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Preparation, characterization, and thermal stability of β-cyclodextrin/soybean lecithin inclusion complex

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ABSTRACT

β-Cyclodextrin (β-CD), which is widely used to increase the stability, solubility, and bioavailability of guests, can form host–guest inclusion complexes with a wide variety of organic molecules. In this study the β-CD/soybean lecithin inclusion complex was prepared. The effect of reaction parameters such as reaction temperature, reaction time and the molar ratio of β-CD/soybean lecithin on inclusion ratio were studied. The inclusion ratio of the product prepared under the optimal conditions of β-CD/soybean lecithin molar ratio 2:1, reaction temperature 60 °C reaction time 2 h was 40.2%. The results of UV–vis, DSC, XRD and FT-IR spectrum indicated the formation of inclusion complex. The thermal stability experiment indicated that the thermal stability of soybean lecithin in inclusion complex was significantly improved compared with free soybean lecithin.

1. Introduction

Cyclodextrins (CDs) are produced by the enzymatic degradation of starch. They are cyclic oligosaccharides composed, for the most, of six, seven or eight α-1,4 linked D-glucopyrannose units forming a rigid torus-shaped conformation (α-, β- and γ-CD) (Benkő & Király, 2012; Challa, Ahuja, Ali, & Khar, 2005). The exterior of the CDs is highly hydrophilic due to the presence of numerous hydroxyl groups while the cavity in the interior of CDs is hydrophobic. Hence, CDs can interact with a variety of hydrophobic compounds to form inclusion complexes (Ambrus et al., 2011; Fernandes, Oliveira, Sztatisz, Szilágyi, & Novák, 2009; Kawasaki, Araki, Nakamura, & Tanada, 2001; Wieslawa & Magdalena, 2009; Zielenkiewicz, Koźbiał, Golankiewicz, & Poznański, 2010). When CDs form inclusion complex with guest molecules, they can affect the physicochemical properties of the guest molecule to a great extent, such as enhancing the solubility and stability of guests (Caliceti et al., 2003; Fontanay, Kedzierewicz, Duval, & Clarot, 2012; Uekama, Hirayama, & Irie, 1998). The process of CDs to entrap guest molecules in their molecular cavities has no formation of chemical bonds and does not change their structure. Such physical interaction can be considered as encapsulation of substances at a molecular scale. Of these host molecules, β-CD is widely employed to enhance the stability, solubility, and bioavailability of guests for its lower price and higher production rate (Chekirou, Benomrane, Lebsir, & Krallafa, 2012; Li et al., 2005).

Lecithin is widely used in the food, pharmaceutical, and cosmetic industries. The percent distributions of lecithin products among the various sectors are: margarine, 25–30%; baking chocolate and ice cream, 25–30%; technical products, 10–20%; cosmetics, 3–5%; and pharmaceuticals, 3% (Wu & Wang, 2003). Soybeans are the predominant plant source of lecithin because of their abundant availability and lecithin's outstanding functionalities (Wu & Wang, 2004). Soybean lecithin can delay aging, regulate blood lipid, lower cholesterol, enhance memory, prevent and treat diabetes (Surh, Jeong, & Vladisavljević, 2008). As a kind of important physiological activator and natural surface active agent, it has been widely used in food, cosmetics, and pharmaceuticals industries. However, soybean lecithin is easy to oxidize and sensitive to heat and light, which restricts its application in industries. Studies have shown that fatty acid composition of soybean lecithin is the dominant factor affecting the stability (Decker, Warner, Richards, & Shahid, 2005; McClements & Decker, 2000; Osborn & Akoh, 2003). There are little reports about improving the stability of soybean lecithin until now.

In this study, we prepared the β-CD/soybean lecithin inclusion complex and obtained the optimal conditions by the optimization of experiment conditions. Then, the inclusion complex was identified by UV–vis, DSC, XRD and FT-IR. We also studied the thermal

Abbreviations: β-CD, β-cyclodextrin; PC, phosphatidylcholine; CDs, cyclodextrins; UV–vis, UV–vis spectroscopy; DSC, differential scanning calorimetry; XRD, X-ray powder diffraction spectra; FT-IR, Fourier transform infrared spectroscopy.

