Carbohydrate Polymers 90 (2012) 1193–1196

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Preparation of the β-cyclodextrin-vitamin C (β-CD-Vc) inclusion complex under high hydrostatic pressure (HHP)

Xiuting Hu, Benxi Wei, Hongyan Li, Chunsen Wu, Yuxiang Bai, Xueming Xu, Zhengyu Jin*, Yaoqi Tian**

The State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi 214122, China

ARTICLE INFO

Article history:
Received 24 May 2012
Received in revised form 11 June 2012
Accepted 12 June 2012
Available online 19 June 2012

Keywords:
High hydrostatic pressure
β-Cyclodextrin
Vitamin C
Inclusion complex

ABSTRACT

In this study, a novel high hydrostatic pressure (HHP) technique was used to prepare the β-cyclodextrin-vitamin C (β-CD-Vc) inclusion complex. The inclusion ratio was positively correlated with the pressure under 300 MPa and remained at above 50.0% when the pressure was more than 300 MPa. Fourier-transform infrared spectroscopy (FI-IR) and UV–visible spectroscopy (UV–vis) analysis showed that characteristic absorption bands and the absorption peak of Vc disappeared in the spectra of the β-CD-Vc inclusion complex. Furthermore, differential scanning calorimetry (DSC) data revealed that only one endothermic peak appeared at about 138 °C in the DSC curve of the β-CD-Vc inclusion complex. These results indicate that the HHP treatment effectively induces the formation of β-CD-Vc inclusion complex.

1. Introduction

High hydrostatic pressure (HHP) is a new non-thermal processing technology. It is widely used in sterilization, protein denaturation, and starch gelatinization (Balny, 2002; Douzals, Marechal, Coquille, & Gervais, 1996.; Hu et al., 2011; Ramaswamy, Shao, & Zhu, 2010). The above-mentioned uses are attributed to the fact that HHP can affect the non-covalent bonds, such as Van der Waals, hydrophobic effects, and hydrogen bonds. These non-covalent interactions are the driving force of forming cyclodextrin (CD) inclusion complexes (Lopata, Darvas, Stadler-Szóke, & Szejtli, 1985). According to Le Chatelier's principle, HHP shifted system toward a state that occupied a smaller volume (Lullien-Pellerin & Balny, 2002). In addition, HHP could induce small molecules penetration into the macromolecule interior (Knorr, Heinz, & Buckow, 2006). These results indicated that HHP might promote the formation of the inclusion complex between host and guest.

Vitamin C (Vc), an essential nutrient for humans, mainly acts as an antioxidant and a cofactor of enzymatic reaction in our bodies. It is highly sensitive to heat, alkali, oxygen, and light. This instability of Vc has brought inconvenience to its preservation and application. Therefore, the hydroxypropyl-β-CD-Vc inclusion complex was prepared to improve the stability of Vc in the previous study (Garnero & Longhi, 2007). Vc also could be encapsulated by β-CD (Bratu, Muresan-Pop, Kacso, & Fărcaş, 2009; Manzanares, Solis, & Rossi, 1996). The encapsulation significantly increased the stability of Vc to high temperature, light, humidity, and oxidation (Feng, Li, & Zhang, 2001; Feng, Zhang, & Zhang, 2001). However, there are no reports on preparing β-CD-Vc inclusion complex under HHP. Therefore, β-CD and Vc were chosen to verify whether HHP could be used to prepare the inclusion complex or not. In this study, the β-CD-Vc inclusion complex was prepared by a HHP method. The complex formation was also identified and analyzed by FT-IR, UV–vis, and DSC.

2. Experimental

2.1. Materials

Vc and β-CD were purchased from Sinopharm Chemical Reagent Co., Ltd. All other chemicals and reagents were of analytical grade unless otherwise stated.

2.2. Preparation of the β-CD-Vc inclusion complex under HHP

β-CD (1.85 g) was dispersed in 100 mL boiled distilled water at 25 °C to produce saturated solution and Vc (0.29 g) was added (the molar ratio of β-CD to Vc was 1:1). The β-CD/Vc mixture solution was vacuum-packed in polyethylene bags and subjected to different pressures (100–600 MPa) at 25 °C for 30 min using a laboratory-scale high-pressure unit (UHPF-800 MPa-3L, New high-tech food machinery company, China). After the HHP treatment, the solution was placed at 4 °C for 24 h and the precipitated β-CD-Vc

* Corresponding author. Tel.: +86 510 85913299; fax: +86 510 85913299.
** Corresponding author. Tel.: +86 510 85328571; fax: +86 510 85328571.
E-mail addresses: jinlab2008@yahoo.com (Z. Jin), yqtian@jiangnan.edu.cn (Y. Tian).

