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journal homepage: <https://www.sciopen.com/journal/2097-0765>Curcumin delivery nanoparticles based on Maillard reaction of *Haematococcus pluvialis* protein/galactose for alleviating acute alcoholic liver damageXinyi Liu^{a,b}, Yukun Song^{a,b}, Shasha Cheng^{a,b,*}, Mingqian Tan^{a,b,*}^a School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China^b National Engineering Research Center of Seafood, Dalian 116034, China

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ABSTRACT

The aim of this study is to investigate the feasibility of Maillard reaction products of *Haematococcus pluvialis* protein and galactose (HPP-GAL) for improving the bioactivities of curcumin (CUR) for alleviating alcoholic liver damage. CUR was embedded into HPP-GAL nanoparticles by the self-assembly of hydrogen bonding and hydrophobic interaction with the particle size around 200 nm. HPP-GAL enhanced the encapsulation efficiency and loading amount of CUR with the value of $(89.21 \pm 0.33)\%$ and $(0.500 \pm 0.004)\%$, respectively. The stabilities of CUR under strong acid, salt ion stability and ultraviolet irradiation conditions were improved by the encapsulation. HPP-GAL-CUR nanoparticles exhibited excellent concentration-dependent *in vitro* antioxidant activities including DPPH and ABTS scavenging rates, and better protective effect on CUR against gastric acid environment as well as longer release of CUR in simulated intestinal fluid. In addition, the HPP-GAL-CUR delivery system possessed liver targeting property due to the existence of GAL, which could effectively alleviate the alcohol-induced liver damage and the inflammation indexes by inhibiting the oxidative stress. Therefore, HPP-GAL-CUR nanoparticles might be a potential candidate system for the prevention of alcoholic liver damage in the future.

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1. Introduction

Alcoholic liver damage has become a healthy issue with worldwide concern, and more than 3 million people die each year due to alcohol abuse according to the statistical data by World Health Organization^[1–2]. In recent years, nutritional interventions has widely attracted attentions as an effective means of prevention from alcoholic liver damage^[3]. A large number of functional substances with biological activities based on antioxidant mechanism have been observed in natural foods^[4]. Curcumin (CUR) is a phenolic pigment extracted from the roots and stems of Zingiberaceae and Araceae pla

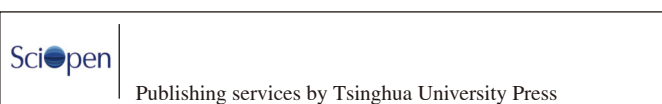
CUR, which has antioxidant, antiviral, anti-inflammatory and other functional activities^[5]. Although oral CUR is safe in theory, its high double bond structure, including carbonyl and phenolic hydroxyl groups leads to its low water solubility, low bioavailability and poor storage stability, which limit its practical application in nutrition and health food^[6].

Encapsulation has been recognized as an important way to improve the water solubility, stability and bioavailability of functional active substances. Nowadays, several delivery carriers such as liposomes, polysaccharides, proteins have been applied to encapsulate CUR. Among these carriers, foodborne proteins attract much attention as wall materials for nanoparticle delivery system due to their amphipathy, biocompatibility, degradability, and environmental friendliness. Proteins such as casein, soy protein isolate, zein and whey protein isolate have been extensively explored in the construction of the nano delivery systems, which could enhance the bioavailability and the stability of food derived bioactive substances

* Corresponding authors at: School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China.

E-mail address: mqtan@dlpu.edu.cn (M.Q. Tan); chengshasha880321@126.com (S.S. Cheng)

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such as CUR, astaxanthin and epigallocatechin gallate^[7]. However, protein-based nanocarriers are easy to aggregate and precipitate under extreme pH value and high ionic strength^[8].

Non-enzymatic Maillard reaction between the reducing-end carbonyl groups of reducing sugars and the amine residues of proteins is an effective methods to improve the protein function and properties such as emulsifying ability, solubility, stability, and antioxidation^[9–10]. Glycosylated proteins via Maillard reaction has been proved to prevent the precipitation of the protein-bioactive complexes and enhance the encapsulation efficiency of protein to bioactives^[11]. Rutin loaded nanoparticles with 98.8% entrapment rate and 7.1% loading rate were prepared using Maillard conjugates from corn protein and hydrolysate carboxymethyl chitosan, which displayed superior stability under different NaCl concentrations and pH levels^[12]. The zein nanoparticles with dextran on the surface through Maillard reaction had 74.6% an 55.1% loading efficiency for insulin and cholic acid, and could prevent the hydrolysis of insulin in digestive fluid and enhance the trans mucus permeability of insulin^[13]. Bovine serum albumin-dextran conjugates prepared through Maillard reaction was applied to construct the CUR loaded nanoparticles by self-assemble, and the delivery system showed better thermal stability and cell antioxidant capacity^[14]. Glycosylated α -lactalbumin-based nanocomplex was developed for CUR via Maillard reaction with dextran, and the environmental and oxidative stabilities of CUR were significantly improved compared to α -lactalbumin^[15]. However, there was less consideration about the targeted delivery of bioactives in the development of nanocarriers based on the glycosylated proteins via Maillard reaction.

Galactosylated ligands can be recognized by the asialoglycoprotein receptors over expressed in hepatic cells via specific receptor-ligands interaction, and galactosylated nanocarriers are attractive for liver-targeted delivery systems to relieve liver damage. Hepatic targeted astaxanthin nanocarriers were achieved based on the Maillard reaction of galactose and whey protein isolate, which could effectively enhance the relief of astaxanthin for nonalcoholic fatty liver disease due to the high affinity between asialoglycoprotein receptors and galactose ligands^[16]. *Haematococcus pluvialis* is a kind of freshwater unicellular green algae and rich in astaxanthin, which has many physiological functions such as strong antioxidant capacity and enhancing immunity^[17]. In addition to the astaxanthin, the high content of protein in *H. pluvialis* is also a valuable resource and has gradually attracted considerable attention due to its excellent emulsifying capacity, emulsifying stability, foaming property and amphipath in recent years^[18–19]. Aqueous protein extracted from *H. pluvialis* residues through ammonium sulfate precipitation has been successfully applied as a stabilizer for Pickering emulsion^[20]. However, there was few research on the application of *H. pluvialis* protein in the construction of nanoparticles delivery systems.

In this study, *H. pluvialis* protein-galactose nanocomplex (HPP-GAL) were prepared by Maillard reaction to develop a delivery system of CUR with high encapsulation efficiency for the alleviation of alcoholic liver damage. Firstly, the degree of Maillard reaction was monitored by browning index and grafting degree, and the products were characterized by Fourier transform infrared spectrometer (FTIR) and fluorescence spectrum. Then, the HPP-GAL-CUR delivery system was characterized and its stability under strong acid, high ion strength and ultraviolet (UV) irradiation were evaluated. In addition, the

DPPH and ABTS free radical scavenging activity of HPP-GAL-CUR nanoparticles and the release characteristics of CUR during *in vitro* simulated digestion were investigated. Finally, the liver targeting ability of HPP-GAL-CUR and its alleviating effect on alcoholic liver damage were evaluated through *in vivo* experiments.

2. Materials and methods

2.1 Materials

The *H. pluvialis* powder was purchased from Kunming Biogenic Co., Ltd. (Yunnan, China). D-Galactose (98% purity) and CUR (98% purity) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). The kits of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (TCHO), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd. (Nanjing, China).

2.2 Extraction of *H. pluvialis* protein

The *H. pluvialis* powder was homogenized after the addition of 2% NaCl solution at 1:20 (*m/V*). After 120 min ultrasonic treatment, the mixture was centrifuged at 7 000 r/min for 15 min at 25 °C (Hitachi high speed refrigerated centrifuge, Hitachi Ltd., Tokyo, Japan). The supernatant was collected, and ammonium sulfate was added to achieve the concentration of 0.662 g/mL. The mixture was stirred until the complete dissolution of ammonium sulfate and centrifuged at 10 000 r/min for 10 min at 4 °C (Cence H1850R centrifuge, Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Hunan, China) to collect the precipitate. Finally, the supernatant was discarded, and the precipitate was dissolved in phosphate-buffered saline (PBS) buffer for further experiments. The protein in the extract was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the protein content was measured using Kjeldahl method.

2.3 Preparation of HPP-GAL by Maillard reaction

GAL powder was added to the HPP solution of 10 mg/mL with a ratio of 1:4 (*m/m*). The pH value of the solution was adjusted to 9 using 1 mol/L NaOH and heated in a water bath at 90 °C for Maillard reaction in a tightly closed container. During the heating process, 1 mL of the solution were sampled every hour for the characterization of Maillard reaction degree.

2.4 Characterization of Maillard reaction degree

The browning index, grafting degree, FTIR and fluorescence spectrum of the sample were measured to evaluate the Maillard reaction degree. Among these, the browning index was measured by ultraviolet spectrophotometer (PerkinElmer, Cambridge, USA) at 420 nm referring to Han's publication^[21]. The free amino groups of the HPP-GAL were analyzed by the *o*-phthalic aldehyde method to determine the grafting degree^[22]. A FTIR spectrometer (thermo science, Massachusetts, USA) was performed on the tablets of the

lyophilized sample and KBr powder at 1:100 ratio in the region of 400 to 4 000 cm^{-1} . Fluorescence emission spectrum of the sample solution (0.25 mg/mL) in the range of 290–450 nm was obtained by a F-2700 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) with a 280 nm excitation wavelength.

2.5 Preparation of HPP-CUR and HPP-GAL-CUR nanoparticles

CUR ethanol solution of 1 mg/mL was prepared, and mixed with HPP or HPP-GAL solution (10 mg/mL) in different proportions of 1:12, 1:15, 1:18, 1:21 (*V/V*). After the stirring at 10 000 r/min for 3 min by an Ultra Turrax T25 high-speed blender (IKA, Staufen, Germany), the ethanol was evaporated by a rotator (EYELA N-1100, Tokyo, Japan) at 37 °C. The solution was centrifuged to remove the free CUR, and the obtained solutions of HPP-CUR and HPP-GAL-CUR nanoparticles were stored in the dark at 4 °C.