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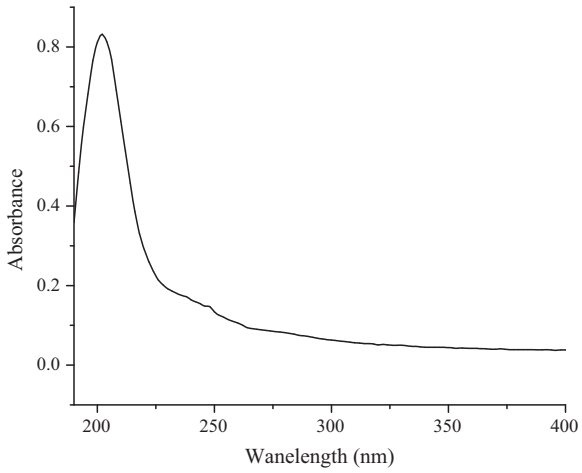

Fig. 1. UV–vis spectra of soybean lecithin.

stability of inclusion complex. This work may provide a pathway to enhance the stability of soybean lecithin through preparing β-CD/soybean lecithin inclusion complex.

2. Material and methods

2.1. Materials

Soybean lecithin (phosphatidylcholine >95%, Epikuron 200) was obtained from Lucas Meyer (Hamburg, Germany) and used without further purification. β-CD was purchased from Boao Biological Technology Co., Ltd. (Shanghai, China). All other chemicals and reagents were of analytical grade.

2.2. Preparation of inclusion complex

β-CD was dispersed in distilled water to produce saturated solution at the desired reaction temperatures. Soybean lecithin was dissolved in anhydrous ethanol and added to the saturated solution of β-CD with the molar ratio of β-CD/soybean lecithin of 0.5:1, 1:1, 2:1 and 3:1, respectively. The β-CD/soybean lecithin mixture solution was stirred and heated at the desired reaction temperatures of 40, 50, 60, and 70 °C for 1, 2, 3, and 4 h, respectively. Then, the solution was placed at 4 °C for 24 h and the precipitated β-CD/soybean lecithin complex was obtained by centrifugation. Finally, the precipitate was washed with anhydrous ethanol to remove soybean lecithin absorbed on the surface and dried in a vacuum oven until the weight kept constant. The complex stored in airtight glass containers was placed away from light until using.

2.3. Determination of soybean lecithin in inclusion complex

0.0102 g soybean lecithin was weighed accurately to a 100 mL volumetric flask and diluted with normal hexane to obtain the soybean lecithin solution (0.102 mg/mL).

The UV–vis absorption spectra of soybean lecithin solution were collected in the range of from 190 to 690 nm. The results showed that soybean lecithin had maximum absorption peak at the wavelength of 202 nm (Fig. 1). Hence, 202 nm was chosen as the detection wavelength.

0.5, 1.0, 1.5, 2.0 and 2.5 mL of soybean lecithin solutions were precisely transferred to 5 mL volumetric flasks and diluted with normal hexane to 5 mL. Absorbance was measured at the wavelength of 202 nm; the regression equation was addressed below:

$$Y = 19.538X + 0.7839 \quad (R = 0.999) \tag{1}$$

where Y is the absorbance of soybean lecithin, X is the concentration of soybean lecithin. The β-CD/soybean lecithin inclusion complex (0.1 g) dissolved in normal hexane was heated at 45 °C for 12 h with stirring throughout. The supernatant containing soybean lecithin was obtained by centrifugation at 4000 × g for 15 min. The above steps were repeated three times. Then the supernatant was diluted with normal hexane to 100 mL (1 mg/mL). Finally, absorbance of the solution was measured at the wavelength of 202 nm.

$$\text{Inclusion ratio} \quad (\%) = \frac{m_1}{m} \times 100\% \tag{2}$$

where m_1 is the quantity of soybean lecithin in inclusion complex calculated by using the Eq. (1), m is the quantity of soybean lecithin that was added to prepare inclusion complex.

2.4. UV–vis spectroscopy (UV–vis)

The UV–vis absorption spectra of soybean lecithin, β-CD, the physical mixture, and the β-CD/soybean lecithin inclusion complex were recorded in the range from 190 to 690 nm to obtain the UV–vis absorption spectra by a Unicam UV-500 spectrophotometer (Thermo Electron, Waltham, USA).

