


Cite this: *RSC Adv.*, 2025, 15, 41850

Received 17th July 2025
Accepted 22nd October 2025
DOI: 10.1039/d5ra05142h
rsc.li/rsc-advances

Preparation, characterization, and antioxidant activity of gallic acid grafted purple sweet potato polysaccharide

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In this study, alkali-soluble purple sweet potato polysaccharide (PSAP) was conjugated with gallic acid (GA) through a free radical grafting reaction. The GA-g-PSAP conjugate's structure was validated using Fourier transform infrared spectroscopy, X-ray diffraction, and scanning electron microscopy. The GA content measured in GA-g-PSAP was 13.5 mg GAE per g, and antioxidant assays confirmed that GA-g-PSAP exhibited stronger radical scavenging activity and higher reducing power than native PSAP.

1. Introduction

Polysaccharides, which are essential active substances required by organisms, exhibit diverse health-promoting properties, including antioxidant,¹ anti-inflammatory,² antitumor,³ anti-diabetes,⁴ and immunoregulatory effects.⁵ Purple sweet potato, a variety of sweet potato (*Ipomoea batatas*), is a rich source of nutrients such as proteins, carbohydrates, dietary fiber, anthocyanins, unsaturated fatty acids, and minerals.⁶ Notably, natural polysaccharides extracted from purple sweet potatoes have demonstrated a range of beneficial effects, including antioxidant, hepatoprotective, antitumor, and anti-inflammatory effects.^{7–9}

In our preliminary work, two crude polysaccharides were obtained from Xu Zishu series purple sweet potatoes using a continuous alkaline extraction process. Furthermore, the antioxidant, immunomodulatory, and intestinal microbiota-regulating effects of these alkali-soluble crude polysaccharides were preliminarily assessed.¹⁰ The chemical structure of alkali-soluble polysaccharides derived from purple sweet potato was analyzed, focusing on key structural characteristics, including monosaccharide composition, linkage sequence, glycosidic bond type, and connectivity modes. The role of alkali-soluble polysaccharides from purple sweet potato in enhancing host immunity, restoring intestinal barriers, and improving gut microbiota pathways to regulate intestinal inflammation has been elucidated.^{11,12} Among these, the purple sweet potato alkali-soluble polysaccharide (PSAP), extracted using a dilute alkaline solution, exhibited a relative molecular weight of 180 kDa. The monosaccharide composition of PSAP includes glucose, rhamnose, mannose, arabinose, and xylose. In contrast

to simple linear water-soluble purple sweet potato polysaccharides, PSAP possesses a complex multibranch structure and a relatively high molecular weight. Additionally, most PSAP molecules display hemicellulose-like structural characteristics and are insoluble in water. Previous research has demonstrated that the poor water solubility of PSAP results in only moderate DPPH radical scavenging activity and reducing power.¹³ To improve its water solubility and increase its application potential, chemical modification of hydroxyl groups within PSAP can be employed, thus improving both its water solubility and biological activity.

In recent years, graft copolymerization reactions mediated by free radicals have been extensively employed in the modification of polysaccharides.^{14,15} Compared to other grafting methods, such as chemical coupling¹⁶ and enzymatic catalysis,¹⁷ free radical mediated methods have the advantages of simple process, mild conditions, economical and environmental protection. In addition, the reaction occurs at room temperature under inert gas protection, which effectively avoids the degradation of polyphenols. Therefore, the yield of graft products is higher than other methods, with fewer by-products and avoiding the production of toxic compounds.¹⁸ Furthermore, this method can also be used for amino free polysaccharides such as sweet potato polysaccharides in this study, which can increase the diversity of natural polyphenol polysaccharide grafts. However, due to the ability of oxygen to capture free radicals and reduce conjugation efficiency, this reaction should occur in inert air. Moreover, the grafting efficiency is influenced by various factors, and the reaction parameters for synthesizing phenolic acid grafted polysaccharides, such as the concentration, temperature, and time of ascorbic acid, polysaccharides, and phenolic acid compounds, should be optimized.

