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Formation and stability of anthocyanins-loaded nanocomplexes prepared with chitosan hydrochloride and carboxymethyl chitosan

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ABSTRACT

Anthocyanins as food additives have attracted increasing interest from the food industries, due to its potential health-related functionalities. However, it is low stability to limit its applications in foods. In this work, nanocomplexes of chitosan hydrochloride (CHC), carboxymethyl chitosan (CMC) and anthocyanins (ACNs) were fabricated through electrostatic interaction to improve the stability of ACNs. At the optimal ratio of 1.2 g CHC to 1.0 g CMC (w/w, 8 mg of ACNs), the ACNs-loaded CHC/CMC nanocomplexes showed high encapsulation efficiency (44.0%) with a preferred particle size (178.1 nm), good stability (+25.6 mV) and acceptable polydispersity index (PDI, 0.315). The content and encapsulation efficiency of individual anthocyanin were estimated by UPLC analysis. The cross-linking interactions between the chitosan derivatives (CHC/CMC) and anthocyanins were confirmed by FT-IR spectroscopy. Furthermore, the spherical structure of ACNs-loaded CHC/CMC nanocomplexes was verified by transmission electron microscopy (TEM). Differential scanning calorimetry (DSC) analyses showed that onset temperature and gelatinization enthalpy of ACNs-loaded CHC/CMC nanocomplexes increased with the introduction of ACNs into the chitosan derivatives. Compared with the ACNs aqueous solution (unencapsulated form), the ACNs-loaded CHC/CMC nanocomplexes (encapsulated form) showed a higher stability when placed in different conventional storage temperature, various L-ascorbic acid (AA) concentration, varying pH or white fluorescent light. The results indicate that these nanocomplexes represent potential food ingredients associated with stable anthocyanins, which exhibits great potential for functional foods and nutraceutical applications.

1. Introduction

Anthocyanins (ACNs) are water-soluble, natural food colorants that exist in a wide range of fruits, vegetables and flowers (Huang et al., 2014). ACNs are responsible for the red, purple, and blue colors of many fruits and flowers as well as derived foods (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009), which allows easy incorporation into food systems. Recently, converging studies have demonstrated that ACNs as a natural pigment affect a wide range of physiology, including cancer (Na, 2012), antioxidative activity (Bornsek et al., 2012; Na, 2012), inflammation (Esposito, Chen, Grace, Komarnytsky, & Lila, 2014), cardiovascular disease (Kruger, Davies, Myburgh, & Lecour, 2014), and vision.

The attractive color and the beneficial bioactivity of ACNs lead to wide usage as food additives for improving both food color and health function. However, ACNs usually show a low chemical stability and a short half-life and relatively low bioavailability for human, since they are easy to be degraded due to be affected by pH (Sui, Dong, & Zhou, 2014; Luna-Vital, Li, West, West, & de Mejia, 2017), temperature (Buckow, Kastell, Terefe, & Versteeg, 2010), ascorbic acid (Chung, Rojanasasithara, Mutilangi, & McClements, 2016; Guldiken, Gibis, Boyacioglu, Capanoglu, & Weiss, 2017), light (Sari, Wijaya, Sajuthi, & Supratman, 2012), oxygen (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2010), enzymes (Piffaut, Kader, Girardin, & Metche, 1994), metallic ions (Buchweitz, Gudi, Carle, Kammerer, & Schulz, 2012). Therefore, the applications of ACNs product as food additives are restricted by its poor stability especially affected by temperature, ascorbic acid, pH and light (Buckow et al., 2010; Chung et al., 2016; Luna-Vital et al., 2017; Sari

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et al., 2012).

In order to increase stability of ACNs and extend its applications in food products, some kind of mitigation are usually used to delay degradation of ACNs, such as low temperature (Reque et al., 2014), low pH (Sui et al., 2014), light avoidance (Sari et al., 2012), co-pigmentation reaction (Sari et al., 2012) and microencapsulation (Oidtmann et al., 2012). Microencapsulation has received increasing interest as a way to stabilize ACNs (Oidtmann et al., 2012). Microencapsulation is a technique that introduces bioactive solid, liquid or gas compounds into a matrix or a polymeric wall system to protect the active ingredients from oxygen, water, light or other conditions (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Encapsulation materials include cyclodextrin (Wilkowska, Ambroziak, Czyżowska, & Adamiec, 2016), protein (Chung, Rojanasasithara, Mutilangi, & McClements, 2015), glucan (Klimaviciute, Navikaite, Jakstas, & Ivanauskas, 2015), hydrocolloid (Navikaite, Simanaviciute, Klimaviciute, Jakstas, & Ivanauskas, 2016), and polymeric nanoparticles (Fang & Bhandari, 2010). The selection of an adequate encapsulation material is important and depends on the target active compound. For instance, several studies have shown that the usage of a negatively charge polysaccharide can improve the stability of ACNs by ionic gelation (Jeong & Na, 2012; Klimaviciute et al., 2015; Navikaite et al., 2016).

Chitosan, a natural polysaccharide obtained from crustacean shells, is an attractive biopolymer for use in a variety of biomedical, pharmaceutical and food applications due to its biodegradability, biocompatibility and non-toxicity (Xia, Liu, Zhang, & Chen, 2011). Chitosan encapsulates bioactive compounds by ionic gelation (Wang, Jung, & Zhao, 2017; Feng et al., 2013; He et al., 2017). Chitosan-based polysaccharide nanoparticles has been shown to improve the stability and enhance the bioavailability of curcumin (Tan, Xie, Zhang, Cai, & Xia, 2016; Anitha et al., 2011; Xiao, Nian, & Huang, 2015). Chitosan hydrochloride (CHC) and Carboxymethyl chitosan (CMC) are two different water-soluble chitosan derivatives with strong positive and negative charges, respectively, on the surface that may be able to improve the stability of anthocyanins by intermolecular electrostatic interaction (He et al., 2017).