2.5. Differential scanning calorimetry (DSC)

Thermal analyses of soybean lecithin, β-CD, the physical mixture and the β-CD/soybean lecithin inclusion complex were performed by a Diamond DSC (Perkin-Elmer, MA, USA). These samples were studied by heating samples in aluminum hermetic pans at a heating rate of 10 °C/min from 30 to 400 °C under ultrahigh-purity nitrogen atmosphere.

2.6. X-ray powder diffraction spectra (XRD)

X-ray diffraction was conducted with a RU200R X-ray diffractometer (Rigaku, Tokyo, Japan) with Cu Kα radiation at 35 kV and 20 mA, a theta-compensating slit, and a diffracted beam monochromator. The XRD spectra of soybean lecithin, β-CD, the physical mixture of soybean lecithin and β-CD, and the β-CD/soybean lecithin inclusion complex were recorded between 5° and 60° (2θ).

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

The FT-IR spectra of soybean lecithin, β-CD, the physical mixture, and the β-CD/soybean lecithin inclusion complex were recorded on a Nicolet 510 spectrophotometer (Thermo Electron, Waltham, USA) using potassium bromide (KBr) disks prepared from powder samples mixed with dry KBr in a ratio of 1:30. Thirty-two scans were taken of each sample recorded from 4000 to 500 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode.

2.8. The thermal stability of inclusion complex

The thermal stability of β-CD/soybean lecithin inclusion complex was measured according to the method of Chen, Chen, Guo, Li, and Li (2007) with some modifications. Two copies of inclusion complex and free soybean lecithin were weighed and sealed in glass bottles. Then, they were placed in oven at 40, 80 °C for 7 days, respectively. At 0, 1, 3, 5 and 7 d, the samples were taken out, dissolved and diluted with normal hexane. The absorbance was measured at 202 nm, and the content of soybean lecithin was