Grafting compounds, particularly phenolic functional groups, onto polysaccharides with functional groups has been

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shown to enhance the biological activities of polysaccharides and expand their potential application areas. Gallic acid (GA) is a prominent polyphenolic compound that has long been part of the human diet. GA and its derivatives, such as octyl gallate, display a wide range of biological activities, such as antioxidant, antibacterial, antitumor, anti-inflammatory, anti-melanogenesis, antiviral, anti-allergic, and neuroprotective properties.¹⁹ Studies have demonstrated that grafting GA onto natural polysaccharides such as chitosan, carboxymethyl chitosan, chitosan oligosaccharides, inulin, cellulose, and lignin not only enhances GA stability but also improves the biological activities of both GA and the polysaccharides.^{20–23} However, there are currently no reported studies on GA-grafted sweet potato polysaccharides.

Therefore, to enhance the antioxidant capacity of PSAP, this study aimed to graft gallic acid onto PSAP based on the Vc/H₂O₂ redox pair system. The structural characteristics and *in vitro* antioxidant activities of the GA-grafted PSAP grafts (GA-g-PSAP) were evaluated. The present study may offer a novel means of improving the antioxidant potential of PSAP.

2. Materials and methods

2.1. Materials and reagents

Purple sweet potato alkali-soluble polysaccharide (PSAP) was prepared in-house at our laboratory. Vitamin C (Vc), gallic acid, Folin–Ciocalteu reagent, DPPH, FeSO₄, salicylic acid, and H₂O₂ were all obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Synthesis of GA grafted PSAP (GA-g-PSAP)

Refer to existing methods,²⁰ GA-g-PSAP was prepared *via* a Vc/H₂O₂ redox pair system. In short, PSAP, VC, and GA (1 : 0.1 : 2) was dissolved in distilled water, and then a slow stream of nitrogen gas was passed through the flask. Next, 5M H₂O₂ solution was added to the reaction system and reacted at room temperature for 12 h. Finally, the reaction system was dialyzed and freeze-dried to obtain the graft product GA-g-PSAP. The grafting ratio of GA-g-PSAP was measured and calculated by the Folin–Ciocalteu method.²⁴

2.3. Characterization of GA-g-PSAP

A sample amount of 2 mg was weighed and mixed with dry KBr (25 mg), then pressed into tablets with a thickness of 1.0 mm. FT-IR spectra were recorded in the frequency range of 4000–400 cm^{−1} using an FT-IR spectrometer (Cary 670 FT-IR, Agilent, USA) with KBr pellets.

An X-ray diffractometer (Bruker, D8 Advance A25, Germany) were used to detect the samples. The Cu K α radiation source operating at a voltage of 40 kV and a current of 40 mA was used, and the diffraction angle (2θ) range was 10°–90°.

SEM images of the sample was taken using a field emission scanning electron microscope (S-48001 FESEM, Hitachi High-Techologies Corporation, Japan) at the accelerating voltage of 15 kV.

2.4. The molecular weight of GA-g-PSAP

According to our previous method,²⁵ the Mw of GA-g-PSAP was conducted based on a high-performance gel permeation chromatography (HPGPC) system (Shimadzu, Kyoto, Japan) outfitted with an RI-10 A differential refractive index detector (Shimadzu, Kyoto, Japan). For each run, 50 μ L of 5 mg mL^{−1} sample solution was injected using tandem columns BRT105-103-101 (Borui Saccharide, Yangzhou, China), followed by elution of the sample under 0.7 mL min^{−1} flow rate using mobile phase (0.2 M NaCl).

2.5. Antioxidant activity of GA-g-PSAP *in vitro*

2.5.1 Determination of DPPH radical scavenging activity.

The DPPH radical scavenging activity was assayed according to the method of Liu *et al.* with some modifications.²⁶ Briefly, 0.2 mL of DPPH solution (0.4 mM) was mixed with 1.0 mL of sample (0.2–1.2 mg mL^{−1}) and 2.8 mL of water. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance of the mixture was recorded at 517 nm. Vitamin C (Vc) was used as the positive control.