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http://dx.doi.org/10.1016/j.carbpol.2012.06.029

complex was recovered by vacuum-filtration. Finally, the precipitate was washed with distilled water to clear Vc absorbed on the surface of β-CD and dried in a vacuum oven at 35 °C until the weight kept constant. All the dry complex powders were stored in an airtight glass desiccator at the room temperature (20 °C). Physical mixtures were prepared by mixing β-CD (1.85 g) and Vc (0.29 g).

2.3. Determination of Vc in the β-CD-Vc inclusion complex

The iodometry method reported by Wu, Zhan, & Xuan (2009) was used to determine the Vc content. Vc was dissolved in the 1% oxalic acid solution to obtain Vc samples with different concentrations (0.05, 0.1, 0.2, 0.4, 0.8, and 1 mg/mL). Then, 10 mL of Vc solution, adding 20 mL 1% oxalic acid solution, were titrated by the I_2/KI solution with 1 mL 1% starch solution as the indicator. The I_2/KI solution volume (Y) was linearly related to the Vc content (X), when the content of Vc was between 5 and 100 mg. The regression equation was addressed below (Eq. (1)):

$$Y = 1.9328X + 0.0397 \quad (R = 0.9987) \tag{1}$$

The β-CD-Vc inclusion complex (1.0 g) was dissolved in 20 mL of 1% oxalic acid solution and incubated at 25 °C for 120 min. The supernatant containing Vc was obtained by centrifugation at 4000 × g for 10 min. The content of Vc in 1% oxalic acid solution was determined using the iodometry method. The inclusion ratio was calculated using Eq. (2).

$$\text{Inclusion ratio}\,(\%) = \left(\frac{m1}{m}\right) \times 100 \tag{2}$$

where m_1 represents Vc content of the complex and m is the maximum inclusion amount of Vc, 155 mg/g β-CD.

2.4. Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra of Vc, β-CD, the physical mixture of Vc and β-CD, and the β-CD-Vc inclusion complex were collected between 4000 and 500 cm^{-1} on a FT-IR spectrophotometer (5DXC FTIR, Nicolet Co., US).

2.5. UV–visible spectroscopy (UV–vis)

The UV–visible absorption spectra of Vc, β-CD, their physical mixture, and the inclusion complex were recorded in the range from 200 to 400 nm to obtain the UV–visible absorption spectra by a UV–visible spectrophotometer (TU-1900, Rayleigh Analytical Instruments, Beijing, China).

2.6. Differential scanning calorimetry (DSC)

Thermal analysis was performed by a Pris1 DSC (Perkin-Elmer, USA). The thermal behaviors of Vc, β-CD, their physical mixture, and the inclusion complex were studied by heating samples in aluminum hermetic pans at a heating rate of 10 °C/min from 30 °C to 300 °C under ultrahigh-purity nitrogen atmosphere.

2.7. Statistic analysis

Statistical analysis was performed using ORIGIN 8.0 program (OriginLab Inc., USA). Data were expressed as means ± standard deviations of at least three determinations on one sample for each time period and analyzed by a one-way analysis of variance (ANOVA).

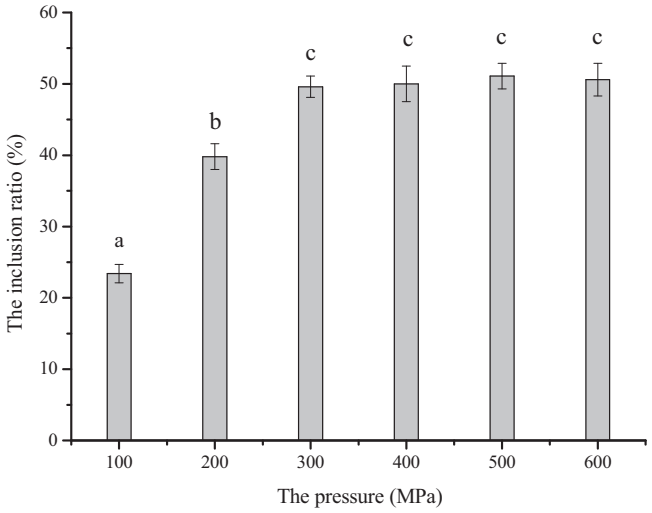

Fig. 1. Effect of the pressure on the inclusion ratio of Vc in β-CD-Vc inclusion complexes.