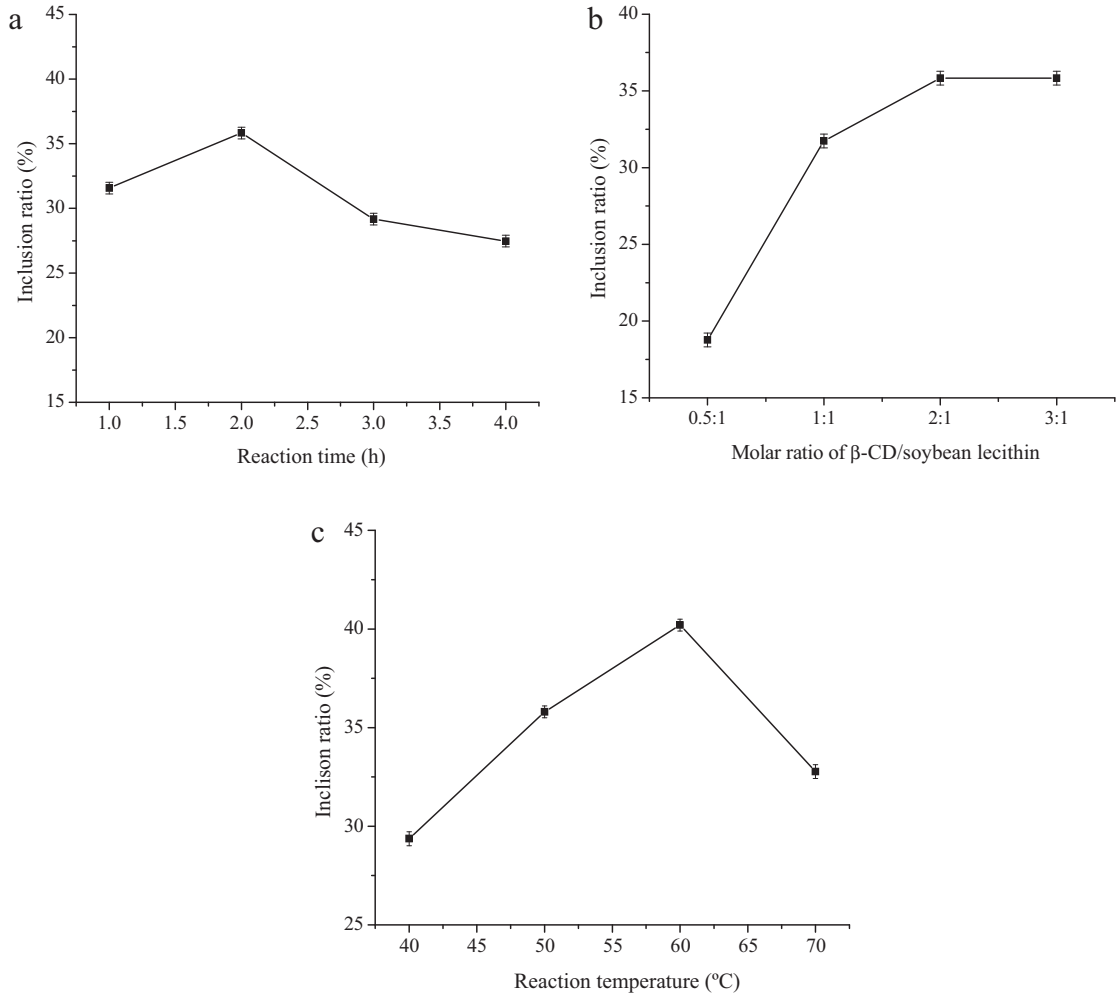

Fig. 2. Effect of reaction parameters on inclusion ratio. (a) Reaction time (reaction temperature 50 °C, molar ration of β-CD/soybean lecithin 2:1); (b) molar ratio of β-CD/soybean lecithin (reaction temperature 50 °C, reaction time 2 h) and (c) reaction temperature (reaction time 2 h, molar ratio of β-CD/soybean lecithin 2:1).

obtained according to Eq. (1). The content loss of soybean lecithin was calculated as follow:

$$\text{The content loss of soybean lecithin } (\%) = \frac{M - M_1}{M} \times 100\% \quad (3)$$

where M is the content of soybean lecithin at 0 d, M_1 is the content of soybean lecithin at 1, 3, 5, 7 d, respectively.

3. Results and discussion

3.1. Effects of reaction parameters on inclusion ratio

The coordination of soybean lecithin to β-CD is obviously a complex reaction system influenced by many factors. The effect of reaction parameters (reaction time, molar ratio, reaction temperature) on the inclusion ratio was studied.

3.1.1. Effect of reaction time

As shown in Fig. 2a, with the lengthening of the reaction time, the inclusion ratio of inclusion complex increased. When the reaction time reached 2 h, the inclusion ratio had the maximum value of 35.8%. However, the inclusion ratio decreased rapidly with the extension of reaction time after 2 h. This result was in accordance with that of Jiang et al. (2007) who also found that long reaction time did not contribute to inclusion ratio. The inclusion was a process that soybean lecithin entered the hydrophobic cavity of β-CD by forming hydrophobic bonds. When the reaction time was too long, it might make soybean lecithin divorce from inclusion complex.

3.1.2. Effect of molar ratio

As shown in Fig. 2b, the inclusion ratio increased significantly with the increase in molar ratio of β-CD/soybean lecithin. When the molar ratio reached 2:1, the inclusion ratio had the maximum value of 35.8%. However, the inclusion ratio essentially kept little change when the molar ratio was over 2:1. The similar trend was also found by Bhandari, D'Arc, and Thi Bich (1998) when they prepared β-CD/lemon oil inclusion complex. The reason might be that soybean lecithin could be wrapped by enough β-CD. The addition of β-CD had little effect on inclusion ratio. Therefore, the optimal molar ratio of β-CD/soybean lecithin was 2:1.

3.1.3. Effect of reaction temperature

As shown in Fig. 2c, the inclusion ratio of inclusion complex rose with the temperature increasing. The inclusion ratio reached a maximum of 40.2% when the temperature was 60 °C. However, the inclusion ratio of inclusion complex declined rapidly when the temperature was over 60 °C. The reason might be that soybean lecithin was easy to oxidative decomposition at high temperature. Therefore, the optimal temperature was 60 °C. The similar results were found by Jin, Zhang, Sun, Li, and Jia (2012) who observed that high temperature did not accelerate the reaction and even an inhibition.