2.5.2 Determination of hydroxyl radical scavenging activity. The hydroxyl radical scavenging activity was determined according to the method of Tang²⁷ with some modifications. The mixture containing 1 mL of sample (0.2–1.2 mg mL^{−1}), 1 mL of 9 mM FeSO₄ and 1 mL of 8.8 mM H₂O₂ in salicylic acid–ethanol solution was shaken vigorously and incubated at 37 °C for 30 min. Then, the absorbance of the reaction mixture was determined at 510 nm. Vc was used as the positive control.

2.6. Cell culture

RAW264.7 cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL of penicillin and 100 μ g mL^{−1} of streptomycin in a humidified atmosphere containing 5% CO₂ at 37 °C.

2.7. Determination of the cytotoxicity of GA-g-PSAP

The cytotoxicity of GA-g-PSAP was determined using cell counting kit-8 (CCK-8). In short, RAW264.7 cells were seeded into a 96 well microplate and incubated for 4 h (37 °C). Subsequently, GA-g-PSAP was treated with different concentrations at 37 °C for 12 h. Finally, CCK-8 solution was added and incubated for 1 h, and each plate was read at 450 nm.

2.8. Stability of GA-g-PSAP

The stability of GA-g-PSAP was evaluated by measuring the changes of GA content in GA-g-PSAP under different storage durations. The GA-g-PSAP was dissolved in deionized water at a concentration of 0.5 mg mL^{−1} and stored under refrigeration (4 °C) for up to 15 days. The GA content at each time point was determined in accordance with the method in Section 2.2.



2.9. Statistical analysis

Data were presented as mean \pm standard derivation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. Graphs were generated using Origin 2021 software.

3. Results and discussion

3.1. Synthesis of GA-g-PSAP

In this study, GA was successfully grafted onto PSAP through free radical mediation. During this process, under the interaction between Vc and H_2O_2 oxidation-reduction, the hydroxyl groups of PSAP could capture free electrons to form polysaccharide free radicals.²⁸ GA acted as a receptor for PSAP large free radicals and bound to PSAP to form graft conjugates.²⁹ The grafting ratio of GA-g-PSAP was determined to be 13.5 mg GAE per g (GA equivalents per g).

The speculatively synthetic mechanisms of phenolic grafted polysaccharides in Vc/ H_2O_2 redox system have been revealed by Liu *et al.*²⁶ In this study, GA was grafted onto PSAP using Vc and H_2O_2 redox system under nitrogen gas. Firstly, Vc could react with H_2O_2 to generate hydroxyl radical (HO^\bullet) which subsequently initiated the grafting process (Fig. 1a). Then, the formed HO^\bullet could abstract hydrogen atom from PSAP molecules with consequent formation of PSAP macro radicals. The GA which were in close vicinity of the reaction site become acceptor of PSAP macro radicals, and thus GA-g-PSAP formed (Fig. 1b).

3.2. Characterization of GA-g-PSAP

The FT-IR spectra of PSAP and GA-g-PSAP are shown in Fig. 2a. The FT-IR spectra of both PSAP and GA-g-PSAP exhibited prominent characteristic peaks at 3271, 2931, 1663, 1414, 1150, 1086 and 1015 cm^{-1} , corresponding to the stretching vibrations of O-H, C-H, COO^- , C-OH, C-O, and C-C groups.³⁰ These results suggest that no major changes in functional groups occurred during the grafting process. Notably, a new absorption band appeared near 1565 cm^{-1} , which was attributed to the typical C=C stretching vibration of the conjugated GA moieties, further confirming the successful grafting of GA onto PSAP chains. Similar results were obtained in other studies.^{31,32}

X-ray diffraction (XRD) is used to obtain information about internal atomic structure and morphology. Normally, crystalline materials exhibit narrow diffraction peaks, while amorphous components display broad peaks. The XRD pattern of PSAP exhibited two broad signal peaks at 16.9° and 20.5° (Fig. 2b), confirming PSAP's amorphous nature. In contrast, GA-g-PSAP displayed three additional sharp, narrow diffraction peaks at 14.3°, 26.5°, and 32.3°, describing to the crystalline peaks of GA.³³ These findings indicate that GA-g-PSAP possesses higher crystallinity than PSAP.