3. Results and discussion

3.1. Preparation of the β-CD-Vc inclusion complex

The results showed that the inclusion ratio of Vc increased with the pressure under 300 MPa and reached the maximum value of about 50.0% when the pressure was in the range of 300–600 MPa (Fig. 1). These results suggest that higher pressure promoted the formation of the β-CD-Vc inclusion complex and HHP was an effective method to prepare the β-CD-Vc inclusion complex.

3.2. FT-IR analysis

The FT-IR spectrum of β-CD showed the prominent absorption bands at 3405 cm^{-1} (for O—H stretching vibration), 2928 cm^{-1} (for C—H stretching vibration), 1640 cm^{-1} (for H—O—H bending), 1156 cm^{-1} (for C—O stretching vibration), and 1028 cm^{-1} (for C—O—C stretching vibration) (Fig. 2a). The FT-IR spectrum of Vc consisted of the prominent absorption bands of stretching vibration of O—H (3536 cm^{-1}, 3410 cm^{-1}, 3315 cm^{-1}, 3028 cm^{-1}), C—H stretching (2916 cm^{-1}), stretching vibration of C=O (1754 cm^{-1}), and of C=C stretching (1673 cm^{-1}) (Fig. 2b). The FT-IR spectrum of the physical mixture showed approximate superimposition of the individual patterns of Vc and β-CD. The majority of the Vc prominent absorption bands were covered by that of β-CD, except that the C=C stretching of Vc was visible in the FT-IR spectrum of the physical mixture (Fig. 2c). However, the FT-IR spectrum of the β-CD-Vc inclusion complex was identical to pure β-CD but had no features similar to pure Vc (Fig. 2d). These results indicated that the β-CD-Vc inclusion complex was obtained by the HHP method and that the five-membered ring of Vc entered the cavity of β-CD during the formation of the inclusion complex.

3.3. UV–vis analysis

The maximum absorption value of Vc in water solution was at 266 nm, which was ascribed to the dihydroxyethyl and lactone groups of Vc (Fig. 3a). β-CD in water had no absorption in the UV (Fig. 3b). The spectrum of the β-CD/Vc physical mixture was identical to that of Vc (Fig. 3c). However, the UV absorption peak of Vc was not observed in the spectrum of the β-CD-Vc inclusion

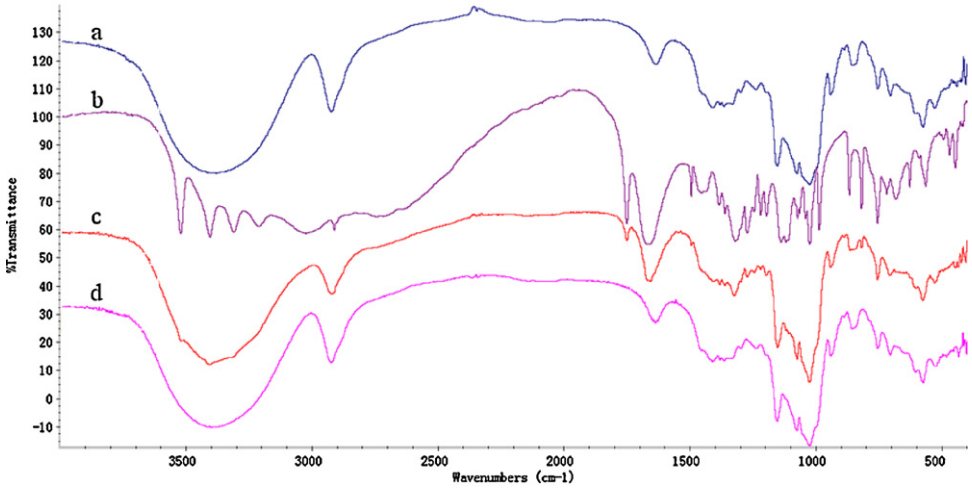

Fig. 2. FT-IR spectra of (a) β-CD, (b) Vc, (c) their physical mixture, and (d) the β-CD-Vc inclusion complex.

