a zinc supplement that may be studied.

Conflict of interest

The authors declare no competing interests.

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References

- Bao, X., Liu, C., Fang, J., & Li, X. (2001). Structural and immunological studies of a major polysaccharide from spores of *Ganoderma lucidum* (Fr.) Karst. *Carbohydrate Research*, 332(1), 67–74. [http://dx.doi.org/10.1016/S0008-6215\(01\)00075-1](http://dx.doi.org/10.1016/S0008-6215(01)00075-1). PMID:11403089.
- Boreiko, C. J. (2010). Overview of health risk assessments for zinc. *Journal of Toxicology and Environmental Health. Part A*, 73(2), 166–174. <http://dx.doi.org/10.1080/15287390903340427>. PMID:20077287.
- Chen, H., Zhang, M., Qu, Z., & Xie, B. (2007). Compositional analysis and preliminary toxicological evaluation of a tea polysaccharide

conjugate. *Journal of Agricultural and Food Chemistry*, 55(6), 2256–2260. <http://dx.doi.org/10.1021/jf0632740>. PMID:17305361.

- Cheng, L., Wang, Y., Zhang, J., Zhu, J., Liu, P., Xu, L., Wei, K., Zhou, H., Peng, L., Zhang, J., Wei, X., & Liu, Z. (2021). Dynamic changes of metabolic profile and taste quality during the long-term aging of Qingzhuan Tea: the impact of storage age. *Food Chemistry*, 359, 129953. <http://dx.doi.org/10.1016/j.foodchem.2021.129953>. PMID:34000695.
- Dong, J., Li, H., & Min, W. (2018). Preparation, characterization and bioactivities of *Athelia rolfsii* exopolysaccharide-zinc complex (AEPS-zinc). *International Journal of Biological Macromolecules*, 113, 20–28. <http://dx.doi.org/10.1016/j.ijbiomac.2018.01.223>. PMID:29454944.
- Gao, Y., Cao, Z., Zhou, F., Zhao, Y., Tang, L., & Zhang, H. (2022). Association between tea drinking and endometrial cancer risk: a meta-analysis. *Food Science and Technology*, 42, e90021. <http://dx.doi.org/10.1590/fst.90021>.
- Guo, L., Ding, W., & Meng, F. (2014). Fabrication and *in vitro* evaluation of folate-modified iron ferrite nanoparticles with high doxorubicin loading for receptors-magnetic-guided drug delivery. *Nano*, 09(2), 1450021. <http://dx.doi.org/10.1142/S1793292014500210>.
- Han, X., Shen, S., Liu, T., Du, X., Cao, X., Feng, H., & Zeng, X. (2015). Characterization and antioxidant activities of the polysaccharides from *Radix Cyathulae officinalis* Kuan. *International Journal of Biological Macromolecules*, 72, 544–552. <http://dx.doi.org/10.1016/j.ijbiomac.2014.09.007>. PMID:25236610.
- Holen, J. P., Johnston, L., Urriola, P. E., Garrett, J. E., & Shurson, G. C. (2020). PSII-16 Comparative digestibility of polysaccharide-complexed zinc and zinc sulfate in diets for gestating and lactating sows. *Journal of Animal Science*, 98(Suppl. 3), 171. <http://dx.doi.org/10.1093/jas/skaa054.303>.
- Iwansyah, A. C., Desnilasari, D., Agustina, W., Pramesti, D., Indriati, A., Mayasti, N. K. I., Andriana, Y., & Kormin, F. B. (2021). Evaluation on the physicochemical properties and mineral contents of *Averrhoa bilimbi* L. leaves dried extract and its antioxidant and antibacterial capacities. *Food Science and Technology*, 41(4), 987–992. <http://dx.doi.org/10.1590/fst.15420>.
- Kayisoglu, S., & Coskun, F. (2021). Determination of physical and chemical properties of kombucha teas prepared with different herbal teas. *Food Science and Technology*, 41(Suppl. 1), 393–397. <http://dx.doi.org/10.1590/fst.12720>.
- Liu, F., Pei, F., Mariga, A. M., Gao, L., Chen, G., & Zhao, L. (2015). Separation and speciation analysis of zinc from *Flammulina velutipes*. *Yao Wu Shi Pin Fen Xi*, 23(4), 630–635. PMID:28911478.
- Liu, S., Yu, Z., Zhu, H., Zhang, W., & Chen, Y. (2016). *In vitro* α-glucosidase inhibitory activity of isolated fractions from water extract of Qingzhuan dark tea. *BMC Complementary and Alternative Medicine*, 16(1), 378. <http://dx.doi.org/10.1186/s12906-016-1361-0>. PMID:27681250.
- Rupérez, P., Ahrazem, O., & Leal, J. A. (2002). Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *Journal of Agricultural and Food Chemistry*, 50(4), 840–845. <http://dx.doi.org/10.1021/jf010908o>. PMID:11829654.
- Wang, K. P., Wang, J., Li, Q., Zhang, Q., You, R., Cheng, Y., Luo, L., & Zhang, Y. (2014). Structural differences and conformational characterization of five bioactive polysaccharides from *Lentinus edodes*. *Food Research International*, 62, 223–232. <http://dx.doi.org/10.1016/j.foodres.2014.02.047>.
- Wang, L., & Li, X. (2019). Preparation, physicochemical property and *in vitro* antioxidant activity of zinc-Hohenbuehelia serotina polysaccharides complex. *International Journal of Biological*