Fig. 3 shows the SEM images of PSAP and GA-g-PSAP. The SEM micrograph of PSAP (Fig. 3a) displayed relatively compact flake structures. Conversely, in the SEM micrographs of GA-g-PSAP (Fig. 3b), the surface appeared rougher and larger, which may be closely associated with the grafted GA. Similar results have been obtained in studies involving other phenolic acid-grafted polysaccharides.³⁴

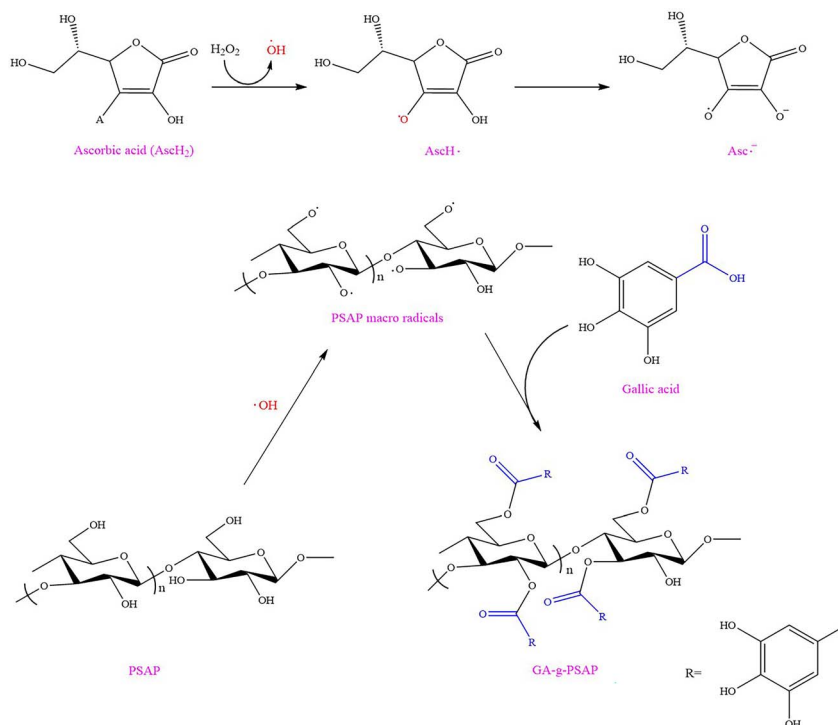


Fig. 1 The proposed mechanism for the synthesis of GA-g-PSAP using Vc/ H_2O_2 redox system under nitrogen gas.