In the present work, a novel nanocomplexes was created using chitosan derivatives (CHC/CMC) to stabilize anthocyanins. The nature of the ionic interactions between anthocyanins and the chitosan derivatives were explored by FT-IR and shown to stabilize the nanocomplexes. The physicochemical properties, including particle size, zeta potential, polydispersity index (PDI) and ACNs encapsulation efficiency, were measured at various ratios of CHC/CMC (at a desired amount of ACNs). UPLC analysis estimated the content and encapsulation efficiency of individual anthocyanin. Formation of the ACNs-loaded CHC/CMC nanocomplexes was confirmed by FT-IR spectroscopy and differential scanning calorimetry (DSC). Transmission electron microscopy (TEM) confirmed that the spherical formation of ACNs-loaded CHC/CMC nanocomplexes. The stabilities of the ACNs-loaded CHC/CMC nanocomplexes were evaluated under different storage temperature, various ascorbic acid (AA) concentration, varying pH or white fluorescent light. This work may expand the food industrial applications of ACNs-loaded CHC/CMC nanocomplexes as improved stability in a high temperature, presence of ascorbic acid, near neutral pH value and light exposure.

2. Materials and methods

2.1. Materials

A blueberry-derived mixture of anthocyanins (cyanidin-3-*O*-glucoside and peonidin-3-glucoside as two main anthocyanin) with a purity of 25% (with 64% total phenolics, 14.5% flavonoids) was obtained from Xi'an Natural Source Plant Engineering Co., Ltd (China). The mixture of anthocyanins was kept in a freezer at −18 °C until further use. Chitosan hydrochloride (CHC, degree of deacetylation 85%) and Carboxymethyl chitosan (CMC, degree of deacetylation 83%) were from Haidebei Marine Bioengineering Co., Ltd (China). L-Ascorbic acid (AA), methanol, sodium phosphate dibasic anhydrous, citric acid monohydrate, hydrochloric acid and formic acid were analytical grade reagents (Alfa Aesar Chemical Co., Ltd, China). Cyanidin-3-*O*-glucoside standard was purchased from Tauto Biotech (China). HPLC grade acetonitrile was purchased from Tedia Co., Inc. (USA).

2.2. Formation of anthocyanins-loaded CHC/CMC nanocomplexes

Anthocyanins-loaded CHC/CMC nanocomplexes were prepared according to a method described previously (He et al., 2017) with slight modifications. Test formulations with varying CHC/CMC ratios (F1–F5) are shown in Table 1 (3.60, 1.80, 1.20, 0.90, or 0.72 g CHC to 1 g CMC). Briefly, the CMC water solution (pH value of 6.0 ± 0.1) and the CHC water solution (pH value of 4.9 ± 0.1) were added into 8 mg of ACNs, respectively. The mixture was stirred continuously for 1 h at room temperature. The insoluble part of the ACNs-loaded CHC/CMC nanocomplexes was separated by centrifugation at 15,000 × g for 20 min. The residual content of ACNs was determined by absorbance with a visible light spectrophotometer.

2.3. Characterization of anthocyanins-loaded CHC/CMC nanocomplexes

2.3.1. Determination of anthocyanins content

The ACNs content was determined based on the method reported previously (He et al., 2017). Samples were diluted in aqueous pH 1.0 (0.025 M) and pH 4.5 (0.4 M) buffers, and the absorbance was measured at 520 nm and 700 nm against distilled water as a blank. The ACNs content (C) was calculated and expressed as cyanidin-3-*O*-glucoside (C3G) equivalent according to the following equation (1).

$$C = \frac{(A_{\mathrm{pH1.0}} - A_{\mathrm{pH4.5}}) \times M_{\mathrm{w}} \times DF \times 1000}{\varepsilon \times l} \quad (1)$$

where $A_{\mathrm{pH1.0}}$ and $A_{\mathrm{pH4.5}}$ are maximum absorbance of a sample diluted with aqueous pH 1.0 and pH 4.5 buffers, respectively; M_{w} is the molecular weight of C3G (449.2 g/mol); DF is the dilution factor; ε is the extinction coefficient (26,900 mol/L*cm); l is the path length (1 cm); and 1000 is the conversion factor from g to mg.

2.3.2. Determination of encapsulation efficiency (EE)

The encapsulation efficiency of the ACNs-loaded CHC/CMC nanocomplexes was determined using methods described previously (He et al., 2017). The ACNs encapsulation efficiency was calculated by the following equation (2):

$$EE(\%) = \frac{Total\ ACNs - Free\ ACNs}{Total\ ACNs} \times 100 \quad (2)$$

where total ACNs was the initial content of anthocyanins added in the formulation (8 mg); and free ACNs was the content of anthocyanins unloaded into the nanocomplexes.

2.3.3. Dynamic light scattering (DLS)

Particle size, zeta potential and polydispersity index (PDI) of the ACNs-loaded CHC/CMC nanocomplexes were analyzed using a DelsaMax Pro Particle Sizing and Zeta Potential Analyzer (Beckman Coulter, UK). The device was programmed to count and calculate the size of particles with diameters of 0.4–10,000 nm. The