2.6 Determination of astaxanthin content, encapsulation efficiency (EE) and loading amount (LA)

For the determination of astaxanthin content in HPP and HPP-GAL and the CUR content in HPP-CUR and HPP-GAL-CUR nanoparticles, 2 mL of ethanol was mixed with 2 mL sample solution of 1 mg/mL and vibrated for 1 min. Then, the mixture was added by 2 mL of *n*-hexane and oscillated on a vortex oscillator for 2 min. After the centrifugation at 3 000 r/min for 3 min at 25 °C, the *n*-hexane layer and the ethanol-aqueous layer were collected and measured at 470 and 425 nm using a UV spectrometer, respectively. The astaxanthin and CUR contents were calculated based on the absorbance value of *n*-hexane layer and ethanol-aqueous layer according to the standard curve of $y = 0.0034x + 0.0056$ and $y = 0.083x + 0.030$ EE (%) and LA (%) using the formula (1) and (2):

$$\text{EE (\%)} = \frac{\text{encapsulated CUR content}}{\text{total CUR input}} \times 100 \quad (1)$$

$$\text{LA (\%)} = \frac{\text{encapsulated CUR content}}{\text{total HPP}} \times 100 \quad (2)$$

2.7 Scanning electron microscope, particle size and polydispersity index (PDI)

CUR, HPP-CUR and HPP-GAL-CUR nanoparticles were lyophilized and uniformly coated on the conductive adhesive, which were observed using a SU8010 scanning electron microscope (SEM, Hitachi, Tokyo, Japan) at a magnification of 10 000 $\times$, 30 000 $\times$ and 50 000 $\times$ after gold sputtering. The particle size and PDI of HPP-CUR and HPP-GAL-CUR nanoparticles were measured at 25 °C using a Marvin Laser Particle Sizer (Zetasizer, Marvin, UK). The multiple scattering effects were avoided by the dilution of sample using deionized water before measurement.

2.8 Spectroscopic characterization

Sample tablets for FTIR analysis were prepared using the mixture of sample and KBr powder at a ratio of 1:100 and scanned in the spectral region of 400 cm^{-1} to 4 000 cm^{-1} .

The fluorescence spectra of samples were measured at excitation wavelengths of 285 and 425 nm by the F-2700 fluorescence spectrometer, and the corresponding scanning range of emission spectrum were 295–450 nm and 435–700 nm, respectively.

The sample solution of 0.1 mg/mL was scanned from 200 to 700 nm by a UV spectrophotometer (Hitachi, Tokyo, Japan) to obtain the ultraviolet absorption spectrum.

The 2θ values of samples were determined by an X-ray diffractometer (XRD-7000s, Shimadzu, Kyoto, Japan) from 10 to 80 at a speed of 10 /min.

2.9 Stability evaluation

In this section, the effects of strong acid, salt ions, UV light and temperature on the stability of CUR were investigated. For strong acid intervention stability, the CUR-ethanol solution, HPP-CUR, and HPP-GAL-CUR were dispersed in hydrochloric acid aqueous solution of pH 2.0 until the concentration of 2.5 mg/mL, which were placed in dark at 25 °C for 30, 60, 90, 120, 150 and 180 min. For ionic stability, the samples were mixed with sodium chloride solutions of 0, 50, 100, 100, 150 and 200 mmol/L at pH 4.0. After 1 h stirring, the mixture was placed in dark for 24 h at 25 °C and measured. For UV stability, 2 mg/mL sample solution was exposed to a UV lamp at 365 nm under 25 °C for 1, 2, 3, 4 and 5 h, respectively. In addition, about 20 mg sample was heated from 40 °C to 600 °C at a rate of 10 °C/min under nitrogen atmosphere to obtain the thermogravimetric analysis (TGA) curves.

2.10 In vitro release characteristics of CUR

The release curve of CUR *in vitro* was determined according to the method in previous publication^[23]. Firstly, 7.5 mL sample and 7.5 mL simulated gastric juice (SGF, pH 2, 1.1 mg/mL pepsin, 40 mmol/L sodium chloride and 0.5% Tween 20) were mixed and incubated for 90 min at 37 °C with stirring at 100 r/min. Then, 15 mL simulated intestinal fluid (SIF, pH 7, 4 mg/mL trypsin and 40 mmol/L sodium chloride) was added to the solution and the pH value of the mixture was adjusted to 7. The mixture was incubated at 37 °C for 2 h with stirring at 100 r/min to simulate the intestinal digestion. During the digestion process, 2 mL solution was sampled at 15, 30, 90, 120, 150, 180 and 210 min for CUR content determination according to the method in section 2.6. The release degree of CUR was calculated referring to the formula (3):

$$\text{Release rate (\%)} = \frac{\text{Weight of released CUR (mg)}}{\text{Weight of CUR added before digestion (mg)}} \quad (3)$$

2.11 Antioxidant activity

DPPH and ABTS free radical scavenging activities were determined to estimate the antioxidant activity of nanoparticles. DPPH and ABTS working solutions were prepared referring to the previous publication^[24]. For DPPH determination, 0.5 mL of sample solution and 0.5 mL of DPPH solution (0.2 mmol/L) was mixed in a cuvette. After 30 min reaction in dark at room temperature, the absorbance was determined at 517 nm. For ABTS determination, 20 μL of sample solution and 150 μL of ABTS solution were mixed well in the well of a 96 well plate. After 30 min reaction at room

temperature, the absorbance was determined at 734 nm. Samples mixed with the solvent as a blank group, and the solvent mixed with DPPH ethanol/ABTS water solution as a control group. The scavenging rate of DPPH/ABTS free radicals was calculated based on the formula (4):

$$\text{DPPH/ABTS scavenging activity (\%)} = \frac{1 - (A_{\text{sample}} - A_{\text{blank}})}{A_{\text{control}}} \times 100 \quad (4)$$

In which A_{control} , A_{blank} and A_{sample} are the absorbance of control group, blank group, and sample group, respectively.

2.12 In vivo experiments

SPF C57BL/6 male black mice were purchased from Liaoning Changsheng Biotechnology Co., Ltd. The mice were 8–10 weeks old with weight about (20.00 ± 0.75) g and kept in light and darkness every 12 h at temperature of (22 ± 2) °C with $(60 \pm 5)\%$ humidity. The animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines EU Directive 2010/63/EU. To characterize the liver-targeting ability of HPP-GAL-CUR, Nile red was selected as a hydrophobic tracer instead of CUR in nanoparticles for biological imaging. Three groups of mice including free Nile red group, Nile red labeled HPP group and Nile red labeled HPP-GAL group were randomly selected, which were fed at an equivalent dose of 1.75 mg/kg body weight. The mice were sacrificed and dissected at 6, 12 and 24 h after gavage, and fluorescence images of mice's organs were obtained by MIIS XFP-BIX living imaging system (Molecular Device Corp., Sunnyvale, USA).

For evaluating the protective effect of CUR loaded nanoparticles on alcohol liver damage, 7 groups of mice including control group (PBS), damage group (PBS + alcohol), free CUR group (1.75 mg/(kg·day) Free CUR + alcohol), HPP group (HPP + alcohol), HPP-GAL group (HPP-GAL + alcohol), HPP-CUR group (1.75 mg/(kg·day) CUR in HPP-CUR + alcohol) and HPP-GAL-CUR group (2.15 mg/(kg·day) CUR in HPP-GAL-CUR + alcohol) were randomly assigned. There were 10 mice in each group. The protein concentration of HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR were all 100 mg/(kg·day). Adaptive feeding was carried out for 3 days with a diet consisting of corn, soybean meal and flour. Then, the mice were continuously given the corresponding samples by intragastric administration for 14 days. After the depriving of food overnight, the mice were given alcohol every 12 h in the next day. To avoid the death of mice, the first and second alcohol treatments were performed with 40% and 50% alcohol, respectively. After 12 h of the second alcohol treatment, the mice were weighted and killed, and the organ indexes were calculated based on the mice's body weight and organ weight. The blood was taken from the posterior eyeball vein and centrifuged at 3 000 r/min for 10 min at 4 °C (Sorvall Legend Micro 17R centrifuge, Thermo Fisher Scientific Co., Ltd., Shanghai, China) to obtain the serum. Liver slices were fixed in 10% formalin solution for hematoxylin-eosin staining and observed using a DM2500 orthotopic microscope (Leica Camera microscope, Wetzlar, Germany). ALT, AST, TCHO and TG levels of the serum and the liver oxidative stress biomarkers of the supernatant of liver homogenate including MDA, GSH, GSH-PX and SOD were measured using commercial test kits^[25].

2.13 Statistical analysis

Each experimental data was repeated at least three times, and the data were expressed as the mean $\pm$ standard deviation (SD). T-test, one-way ANOVA and Bonferroni post-processing analysis were performed using SPSS from social science software version 26.0 (Chicago, IL, USA). The curves were made using origin 2022 software (Origin Lab, Northampton, Ma, USA).

3. Results and discussion

3.1 Characterization of Maillard reaction degree

The protein content of the HPP sample was $(27.34 \pm 3.51)\%$, and the SDS-PAGE analysis in Fig. S1 showed obvious bands at 27–30, 32–35, 42–47 and 55–63 kDa, which might be attributed to subunits of phycoerythrin and phycocyanin according to the previous researches of red algal^[26–28]. The Maillard reaction between HPP and GAL was carried out according to the protocol in Fig. 1A. The browning index can be reflected by the absorbance at 420 nm and shown in Fig. 1B. In the first 4 h, the browning index increased significantly as the increase of reaction time, which indicated the occurrence of glycosylation of HPP during Maillard reaction. Zhong et al.^[29] found similar result in the study of Maillard conjugates of soy protein isolates and prebiotic oligosaccharides. However, the browning index decreased slightly after 5 h, which might be due to the formation of by-products of Maillard reaction between crude protein and GAL. Fig. 1C shows the grafting degree of HPP under heating conditions. With the progress of Maillard reaction, the degree of glycosylation increased and reached the maximum value of 24.61% after 5 h. The Maillard reaction between the carboxyl group of saccharide and the amino group of protein leads to the formation of Schiff base^[30]. The increase of the browning index and the degree of glycosylation indicated the occurrence of Maillard reaction between HPP and GAL. To minimize the production of Maillard by-products, the HPP-GAL after 4 h Maillard reaction was used in the subsequent experiment.

Fig. 1D shows the FTIR spectrum of HPP-GAL nanocomplex with different Maillard reaction times. The characteristic peaks at 3 405 and 1 075 cm^{-1} belonged to O–H stretching vibration and C–O stretching vibration, and their intensities increased with the increase of reaction time. In addition, the peaks at 1 647 and 1 539 cm^{-1} were ascribed to the amide I (80% C=O stretching) and amide II (60% N–H bending and 40% C–N stretching) bands, which were characteristic peaks of the protein and their intensity decreased obviously. The decrease in the characteristic peaks of HPP suggested the covalent binding between HPP and GAL, which depleted the amine group of the protein.

Fig. 1E shows the changes in fluorescence spectroscopy of HPP during the Maillard reaction. At the excitation wavelength of 280 nm, the maximum emission intensity of HPP was observed at 333 nm. With the progress of Maillard reaction, the fluorescence intensity of HPP-GAL gradually decreased and the peak was red shifted, which could be explained by the shielding effect of GAL, and the shielding effect was more obvious with higher grafting degree.