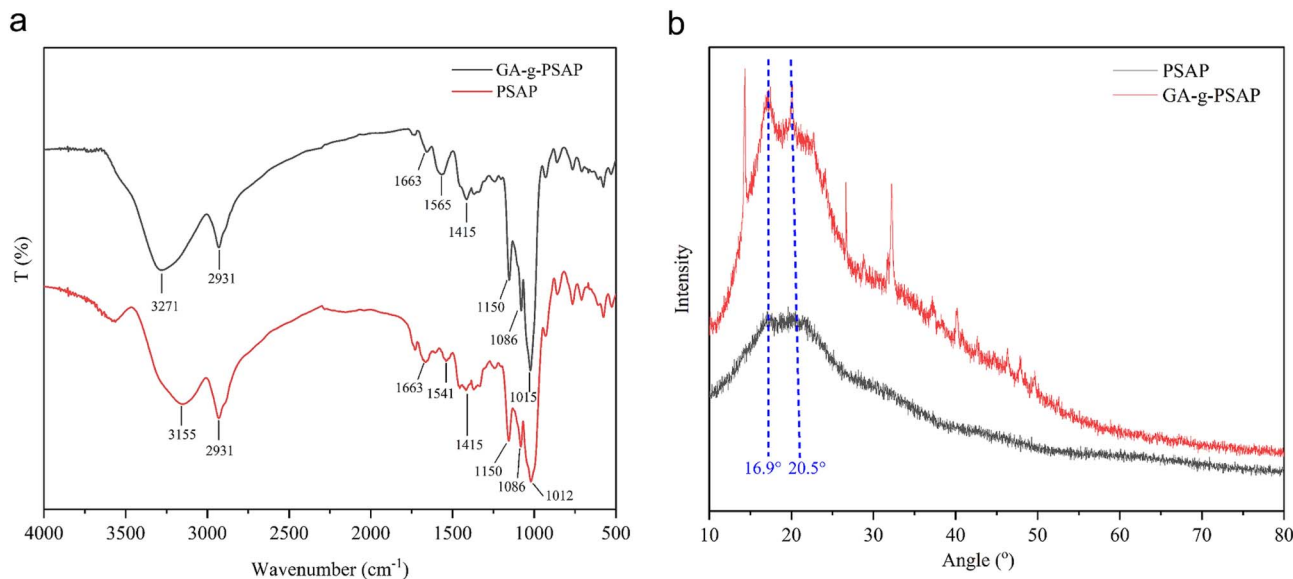


Fig. 2 FT-IR spectra (a) and typical XRD patterns (b) of PSAP and GA-g-PSAP.

3.3. The molecular weight of GA-g-PSAP

As showed in Table 1, the molecular weight of GA-g-PSAP complex (78.30 kDa) was lower as compared with that of PSAP (180 kDa), which indicated the partial degradation of PSAP caused by 'OH during the synthesis of the complex, which was common in the grafting reactions.³⁵

Table 1 The molecular weight of GA-g-PSAP and PSAP^a

Samples	GA-g-PSAP	PSAP ¹¹
M_w (kDa)	78.30	180

^a M_w are weight-average molecular mass.

3.4. Antioxidant activity of GA-g-PSAP

The stable DPPH radical scavenging model is widely employed to evaluate and screen the antioxidant activity of various substance extracts.³⁶ As shown in Fig. 4a, the DPPH radical scavenging activities of PSAP and GA-g-PSAP increased in a concentration-dependent, reaching 9.06% and 32.91% at 1.2 mg mL⁻¹, respectively. Clearly, PSAP exhibited significantly lower scavenging activity than GA-g-PSAP. Nevertheless, both

the polymers demonstrated weaker DPPH radical scavenging effects than gallic acid and Vc at the same concentrations.

Hydroxyl radicals, which exhibit high chemical reactivity, can attack cells, damaging biomolecules such as DNA.³⁷ The scavenging activities of all samples increased with the increase in concentrations. At a concentration of 1.2 mg mL⁻¹, the scavenging rates of PSAP and GA-g-PSAP were 6.21% and 61.86%, respectively (Fig. 4b). Additionally, Vc showed superior