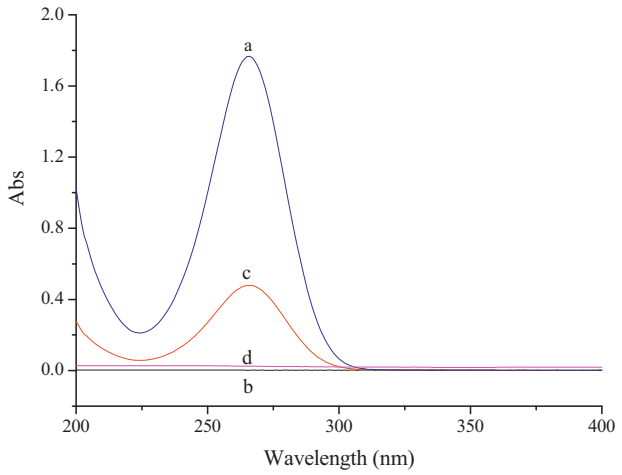

Fig. 3. UV–visible absorption spectra of (a) Vc, (b) β-CD, (c) the β-CD/Vc physical mixture, and (d) the β-CD-Vc inclusion complex.

complex (Fig. 3d). These results indicated that Vc was capable of forming the inclusion complex with β-CD under HHP and that the dihydroxyethyl and lactone groups were inside the cavity of β-CD in the β-CD-Vc inclusion complex.

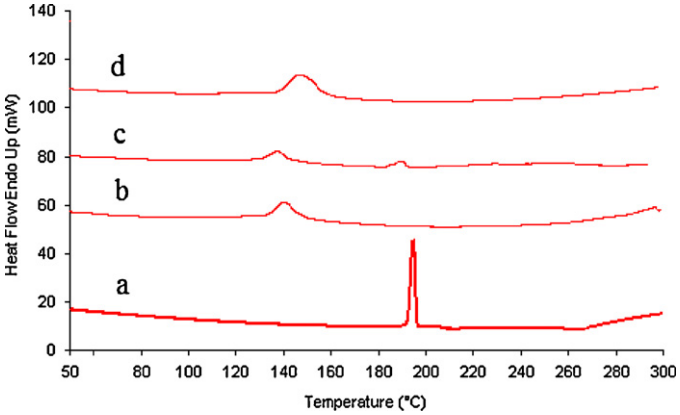

Fig. 4. DSC curves of (a) Vc, (b) β-CD, (c) the β-CD/Vc physical mixture, and (d) the β-CD-Vc inclusion complex.

3.4. DSC analysis

The thermogram of Vc showed an endothermic peak at about 197 °C (Fig. 4a). A wide endothermic peak at about 133 °C was presented in the thermogram of β-CD (Fig. 4b). The DSC curve of the β-CD/Vc physical mixture showed a superimposition of the two individual components, since both of peaks at about 133 °C and 197 °C were observed in the thermogram (Fig. 4c). However, a different pattern was observed in the thermogram of the inclusion complex (Fig. 4d). The endothermic peak at about 133 °C originally in the β-CD was slightly shifted to a higher temperature of about 138 °C for the inclusion complex. This indicated an interaction between Vc and β-CD. The exothermic peak of Vc was not present in the DSC scan of the β-CD-Vc inclusion complex, indicating that Vc was inside the β-CD cavity.

4. Conclusions

This work made it clear that the β-CD-Vc inclusion complex could be prepared by the HHP treatment according to the results of FT-IR, UV–vis, and DSC. When the β-CD-Vc inclusion complex was formed, the dihydroxyethyl and lactone groups of Vc completely entered the cavity of β-CD under the HHP treatment. It was also concluded that that the pressure of 300 MPa was the most suitable for preparing the β-CD-Vc inclusion complex.

Acknowledgments

This study was financially supported by the Fundamental Research Funds for the Central Universities (Nos. JUDCF11027 and JUSRP11225), Project of the State Key Laboratory of Food Science and Technology, Jiangnan University (No. SKLF-ZZB-201206), and the National Key Technology R&D Program for the 12th Five-Year Plan (Nos. 2012BAD37B01, 2012BAD37B02 and 2012BAD37B06).

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