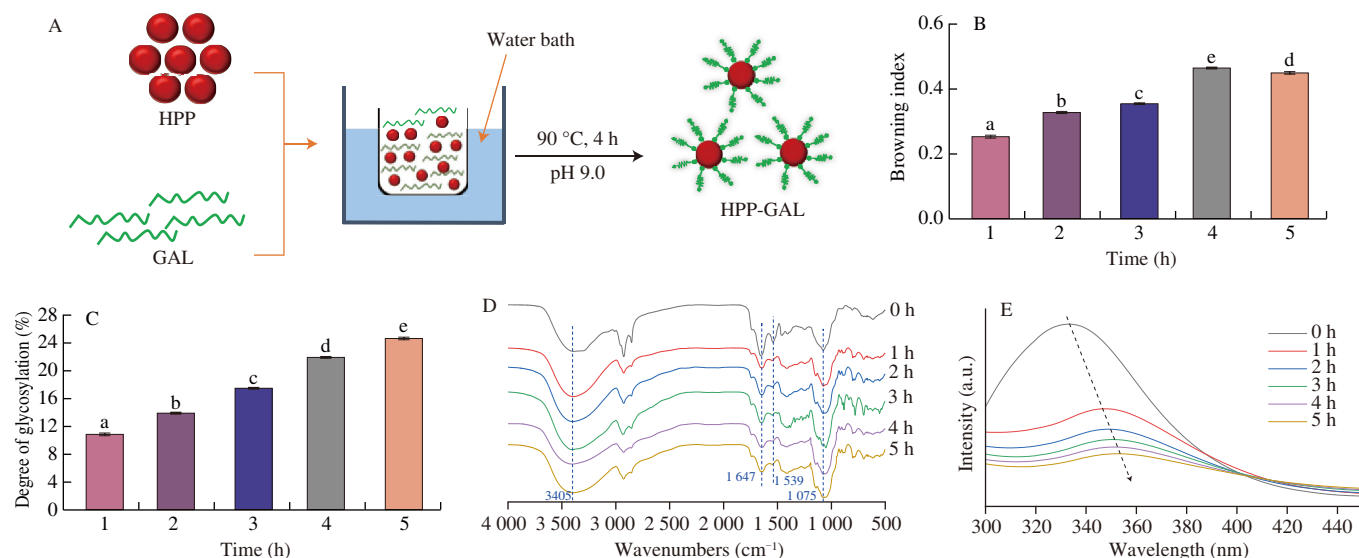


Fig. 1 (A) Schematic diagram for the preparation of HPP-GAL; (B) Browning index; (C) Degree of glycosylation; (D) FTIR spectra and (E) FL spectra of HPP-GAL during Maillard reaction. Different letters indicated that the difference was statistically significant ($P < 0.05$).

3.2 EE and LA of CUR in HPP-GAL particles

The astaxanthin content in HPP and HPP-GAL shown in Fig. 2A were (0.58 ± 0.01) and (0.56 ± 0.07) mg/mL. Both the HPP and HPP-GAL particles were rich in astaxanthin, and there was little influence of Maillard reaction on the astaxanthin content. Schematic diagram of the preparation for HPP-CUR and HPP-GAL-CUR nanoparticles was shown in Fig. 2B. Then, the influence of the mass ratios of CUR to HPP or HPP-GAL on the EE and LA of CUR in the delivery particles were investigated, and the results were shown in Figs. 2C and D. When the ratio of CUR:HPP or

CUR:HPP-GAL changed from 1:12 to 1:18, the EE values of CUR increased from $(36.65 \pm 0.95)\%$ and $(54.99 \pm 0.71)\%$ to $(76.90 \pm 0.12)\%$ and $(89.21 \pm 0.24)\%$, respectively. However, the continuous increase of HPP and HPP-GAL proportion could cause the decrease of CUR encapsulation efficiency, which might be due to the limited encapsulation capability of HPP and HPP-GAL. The LA value of CUR showed a similar trend as the change of HPP and HPP-GAL ratio, and reached the maximum values of $(0.43 \pm 0.01)\%$ and $(0.500 \pm 0.004)\%$ at 1:18 ratio of CUR to HPP/HPP-GAL. In addition, the EE and LA values of the delivery particles based on HPP-GAL were higher than that based on HPP.

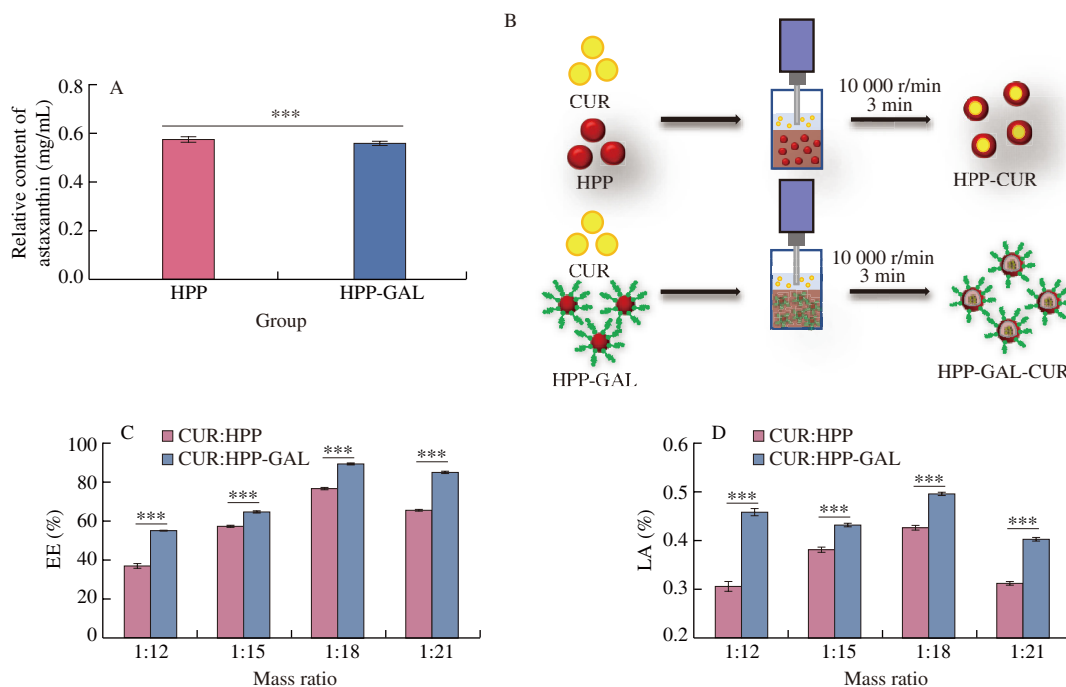


Fig. 2 (A) Relative content of astaxanthin of HPP and HPP-GAL; (B) Schematic diagram of preparation of nanoparticles; (C) EE and (D) LA of HPP-CUR and HPP-GAL-CUR nanoparticles with different CUR:HPP mass ratio. SEM images of HPP-CUR nanoparticles at different magnifications ((E) 10 000 $\times$, (F) 30 000 $\times$ and (G) 50 000 $\times$) and HPP-GAL-CUR ((H) 10 000 $\times$, (I) 30 000 $\times$ and (J) 50 000 $\times$); (K) Particle size and (L) PDI of HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

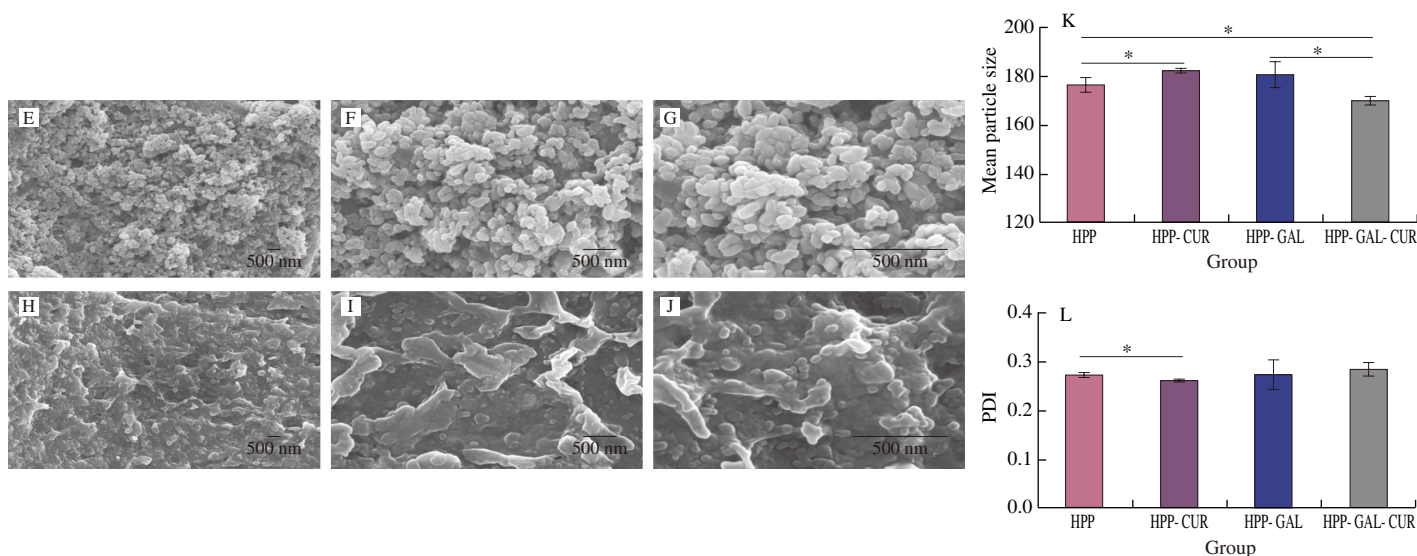


Fig. 2 (Continued)

The enhanced EE and LA values of HPP-GAL might be assigned to the improvement in the emulsification and amphipathy of HPP through Maillard reaction.

3.3 Morphology, particle size and PDI of HPP-CUR and HPP-GAL-CUR particles

SEM images of HPP-CUR and HPP-GAL-CUR particles are shown in Figs. 2E–J. It was noted that the HPP-CUR particles were spindle and spherical with smooth surface and particle size of (131.74 ± 10.67) nm in Figs. 2E–G. The nanoparticles were aggregated, which might be caused by the loss of water in the sample during freeze-drying process. HPP-GAL-CUR nanoparticles were spherical and had strong adhesion as shown in Figs. 2H–J, which could be attributed to the enhanced adhesion and emulsification capacity of proteins by Maillard reaction modification^[31]. The particle size is (93.25 ± 9.65) nm, and the size is relatively uniform. Compared with Fig. S2, the flat needle structure of CUR was not observed in SEM images of HPP-CUR and HPP-GAL-CUR.

The hydrodynamic diameters and PDI of HPP, HPP-GAL, HPP-CUR and HPP-GAL-CUR are shown in Figs. 2K and L. Compared with HPP nanoparticles, the particle size of CUR-loaded HPP nanoparticles was relatively larger. This might be due to the expansion of nanoparticles caused by CUR loading into the hydrophobic core of HPP. However, the nanoparticle size of HPP-GAL-CUR was slightly smaller than HPP-GAL nanoparticles, which might be due to the hydrogen bonding and hydrophobic interaction between CUR and HPP-GAL^[32]. As shown in Fig. 2L, the PDI of HPP, HPP-CUR, HPP-GAL and HPP-GAL-CUR were 0.273 ± 0.005 , 0.261 ± 0.003 , 0.274 ± 0.032 and 0.284 ± 0.014 , and the nanoparticles still maintained good dispersion after the loading of CUR.