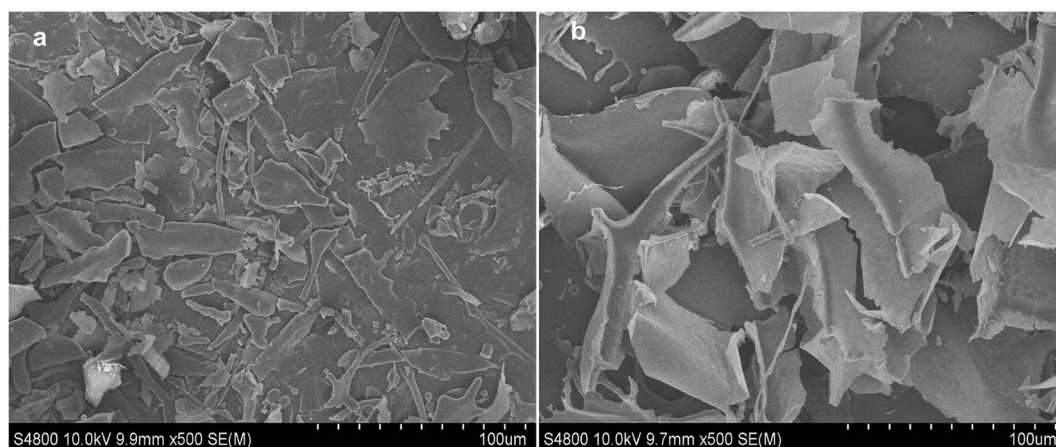


Fig. 3 SEM images of PSAP (a) and GA-g-PSAP (b) samples. The magnification of regions marked by uppercase and lowercase letters was 500 \times .

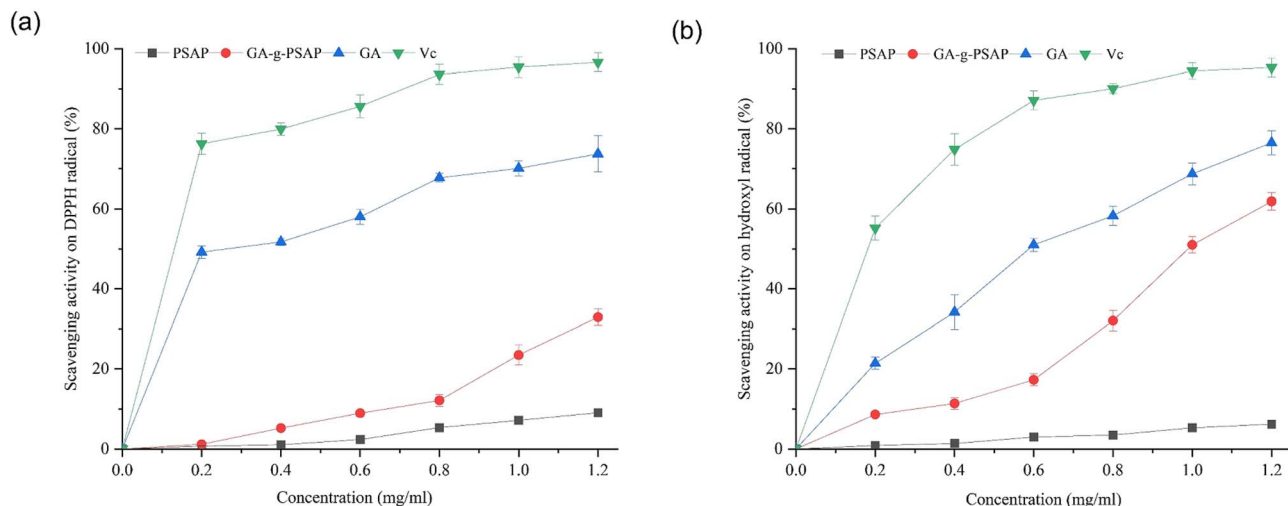


Fig. 4 DPPH radical (a) and hydroxyl radical (b) scavenging activities of PSAP, GA-g-PSAP, GA, and Vc. Data are presented as mean \pm SD from triplicate experiments.