- Macromolecules*, 121, 862-869. <http://dx.doi.org/10.1016/j.ijbiomac.2018.10.118>. PMID:30342126.
- Xu, N., Ren, Z., Zhang, J., Song, X., Gao, Z., Jing, H., Li, S., Wang, S., & Jia, L. (2017). Antioxidant and anti-hyperlipidemic effects of mycelia zinc polysaccharides by *Pleurotus eryngii* var. *tuoliensis*. *International Journal of Biological Macromolecules*, 95, 204-214. <http://dx.doi.org/10.1016/j.ijbiomac.2016.11.060>. PMID:27871790.
- Yao, Q., Lin, Q., Yan, S. A., Huang, M., & Chen, L. (2021). Dietary risk assessment of fluoride, lead, chromium, and cadmium through consumption of Tieguanyin tea and white tea. *Food Science and Technology*, 41(3), 782-789. <http://dx.doi.org/10.1590/fst.69220>.
- Zhang, M., Zhao, H., Shen, Y., Wang, Y., Zhao, Z., & Zhang, Y. (2020). Preparation, characterization and antioxidant activity evaluation in vitro of *Fritillaria ussuriensis* polysaccharide-zinc complex. *International Journal of Biological Macromolecules*, 146, 462-474. <http://dx.doi.org/10.1016/j.ijbiomac.2020.01.002>. PMID:31923487.
- Zhang, Y., Khan, M. Z. H., Yuan, T., Zhang, Y., Liu, X., Du, Z., & Zhao, Y. (2019). Preparation and characterization of *D. opposita* Thunb polysaccharide-zinc inclusion complex and evaluation of anti-diabetic activities. *International Journal of Biological Macromolecules*, 121, 1029-1036. <http://dx.doi.org/10.1016/j.ijbiomac.2018.10.068>. PMID:30342130.
- Zhao, C., Tang, G., Cao, S., Xu, X., Gan, R., Liu, Q., Mao, Q., Shang, A., & Li, H. (2019). Phenolic profiles and antioxidant activities of 30 tea infusions from green, black, oolong, white, yellow and dark teas. *Antioxidants*, 8(7), 215. <http://dx.doi.org/10.3390/antiox8070215>. PMID:31295859.
- Zheng, L., Liu, M., Zhai, G.-Y., Ma, Z., Wang, L.-Q., & Jia, L. (2015). Antioxidant and anti-ageing activities of mycelia zinc polysaccharide from *Pholiota nameko* SW-03. *Journal of the Science of Food and Agriculture*, 95(15), 3117-3126. <http://dx.doi.org/10.1002/jsfa.7048>. PMID:25511755.
- Zheng, L., Zhai, G., Zhang, J., Wang, L., Ma, Z., Jia, M., & Jia, L. (2014). Antihyperlipidemic and hepatoprotective activities of mycelia zinc polysaccharide from *Pholiota nameko* SW-02. *International Journal of Biological Macromolecules*, 70, 523-529. <http://dx.doi.org/10.1016/j.ijbiomac.2014.07.037>. PMID:25077837.
- Zhou, H., Wang, S., Wang, Z., Xie, W., Wang, C., & Zheng, M. (2022a). Preparation, characterization and antioxidant activity of polysaccharides selenides from Qingzhuan Dark Tea. *Food Science and Technology*, 42, e108421. <http://dx.doi.org/10.1590/fst.108421>.
- Zhou, H., Wang, Z., Ma, H., Wang, S., Xie, W., Chen, Y., Xie, C., Guo, A., Wang, C., & Zheng, M. (2022b). Characterisation and antioxidant activity of polysaccharide iron (III) complex in Qingzhuan Dark Tea. *Food Science and Technology*, 42, e119421. <http://dx.doi.org/10.1590/fst.119421>.