3.4 Spectra acquisition

The FTIR spectra of CUR, HPP, HPP-GAL, HPP-CUR and HPP-GAL-CUR are shown in Fig. 3A. The spectral band of CUR at

3510 cm^{-1} was corresponded to the stretching vibration of phenol hydroxyl group, and the peaks at 1428 , 1280 , and 1155 cm^{-1} were telescopic vibrations of the aromatic rings, part of “keto” and the inter-ring chain, respectively. HPP, HPP-CUR, HPP-GAL and HPP-GAL-CUR all had the characteristic peaks of protein such as C–H stretching at 2960 cm^{-1} , amide II band at 1535 cm^{-1} and amide I band stretching at 1654 cm^{-1} . The characteristic peaks of CUR were not found in the spectra of HPP-CUR and HPP-GAL-CUR nanoparticles due to the encapsulation.

Fig. 3B records XRD spectra of CUR, HPP, HPP-CUR, HPP-GAL, and HPP-GAL-CUR to evaluate the changes in the crystalline structure of CUR. The free CUR showed sharp peaks at the 2θ of 16.88 , 20.86 , 23.04 , 24.46 and 25.42 , which suggested the crystal structure of CUR. HPP, HPP-CUR, HPP-GAL and HPP-GAL-CUR showed broad peaks in the 2θ range of 19.74 to 22.62 , and no sharp peak was found. In addition, there was no characteristic peak related to CUR observed in the XRD spectra of HPP-CUR and HPP-GAL-CUR, which might demonstrate the successful loading of CUR.

Fig. 3C shows the UV absorption spectrum of CUR, HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR. The characteristic absorption peaks of CUR, HPP and HPP-GAL were at 420 , 490 , and 429 nm . After the loading of CUR, the maximum absorption peak of HPP-CUR was at 480 nm and that of HPP-GAL-CUR was at 443 nm . Compared to free CUR, the enhanced absorption intensities of HPP-CUR and HPP-GAL-CUR revealed the hydrophobic interaction between HPP or HPP-GAL and CUR^[33].

As shown in Fig. 3D, the fluorescence spectrum of CUR in ethanol showed a maximum peak at 544 nm at the excitation wavelength of 425 nm . Due to its low solubility in water, the fluorescence of CUR in water was weak. However, the encapsulation of CUR by HPP and HPP-GAL enhanced its fluorescence intensity at 544 nm due to the improved solubility observed in Fig. S3. In addition, the fluorescence intensity of HPP-GAL-CUR was higher than that of HPP-CUR, which might be related to its higher ability to encapsulate CUR^[34]. CUR loading also led to the decrease in intrinsic fluorescence of HPP and HPP-GAL as shown in Fig. S4.

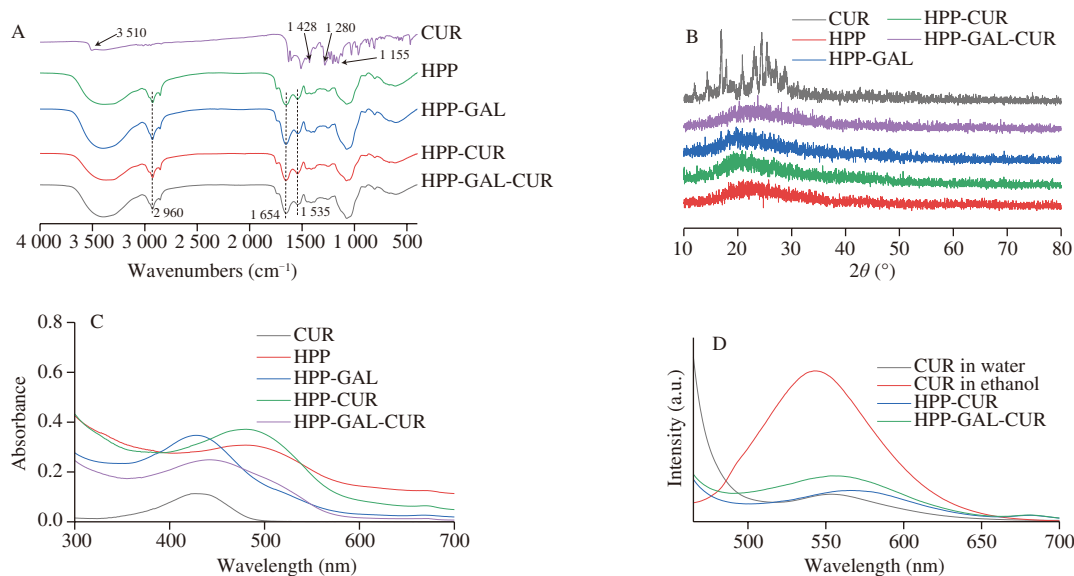


Fig. 3 (A) FTIR spectra, (B) XRD spectra, (C) UV spectra and (D) fluorescence spectra of CUR, HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR.

3.5 Stability evaluation

Fig. 4A shows the CUR contents of free CUR, HPP-CUR, and HPP-GAL-CUR under strong acid. Free CUR decomposed rapidly in strong acid environments and the retention rate was only ($28.36 \pm 0.02\%$) after 30 min. However, the retention rates of CUR in HPP-CUR and HPP-GAL-CUR particles after 30 min were ($66.51 \pm 1.26\%$) and ($99.06 \pm 0.83\%$), respectively. After 3 h, the retention rate of free CUR was only ($12.42 \pm 0.02\%$), and HPP-CUR and HPP-GAL-CUR could still retain ($54.91 \pm 0.43\%$) and ($71.81 \pm 1.25\%$) of CUR. Therefore, HPP-CUR and HPP-GAL-CUR nanoparticles could effectively protect CUR from strong acid damage, and the protection of HPP-GAL-CUR nanoparticles was more effective than HPP-CUR nanoparticles.

Fig. 4B shows the changes in the CUR stability of free CUR, HPP-CUR, and HPP-GAL-CUR nanoparticles under different ionic strength conditions of 50, 100, 150 and 200 mmol/L. The CUR retention rate of free CUR, HPP-CUR, and HPP-GAL-CUR all gradually decreased with the increase of salt ion concentration. After the treatment of 150 mmol/L salt solution, the CUR retention rate of HPP-CUR and HPP-GAL-CUR were ($52.80 \pm 0.41\%$) and ($57.55 \pm 0.74\%$), respectively. The ionic stability of HPP-GAL-CUR was better than free CUR and HPP-CUR, which reflected the release degree of active substances during gastrointestinal digestion. Therefore, it was speculated that HPP-GAL-CUR would show better release of CUR in the following simulated digestion experiments.

The UV stability of free CUR, HPP-CUR and HPP-GAL-CUR are shown in Fig. 4C. Compared with free CUR, HPP-CUR and HPP-GAL-CUR had obvious protective effects on CUR. After 5 h UV irradiation, the CUR retention rates of HPP-CUR and HPP-GAL-CUR were ($54.02 \pm 1.21\%$) and ($58.18 \pm 1.31\%$), which was higher than that of free CUR with a value of ($47.62 \pm 3.74\%$). The improvement in UV stability of HPP-CUR and HPP-GAL-CUR might be due to the existence of astaxanthin in HPP, which could absorb the UV light and protect the CUR.

The TGA curves of free CUR, HPP-CUR and HPP-GAL-CUR are shown in Fig. 4D. From the TGA curves, three weight loss stages

of CUR could be observed. When the temperature raised from 40 °C to 200 °C, the total mass of all samples had a slight loss, which was due to the evaporation of free water. Further increase from 200 °C to 400 °C led to rapid weight loss of free CUR, HPP-CUR and HPP-GAL-CUR, which might be due to the backbone breaks of samples^[35]. When the temperature was higher than 400 °C, the degradation rate of samples gradually slowed down, which might be explained by the disappearance of functional groups under high temperature. At the second stage, the weight loss rates of CUR, HPP-CUR and HPP-GAL-CUR were 57.34%, 35.55% and 36.38%, indicating that the embedding prevented the decomposing of CUR under high temperature.

Therefore, HPP-CUR and HPP-GAL-CUR effectively improved the stability of CUR under strong acids, salt ions, UV irradiation and high temperature conditions.

3.6 In vitro release characteristics of CUR in HPP-CUR and HPP-GAL-CUR nanoparticles

The CUR release characteristics of HPP-CUR and HPP-GAL-CUR nanoparticles during simulated gastrointestinal digestion were studied as the protocol shown in Fig. 4E. The corresponding results are shown in Figs. 4F and G. In SGF, ($15.73 \pm 0.88\%$) CUR was rapidly released from the HPP-CUR nanoparticle in the first 30 min, possibly caused by the CUR partially absorbed or wrapped on the nanoparticle surface^[25]. However, the CUR release rate of HPP-GAL-CUR was only ($4.67 \pm 0.39\%$) within the first 30 min, which might be assigned to the existence of hydrogen bond and hydrophobic interaction between the CUR and HPP-GAL^[36]. Then, the CUR release rates of HPP-CUR and HPP-GAL-CUR after 90 min were ($17.06 \pm 0.65\%$) and ($6.08 \pm 1.12\%$), respectively. It indicated that the HPP-GAL-CUR nanoparticles reduced the release of CUR during gastric digestion. In SIF, both HPP-CUR and HPP-GAL-CUR showed rapid release of CUR after 60 min with the release rates of ($97.01 \pm 0.93\%$) and ($93.12 \pm 1.37\%$), which was attributed to the release of CUR encapsulated inside the nanoparticles^[37]. After 2 h of intestinal digestion, the CUR release rate of HPP-CUR reached