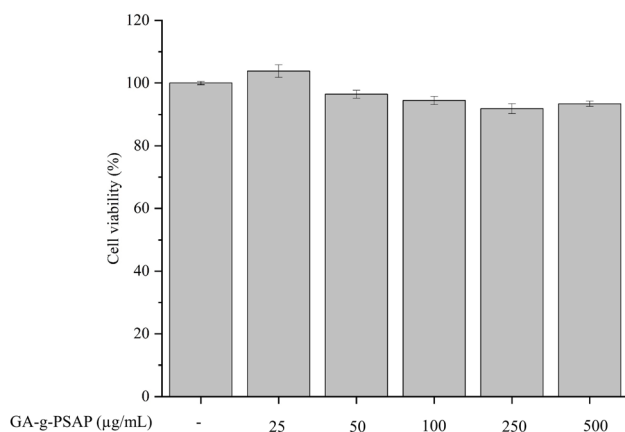


Fig. 5 Effect of GA-g-PSAP (25–500 $\mu\text{g mL}^{-1}$) on the cell viability of RAW264.7 cells.

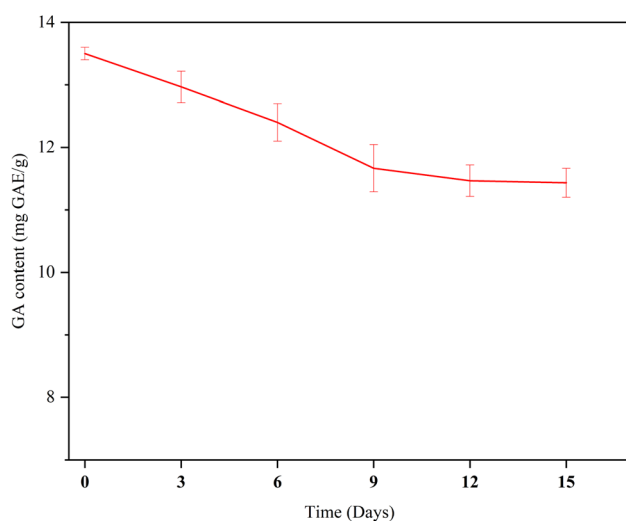


Fig. 6 Stability of gallic acid-PSAP conjugate (GA-g-PSAP) at 4 °C.

hydroxyl radical scavenging activities compared to both PSAP and GA-g-PSAP.

3.5. Cytotoxicity of GA-g-PSAP on RAW264.7 cells

In order to evaluate the cytotoxicity of GA-g-PSAP, RAW264.7 cells were treated with different concentrations (25–500 $\mu\text{g mL}^{-1}$) of GA-g-PSAP. As depicted in Fig. 5, the GA-g-PSAP showed no cytotoxic effects on RAW264.7 cells at the tested concentrations after 24 h of treatment. The cell viability of the normal control group was set as 100%, and the relative cell viability of GA-g-PSAP treatment groups were over 90%.

3.6. Stability of GA-g-PSAP

PSAP is covalently bound to GA, which plays a positive role in the stability of GA-g-PSAP. In the present work, we evaluated the stability of GA-g-PSAP under refrigeration (4 °C) condition by detecting the GA content of grafts at different time points (Fig. 6). The results showed that the GA content slightly decreased with the extension of storage time. On the 15th day, the GA content was 11.01 mg GAE per g, indicating that GA-g-PSAP had good stability within a certain period of time.

4. Conclusion

In this work, the modification of PSAP with gallic acid was achieved by using the Vc/ H_2O_2 redox pair system under an inert atmosphere. The spectroscopic results of FT-IR and XRD both confirmed the successful grafting. Moreover, GA-g-PSAP showed higher antioxidant activity than PSAP, suggesting the antioxidant ability of PSAP could be enhanced by grafting with antioxidant molecules. Collectively, our results suggest that GA-g-PSAP could be used in food and pharmaceutical industries as a novel antioxidant.



Author contributions

Wenting Zhang: writing – original draft, investigation. Ruixue Yue and Hao Guo: formal analysis. Yi Zhang and Chen Ma: software. Hong Zhu: validation. Shaoying Deng: methodology. Jian Sun: writing – review & editing.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Data availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

This research was funded by the Earmarked Fund for CARS-10-Sweetpotato (CARS-10) and Xuzhou Science and Technology Project (KC23074).

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