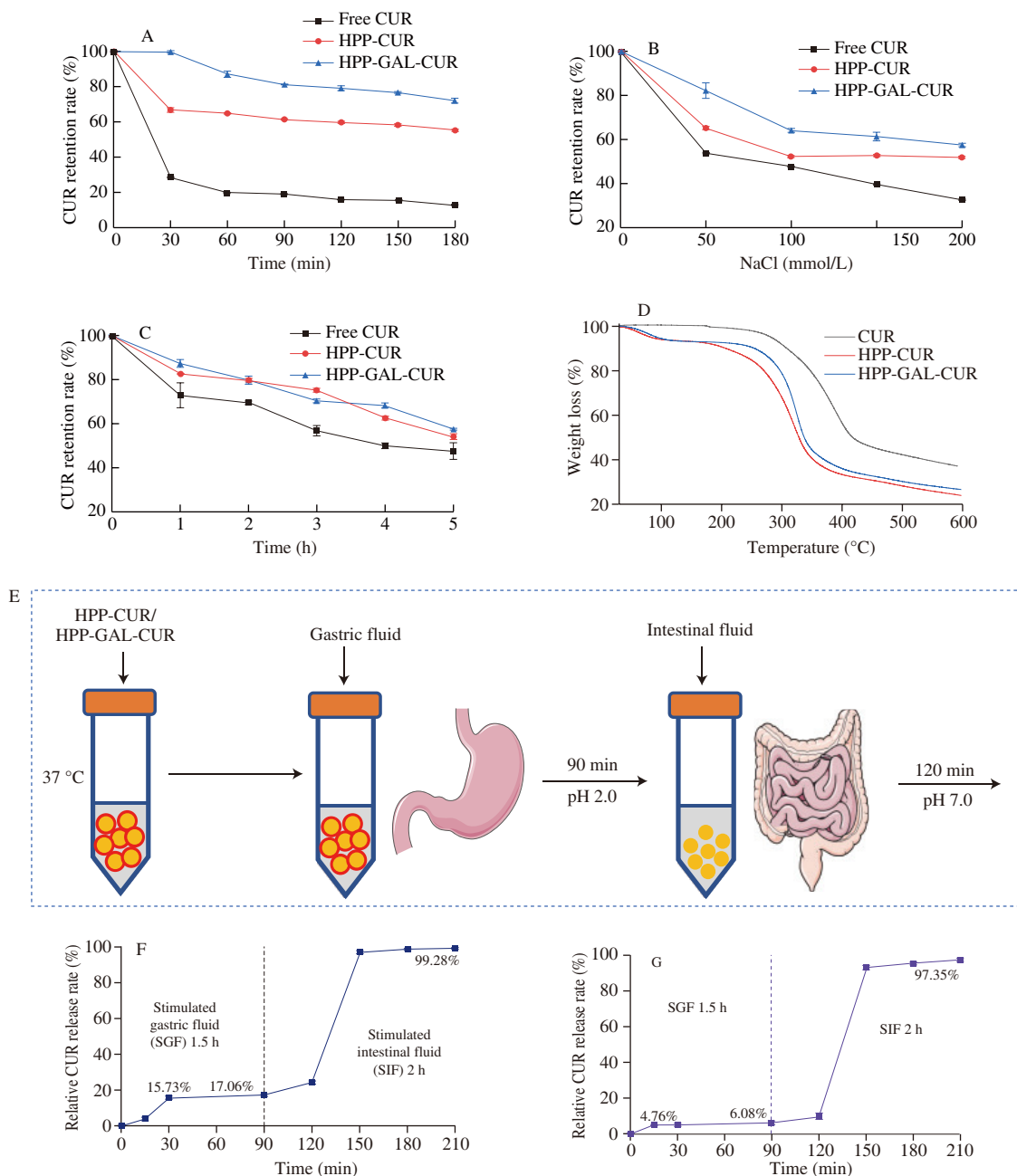


Fig. 4 Stability of HPP-GAL-CUR nanoparticles at (A) pH 2.0, (B) ionic strength stability, (C) UV irradiation and (D) TGA curves. (E) Schematic diagram of simulated digestion *in vitro*. *In vitro* release profile of (F) HPP-CUR and (G) HPP-GAL-CUR nanoparticles in simulated gastric fluid and intestinal fluid.

(99.28 ± 0.47)%, which was almost completely released. However, the release rate of HPP-GAL-CUR was (97.35 ± 1.04)%, which might need more time for complete release. These results suggested that HPP-GAL could provide better protection for CUR and might be effective delivery carriers for hydrophobic active compounds.

3.7 Antioxidant activity of HPP-CUR and HPP-GAL-CUR

The antioxidant activities of HPP and HPP-GAL before and after the embedding of CUR were estimated by DPPH and ABTS radical scavenging experiments. The DPPH and ABTS scavenging activities of the four samples all increased with the concentration as shown in Fig. 5. When the concentration was 1 mg/mL, the DPPH scavenging

rates of HPP, HPP-GAL, HPP-CUR and HPP-GAL-CUR were (59.95 ± 12.07)%, (35.88 ± 9.21)%, (70.33 ± 13.26)% and (72.970 ± 6.025)%, and the ABTS scavenging rates were (26.11 ± 2.78)%, (19.99 ± 0.85)%, (42.32 ± 1.81)% and (49.87 ± 2.06)%, respectively. The antioxidant activity of HPP and HPP-GAL was ascribed to the astaxanthin in HPP, and the scavenging rate of HPP-GAL was reduced after Maillard reaction. Besides, the antioxidant capacities of HPP-CUR and HPP-GAL-CUR was increased after the CUR embedding due to the scavenging free radical's ability of CUR. In addition, the HPP-CUR and HPP-GAL-CUR nanoparticles improved the solubility of CUR in water, which also enhanced the antioxidant activity of nanoparticles^[38]. The higher scavenging rate of HPP-GAL-CUR than HPP-CUR was probably due to its higher EE and LA values of CUR.

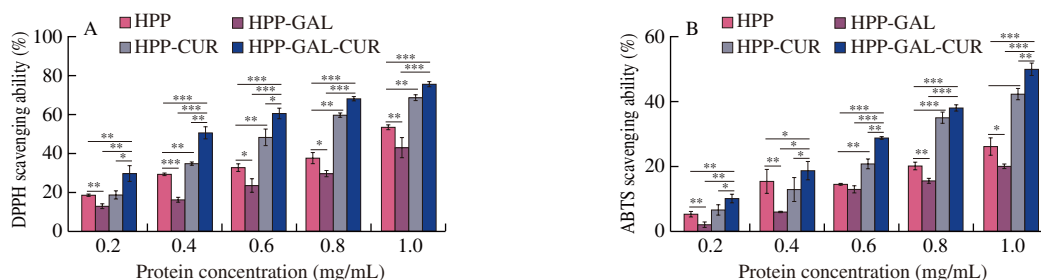


Fig. 5 (A) DPPH and (B) ABTS scavenging ability of HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR nanoparticles. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.8 The liver-targeting characteristics of HPP-GAL-CUR

The liver targeting properties of HPP-GAL-CUR in mice were evaluated by fluorescence imaging using Nile Red instead of CUR. Fig. 6A shows the optical images of major organs in healthy mice treated with Free-Nile Red. As shown in Fig. 6B, the fluorescence signal of the Free-Nile Red group and the HPP-Nile Red group was only observed in stomach and intestine during the 24 h digestion. However, the fluorescence signal was observed in liver for HPP-GAL-Nile Red group after 6 and 12 h of oral administration, which revealed the liver-targeting characteristics of HPP-GAL-CUR due to the presence of galactose^[39]. Asialoglycoprotein receptors expressed in hepatic cells can recognize galactosylated ligands via specific receptor-ligands interaction^[39]. Hepatic targeted astaxanthin nanocarriers were achieved using Maillard reaction products of galactose and whey protein isolate based on the specific recognition

between asialoglycoprotein receptors and galactosylated ligands^[16]. Galactosylated BSA nanoparticles loaded with CUR was constructed and could improve the CUR solubility and the cell internalization ability of nanoparticles^[40].

After gavage, the fluorescence in the stomachs of all three groups gradually decreased over time as shown in Fig. 6C. The fluorescence intensity of Free-Nile Red in the intestine only decreased after 24 h, while that of HPP-Nile Red group in the intestine gradually decreased during the whole 24 h as shown in Fig. 6D. The fluorescence intensities of HPP-GAL-Nile Red in the intestine at 12-h and 24-h were higher than HPP-Nile red, which indicated more persistent release of HPP-GAL-CUR and was consistent with the release curve in Fig. 4G. As shown in Fig. 6E, the liver fluorescence signal of HPP-GAL-Nile red group was 10.23 ± 0.24 at 6-h, which decreased to 1.56 ± 0.10 and 0 at 12-h and 24-h, indicating that the HPP-GAL-CUR nanoparticles could be metabolized from liver.

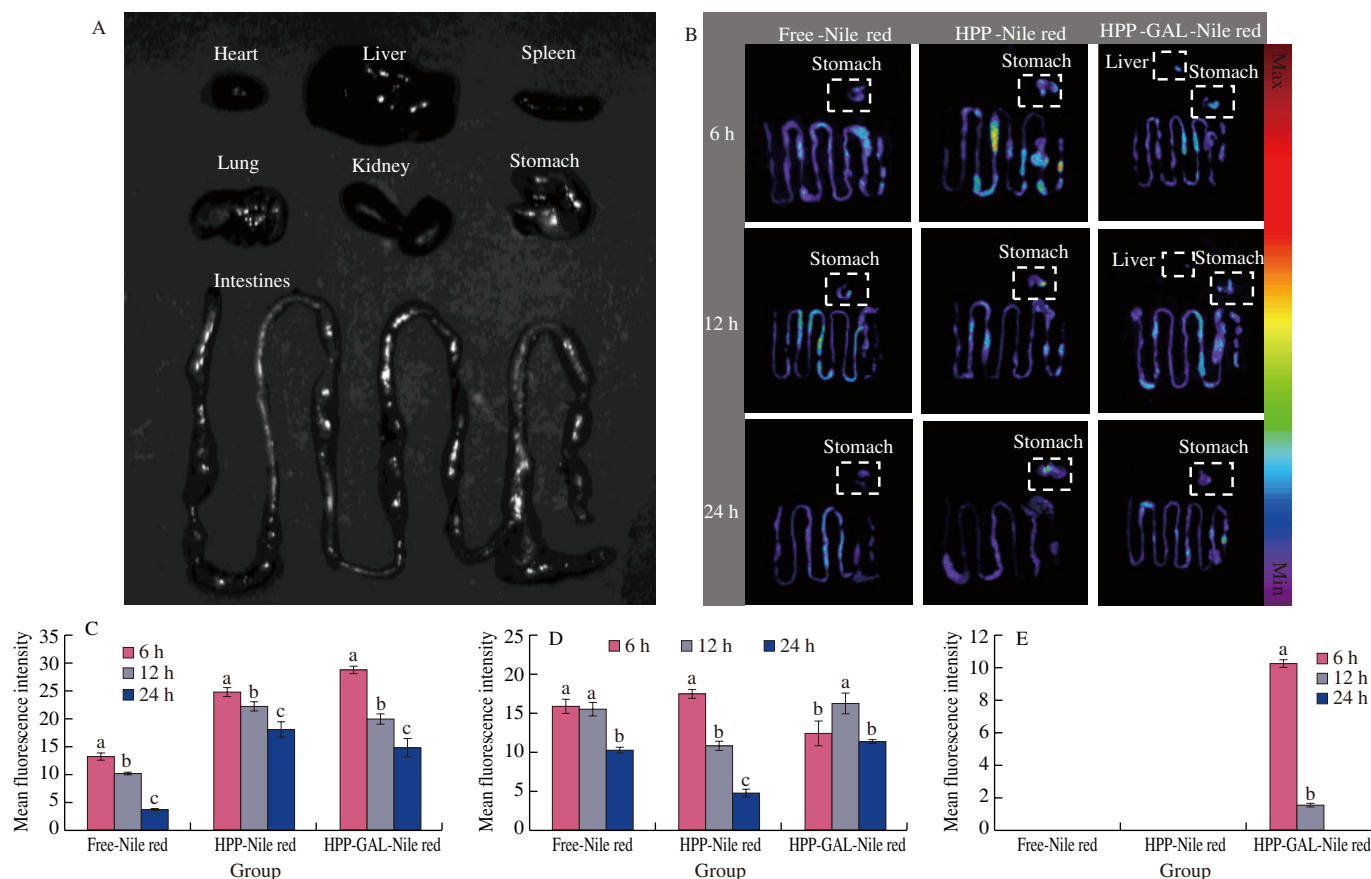


Fig. 6 (A) Bright field images and (B) pseudo color images of major organs of mice, and mean fluorescence intensity of (C) stomach, (D) intestine and (E) liver in each group. Different letters indicated that the difference was statistically significant, $P < 0.05$.

3.9 Protective effect of HPP-GAL-CUR on acute alcoholic liver damage

The effects of free CUR, HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR on the acute alcoholic liver damage of mice were conducted according to the protocol in Fig. 7A. Fig. 7B shows the liver appearance of mice with different treatments. The liver of control group was rosy and bright with luster, while the liver of ALD group was dull and lackluster with tissue necrosis after alcohol treatment. Compared with ALD group, the apparent color of the liver treated by the free CUR was slightly improved, but there was still some tissue necrosis. There were no apparent tissue necrosis in the livers of HPP and HPP-GAL groups, but the color were slightly dimmer. The color of the livers of HPP-CUR and HPP-GAL-CUR groups were bright red and there were no tissue necrosis, and there were no obvious difference in apparent appearance of liver between the HPP-CUR group and HPP-GAL-CUR group. Fig. 7C shows the changes in the body weight of mice during 16 days. Overall, the body weight was relatively stable during the first 14 days, which demonstrated the good biocompatibility of HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR samples. From the 15th day, the mice in each group showed significant weight loss, indicating that the treatment of CUR, HPP, HPP-GAL, HPP-CUR and HPP-GAL-CUR did not alleviate the weight loss induced by alcohol.

Figs. 7D–I shows the organ indexes of liver, stomach, spleen, kidney, heart, and lung in mice with different treatments. When the body is healthy, the ratio of each organ index is relatively stable, but

the organ index increases when the organ is congested, edema or hyperplasia and hypertrophy. As shown in Fig. 7D, the ALD group displayed significant raise in the organ index of liver contrast to the control group, which demonstrated the liver swelling or congestion induced by the alcohol treatment. Compared with ALD group, free CUR could slightly alleviate the liver swelling, but HPP, HPP-GAL, HPP-CUR and HPP-GAL-CUR nanoparticles could significantly alleviate the increase of liver index induced by alcohol. As shown in Figs. 7E–G, decreases in stomach, spleen and kidney indexes were observed after alcohol treatment, and free CUR, HPP, HPP-GAL, HPP-CUR and HPP-GAL-CUR all showed protective effects on the decrease of stomach index. For the spleen index and kidney indexes, HPP-CUR and HPP-GAL-CUR groups showed better improvements in the damage induced by alcohol treatment. As shown in Figs. 7H and I, there was no significant change in the heart and lung indexes after the alcohol treatment.

Liver tissue of control group in Fig. 8A showed compact hepatocytes without adverse conditions. As shown in Fig. 8B, alcohol treatment caused severe liver damage, including granulocyte aggregation and cell swelling. For the free CUR treatment, significant accumulation of granulocytes and cell swelling were still observed in Fig. 8C, and free CUR could not completely alleviate the liver damage induced by alcohol. As shown in Figs. 8D and E, HPP and HPP-GAL could partly inhibit the cell swelling, but the granulocyte accumulation was still observed. In the Fig. 8F, there was almost no cell swelling and only a slight accumulation of granulocytes in the liver of HPP-CUR group. For the HPP-GAL-CUR group as shown in

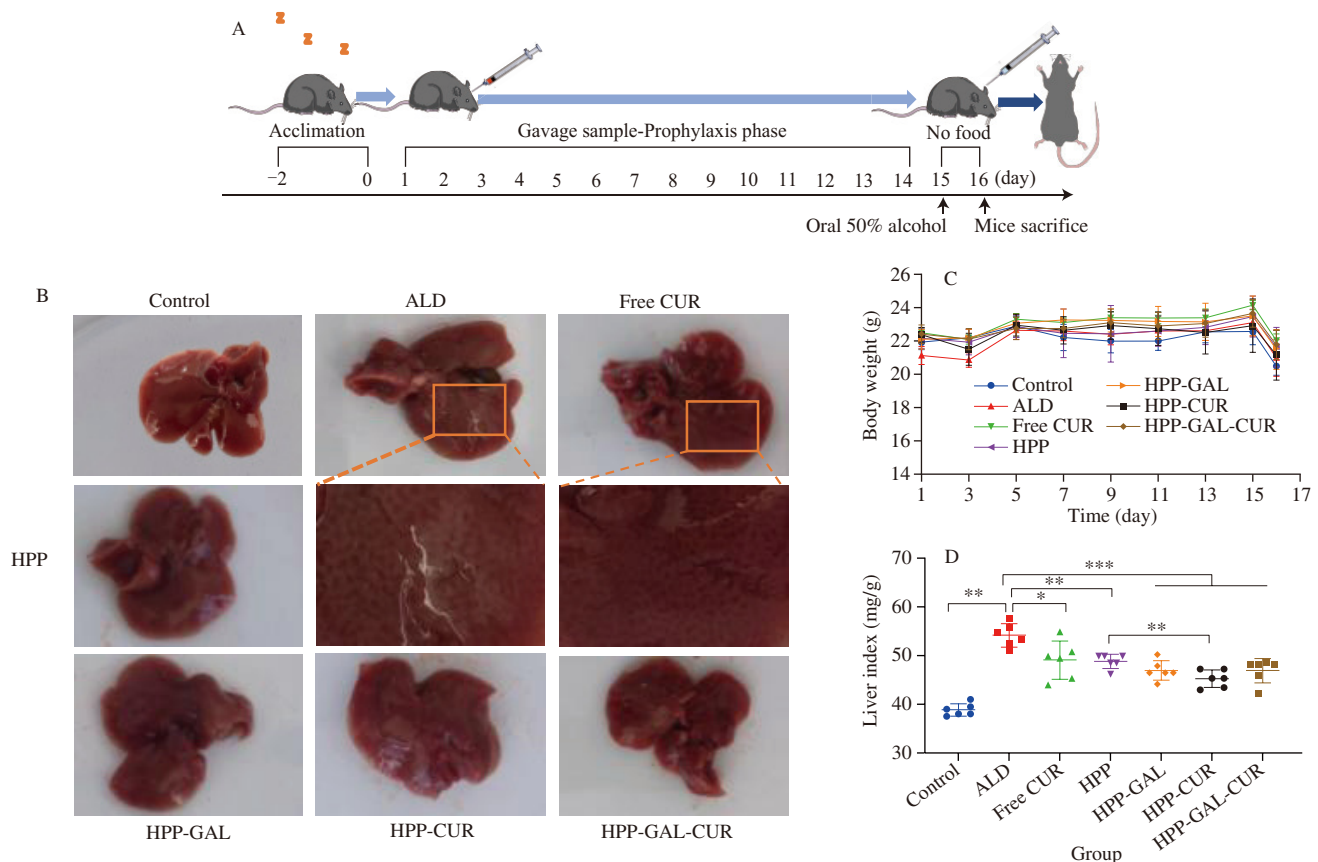


Fig. 7 (A) Schematic diagram of *in vivo* evaluation of HPP-GAL-CUR against ALD in mice, (B) liver photos of each group, and (C) body weight changes in mice during the experiment. Organ indexes of each group including (D) liver, (E) stomach, (F) spleen, (G) kidney, (H) heart and (I) lung. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

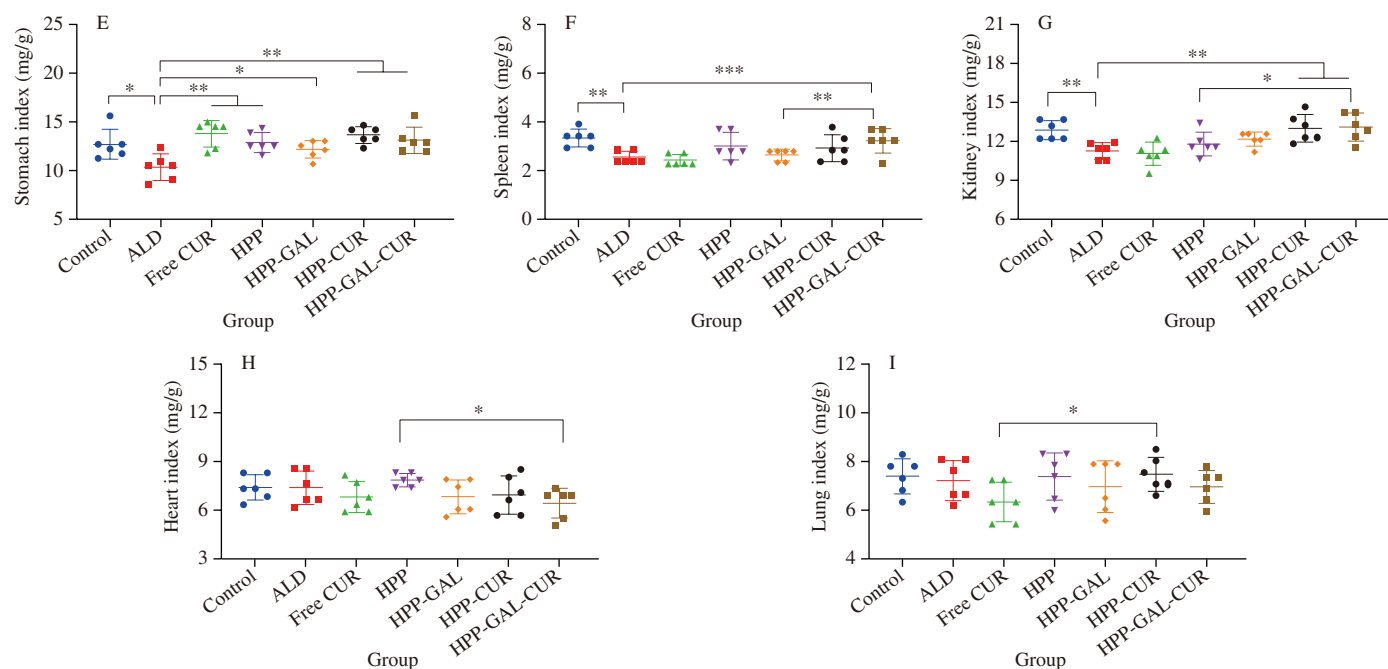


Fig. 7 (Continued)

Fig. 8G, there was no site of inflammation, and the prevention effect of HPP-GAL-CUR against the alcoholic liver damage was the best.

Next, as shown in Figs. 8H and I, the biomarkers AST and ALT of liver damage were determined. Different from the control group, the AST and ALT levels in serum were significantly increased after the alcohol treatment, indicating the successful establishment of the acute alcoholic liver model. There was no significant alleviation on the increase of ALT and AST for Free-CUR. However, HPP and HPP-GAL treatments partly inhibited the ALT and AST increase induced by alcohol treatment, and HPP-CUR and HPP-GAL-CUR showed better inhibition effect than HPP and HPP-GAL. Notably, the preventive effect of HPP-GAL-CUR on the ALT and AST increase in serum was the best among all the treatments. As shown in Figs. 8J and K, the TG and TCHO contents in serum significantly increased after the alcohol intervention, and the treatments of free CUR, HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR all could reduce the increase of

TG and TCHO levels. Among these groups, HPP-GAL-CUR showed the best inhibition for the accumulation of lipids in liver. MDA levels, GSH content, GSH-PX and SOD activity in Figs. 8L–O were determined to evaluate the alleviating effect of Free-CUR, HPP, HPP-GAL, HPP-CUR, and HPP-GAL-CUR on oxidative stress. Alcohol damage led to severe oxidation of liver lipids and increased the MDA level. The treatments of free CUR, HPP, HPP-GAL, HPP-CUR and HPP-GAL-CUR could inhibit the increase of MDA level and HPP-GAL-CUR displayed the most significant inhibition effect. Moreover, alcohol treatment significantly decreased the levels of GSH, GSH-PX and SOD activity, which were intracellular antioxidant and essential endogenous antioxidant enzymes. Free-CUR, HPP, HPP-GAL, HPP-CUR and HPP-GAL-CUR groups all showed increase in the levels of GSH, GSH-PX and SOD activity, and HPP-GAL-CUR displayed the most significant improvement on the reduction of the above 3 indicators.

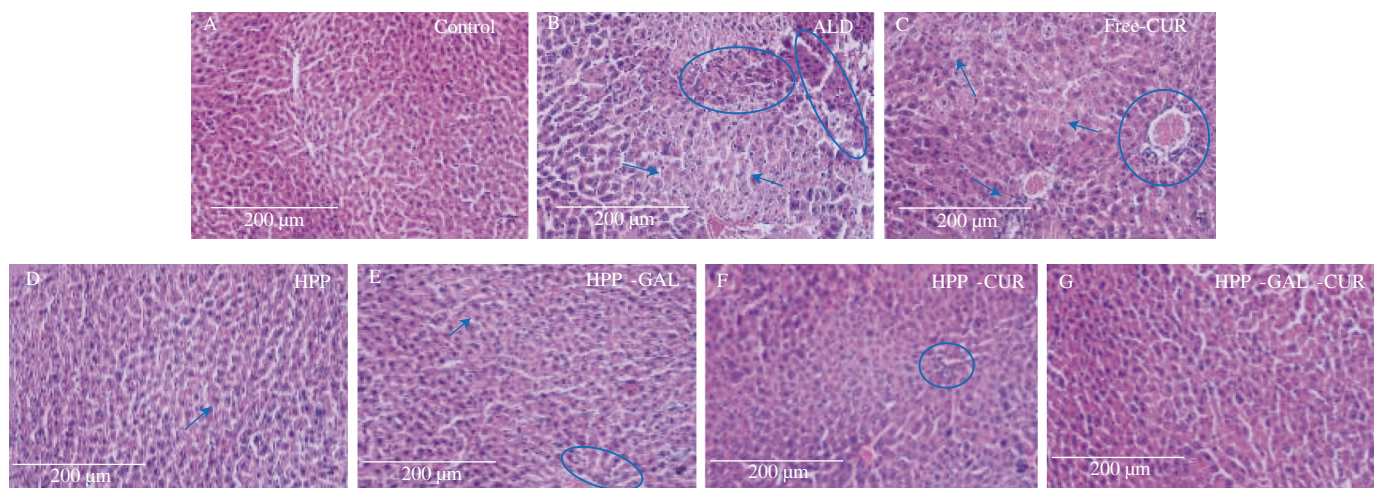


Fig. 8 Hematoxylin-eosin staining histological section imaging of liver in (A) Control, (B) ALD, (C) Free-CUR, (D) HPP, (E) HPP-GAL, (F) HPP-CUR and (G) HPP-GAL-CUR. Arrows represent sites of cell swelling and circles represent sites of granulocyte accumulation. The expression levels of serum (H) ALT, (I) AST, (J) TG, (K) TCHO and liver (L) MDA, (M) GSH, (N) SOD and (O) GSH-PX in each group of mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n = 3$.

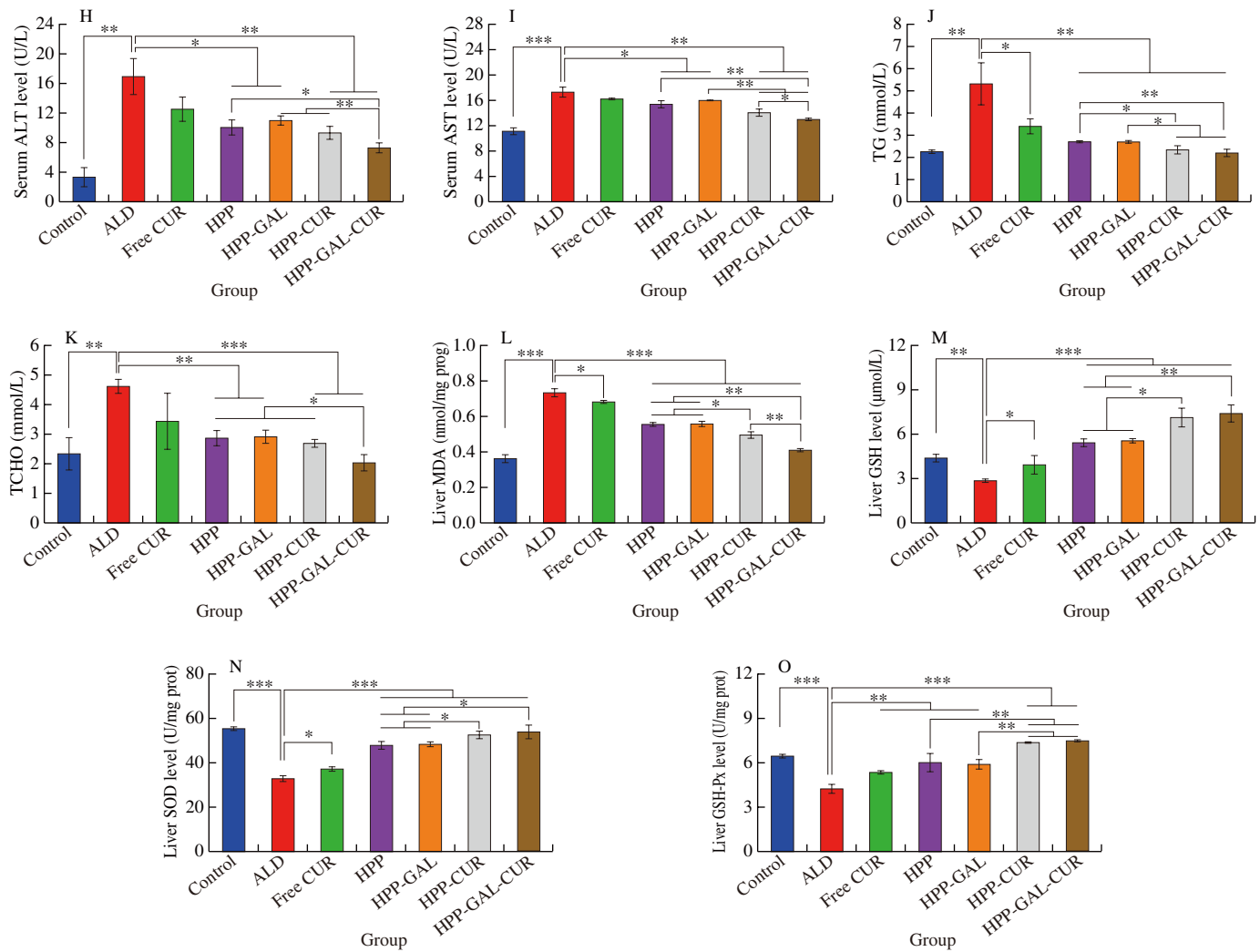


Fig. 8 (Continued)

In brief, the HPP-GAL-CUR nanoparticles were better than the free CUR, HPP and HPP-GAL to against acute alcoholic liver damage, which might be ascribed to the liver targeting feature of GAL and the continuous release of CUR to improve the bioavailability.

4. Conclusion

In this study, HPP-GAL nanocomplex were successfully prepared by Maillard reaction and used to construct CUR delivery system for alleviating acute alcoholic liver damage. The CUR was encapsulated in HPP-GAL particles through hydrogen bonding and hydrophobic interaction with improved encapsulation efficiency and loading capacity. HPP-GAL-CUR nanoparticle displayed good in vitro antioxidant activity and CUR release in simulated intestinal digestion. Moreover, HPP-GAL-CUR nanoparticles showed liver targeting ability and could be accumulated in the liver based on in vivo experiments due to the existence of GAL residues. Compared with Free-CUR and HPP, the HPP-GAL-CUR nanoparticles were more effective in alleviating the alcoholic liver damage and the inflammatory response by inhibiting the oxidative stress. Therefore, HPP-GAL-CUR will a potential liver-targeted delivery system of CUR against alcoholic liver damage.

Conflicts of interest

Mingqian Tan is an editorial board member for *Food Science and Human Wellness* and was not involved in the editorial review or the decision to publish this article. There are no conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.26599/FSHW.2022.9250211>.

References

- [1] J.S. Bajaj, Alcohol, liver disease and the gut microbiota, *Nat. Rev. Gastroenterol. Hepatol.* 16(4) (2019) 235-246. <https://doi.org/10.1038/s41575-018-0099-1>.

- [2] H.A. Zhao, N. Cheng, L.L. He, et al., Antioxidant and hepatoprotective effects of *A. cerana* honey against acute alcohol-induced liver damage in mice, *Food. Res. Int.* 101 (2017) 35–44. <https://doi.org/10.1016/j.foodres.2017.08.014>.
- [3] J.Y. Ma, V. Kumar, R.I. Mahato, Nanoparticle delivery of novel PDE4B inhibitor for the treatment of alcoholic liver disease, *Pharmaceutics* 14(9) (2022) 1894. <https://doi.org/10.3390/pharmaceutics14091894>.
- [4] F.L. Yen, T.H. Wu, C.W. Tzeng, et al., Curcumin nanoparticles improve the physicochemical properties of curcumin and effectively enhance its antioxidant and antihepatoma activities, *J. Agric. Food Chem.* 58(12) (2010) 7376–7382. <https://doi.org/10.1021/jf100135h>.
- [5] P. Anand, A.B. Kunnumakkara, R.A. Newman, et al., Bioavailability of curcumin: problems and promises, *Mol. Pharmaceutics* 4(6) (2007) 807–818. <https://doi.org/10.1021/mp700113r>.
- [6] Z. Li, Q. Lin, D.J. McClements, et al., Curcumin-loaded core-shell biopolymer nanoparticles produced by the pH-driven method: physicochemical and release properties, *Food Chem.* 355 (2021) 129686. <https://doi.org/10.1016/j.foodchem.2021.129686>.
- [7] F.P. Chen, B.S. Li, C.H. Tang, Nanocomplexation between curcumin and soy protein isolate: influence on curcumin stability/bioaccessibility and *in vitro* protein digestibility, *J. Agric. Food Chem.* 63(13) (2015) 3559–3569. <https://doi.org/10.1021/acs.jafc.5b00448>.
- [8] M. Nooshkam, M. Varidi, Maillard conjugate-based delivery systems for the encapsulation, protection, and controlled release of nutraceuticals and food bioactive ingredients: a review, *Food Hydrocoll.* 100 (2020) 105389. <https://doi.org/10.1016/j.foodhyd.2019.105389>.
- [9] D.Y. Kim, W.S. Shin, Characterisation of bovine serum albumin-fucoidan conjugates prepared via the Maillard reaction, *Food Chem.* 173 (2015) 1–6. <https://doi.org/10.1016/j.foodchem.2014.09.167>.
- [10] X.P. Liang, C.C. Ma, X.J. Yan, et al., Structure, rheology and functionality of whey protein emulsion gels: effects of double cross-linking with transglutaminase and calcium ions, *Food Hydrocoll.* 102 (2020) 105569. <https://doi.org/10.1016/j.foodhyd.2019.105569>.
- [11] W.J. Chen, R.L. Lv, A.I. Muhammad, et al., Fabrication of (–)-epigallocatechin-3-gallate carrier based on glycosylated whey protein isolate obtained by ultrasound Maillard reaction, *Ultrason. Sonochem.* 58 (2019) 104678. <https://doi.org/10.1016/j.ultsonch.2019.104678>.
- [12] Y. Han, L. X. Wang, W.P. Jiang, et al., An enhanced stability nanoparticle preparation by corn protein hydrolysate-carboxymethyl chitosan maillard conjugates loaded with rutin, *J. Food Sci.* 84(7) (2019) 1829–1835. <https://doi.org/10.1111/1750-3841.14616>.
- [13] X.Y. Bao, K. Qian, P. Yao, Insulin-and cholic acid-loaded zein/casein-dextran nanoparticles enhance the oral absorption and hypoglycemic effect of insulin, *J. Mater. Chem. B* 9(31) (2021) 6234–6245. <https://doi.org/10.1039/D1TB00806D>.
- [14] Y.T. Fan, J. Yi, Y.Z. Zhang, et al., Fabrication of curcumin-loaded bovine serum albumin (BSA)-dextran nanoparticles and the cellular antioxidant activity, *Food Chem.* 239 (2018) 1210–1218. <https://doi.org/10.1016/j.foodchem.2017.07.075>.
- [15] J. Yi, Y. T. Fan, Y.Z. Zhang, et al., Glycosylated α -lactalbumin-based nanocomplex for curcumin: physicochemical stability and DPPH-scavenging activity, *Food Hydrocoll.* 61 (2016) 369–377. <https://doi.org/10.1016/j.foodhyd.2016.05.036>.
- [16] T.T. Che, Y.S. Song, W.T. Su, et al., Hepatic parenchymal cell and mitochondrial-targeted astaxanthin nanocarriers for relief of high fat diet-induced nonalcoholic fatty liver disease, *Food Funct.* 14 (2023) 2908–2920. <https://doi.org/10.1039/D2FO04036K>.
- [17] R. Singh, P. Parihar, M. Singh, et al., Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: current status and future prospects, *Front. Microbiol.* 8 (2017) 515. <https://doi.org/10.3389/fmicb.2017.00515>.
- [18] F. Ba, A. V. Ursu, C. Laroche, et al., *Haematococcus pluvialis* soluble proteins: extraction, characterization, concentration/fractionation and emulsifying properties, *Bioresour. Technol.* 200 (2016) 147–152. <https://doi.org/10.1016/j.biortech.2015.10.012>.
- [19] Y.P. Zhu, X.Y. Zhao, X.W. Zhang, et al., Extraction, structural and functional properties of *Haematococcus pluvialis* protein after pigment removal, *Int. J. Biol. Macromol.* 140 (2019) 1073–1083. <https://doi.org/10.1016/j.ijbiomac.2019.08.209>.
- [20] Z.H. Yin, M.W. Wang, M. Y. Zeng, Novel Pickering emulsion stabilized by natural fiber polysaccharide-protein extracted from *Haematococcus pluvialis* residues, *Food Hydrocoll.* 134 (2023) 108048. <https://doi.org/10.1016/j.foodhyd.2022.108048>.
- [21] Y.N. Han, B. Hu, M.Y. Wang, et al., PH-sensitive tumor-targeted hyperbranched system based on glycogen nanoparticles for liver cancer therapy, *Appl. Mater.* 18 (2020) 100521. <https://doi.org/10.1016/j.apmt.2019.100521>.
- [22] L. Sheng, G.Y. Tang, Q. Wang, et al., Molecular characteristics and foaming properties of ovalbumin-pullulan conjugates through the Maillard reaction, *Food Hydrocoll.* 100 (2020) 105384. <https://doi.org/10.1016/j.foodhyd.2019.105384>.
- [23] X.D. Zhang, X. Zhao, S.S. Tie, et al., A smart cauliflower-like carrier for astaxanthin delivery to relieve colon inflammation, *J. Controlled Release* 342 (2022) 372–387. <https://doi.org/10.1016/j.jconrel.2022.01.014>.
- [24] V. Mareček, A. Mikiška, D. Hampel, et al., ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt, *J. Cereal Sci.* 73 (2017) 40–45. <https://doi.org/10.1016/j.jcs.2016.11.004>.
- [25] K. Wang, J. Xu, Y. Liu, et al., Self-assembled *Angelica sinensis* polysaccharide nanoparticles with an instinctive liver-targeting ability as a drug carrier for acute alcoholic liver damage protection, *Int. J. Pharm.* 577 (2020) 118996. <https://doi.org/10.1016/j.ijpharm.2019.118996>.
- [26] F.B. Pimentel, M. Cermeño, T. Kleekayai, et al., Effect of *in vitro* simulated gastrointestinal digestion on the antioxidant activity of the red seaweed *Porphyra dioica*, *Food. Res. Int.* 136 (2020) 109309. <https://doi.org/10.1016/j.foodres.2020.109309>.
- [27] A. Vázquez-Suárez, F. Lobos-González, A. Cronshaw, et al., The γ^{33} subunit of *R*-phycoerythrin from *Gracilaria chilensis* has a typical double linked phycocouobilin similar to γ subunit, *PLoS One* 13(4) (2018) e0195656. <https://doi.org/10.1371/journal.pone.0195656>.
- [28] M.R. Zhao, L. Sun, S.C. Sun, et al., The 42.1 and 53.7 kDa bands in SDS-PAGE of *R*-phycoerythrin from *Polysiphonia urceolata*, *Int. J. Biol. Macromol.* 60 (2013) 405–411. <https://doi.org/10.1016/j.ijbiomac.2013.06.009>.
- [29] S.R. Zhong, M.F. Li, Z.H. Zhang, et al., Novel antioxidative wall materials for lactobacillus casei microencapsulation via the Maillard reaction between the soy protein isolate and prebiotic oligosaccharides, *J. Agric. Food Chem.* 69(46) (2021) 13744–13753. <https://doi.org/10.1021/acs.jafc.1c02907>.
- [30] H.Y. Chen, P. Wang, F.F. Wu, et al., Preparation of phosvitin-dextran conjugates under high temperature in a liquid system, *Int. J. Biol. Macromol.* 55 (2013) 258–263. <https://doi.org/10.1016/j.ijbiomac.2013.01.018>.
- [31] X.H. Sun, H. Wang, S.N. Li, et al., Maillard-type protein-polysaccharide conjugates and electrostatic protein-polysaccharide complexes as delivery vehicles for food bioactive ingredients: formation, types, and applications, *Gels* 8(2) (2022) 135. <https://doi.org/10.3390/gels8020135>.
- [32] J.M. Li, Z. Chen, Fabrication of heat-treated soybean protein isolate-EGCG complex nanoparticle as a functional carrier for curcumin, *LWT-Food Sci Technol.* 159 (2022) 113059. <https://doi.org/10.1016/j.lwt.2021.113059>.
- [33] Z.S. Li, Y.T. Wang, Y.Q. Pei, et al., Curcumin encapsulated in the complex of lysozyme/carboxymethylcellulose and implications for the antioxidant activity of curcumin, *Food. Res. Int.* 75 (2015) 98–105. <https://doi.org/10.1016/j.foodres.2015.05.058>.
- [34] J. Yang, J.Q. Lin, X.X. Chen, et al., Mesona chinensis polysaccharide/zein nanoparticles to improve the bioaccessibility and *in vitro* bioactivities of curcumin, *Carbohydr. Polym.* 295 (2022a) 119875. <https://doi.org/10.1016/j.carbpol.2022.119875>.
- [35] J. Gómez-Estaca, M.P. Balaguer, G. López-Carballo, et al., Improving antioxidant and antimicrobial properties of curcumin by means of encapsulation in gelatin through electrohydrodynamic atomization, *Food Hydrocoll.* 70 (2017) 313–320. <https://doi.org/10.1016/j.foodhyd.2017.04.019>.
- [36] R. Meng, Z.Z. Wu, Q.T. Xie, et al., Preparation and characterization of zein/carboxymethyl dextrin nanoparticles to encapsulate curcumin: physicochemical stability, antioxidant activity and controlled release properties, *Food Chem.* 340 (2021) 127893. <https://doi.org/10.1016/j.foodchem.2020.127893>.
- [37] S. Das, A.K. Chaudhari, V.K. Singh, et al., High speed homogenization assisted encapsulation of synergistic essential oils formulation: characterization, *in vitro* release study, safety profile, and efficacy towards mitigation of aflatoxin B1 induced deterioration in rice samples, *Food Chem. Toxicol.* 169 (2022) 113443. <https://doi.org/10.1016/j.fct.2022.113443>.
- [38] S.T. Chen, J. Wu, Q. Tang, et al., Nano-micelles based on hydroxyethyl starch-curcumin conjugates for improved stability, antioxidant and anticancer activity of curcumin, *Carbohydr. Polym.* 228 (2020) 115398. <https://doi.org/10.1016/j.carbpol.2019.115398>.
- [39] W.D. Teng, L.Y. Zhao, S.T. Yang, et al., The hepatic-targeted, resveratrol loaded nanoparticles for relief of high fat diet-induced nonalcoholic fatty liver disease, *J. Controlled Release* 307 (2019) 139–149. <https://doi.org/10.1016/j.jconrel.2019.06.023>.
- [40] Y.K. Huang, L. Hu, S. Huang, et al., Curcumin-loaded galactosylated BSA nanoparticles as targeted drug delivery carriers inhibit hepatocellular carcinoma cell proliferation and migration, *Int. J. Nanomed.* 13 (2018) 8309. <https://doi.org/10.2147/IJN.S184379>.