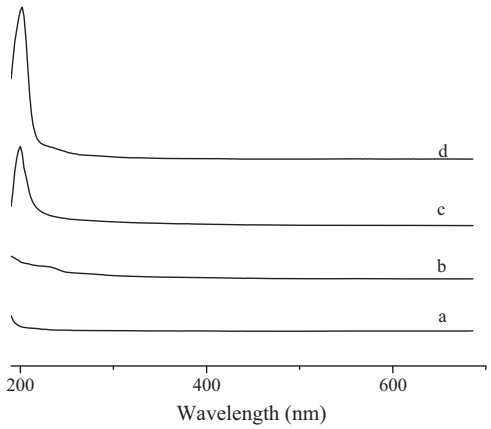

Fig. 3. UV–vis spectra. (a) β-CD; (b) β-CD/soybean lecithin inclusion complex; (c) β-CD/soybean lecithin physical mixture and (d) soybean lecithin.

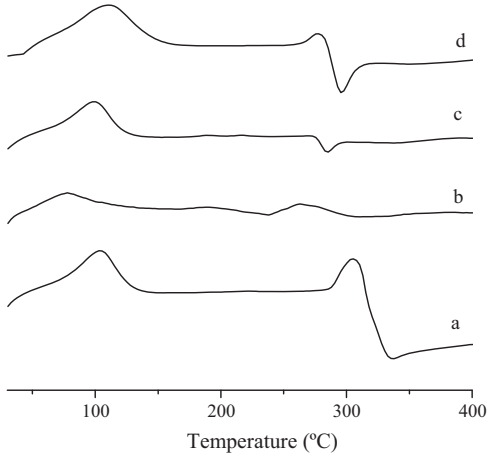

Fig. 4. DSC curves. (a) β-CD; (b) soybean lecithin; (c) β-CD/soybean lecithin physical mixture and (d) β-CD/soybean lecithin inclusion complex.

3.2. UV–vis analysis

β-CD had no maximum absorption peak in the UV–vis absorption spectra (Fig. 3a). The maximum absorption value of soybean lecithin was at 202 nm (Fig. 3d), which mainly attributed to unsaturated fatty acyl group. The spectrum of the β-CD/soybean lecithin physical mixture was consistent with that of soybean lecithin (Fig. 3c), while the β-CD/soybean lecithin inclusion complex had no significant absorption peak in the UV–vis absorption spectra (Fig. 3b). The reason was mainly that the unsaturated acyl group of soybean lecithin was wrapped into the cavity of β-CD. These results indicated that soybean lecithin had formed the inclusion complex with β-CD.

3.3. DSC analysis

As shown in Fig. 4, the thermogram of β-CD showed a wide endothermic peak at about 110 °C, which referred to the water releasing. There was another endothermic peak at about 302 °C, which was mainly related to the phase transition of β-CD (Fig. 4a) (Serafini et al., 2012; Yáñez et al., 2012). Soybean lecithin exhibited an endothermal effect peaked at about 80 °C, attributable to the fusion of its crystalline portion. Another endothermic peak at 265 °C presented in the thermogram of soybean lecithin (Fig. 4b), which was attributed to the decomposition of soybean lecithin (Cirri & Maestrelli, 2009). The thermogram of the physical mixture was similar to the superimposition of the thermograms of individual

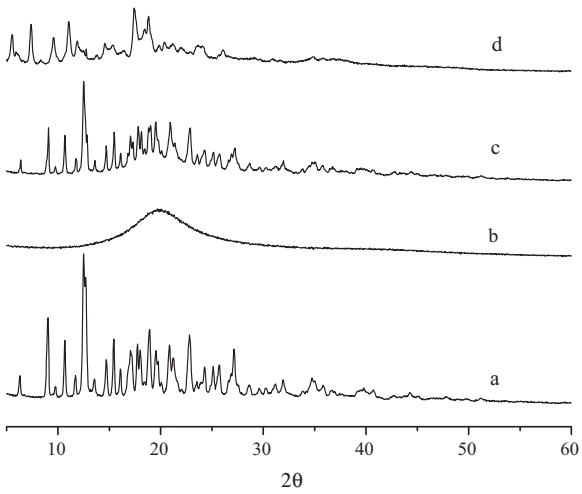

Fig. 5. XRD spectra. (a) β-CD; (b) soybean lecithin; (c) β-CD/soybean lecithin physical mixture and (d) β-CD/soybean lecithin inclusion complex.

β-CD and soybean lecithin (Fig. 4c). However, a difference pattern was observed in the thermogram of inclusion complex. The thermogram of inclusion complex was coincident with that of β-CD, the endothermic peak of soybean lecithin had merged to that of β-CD in the DSC curve of inclusion complex. In addition, the endothermic peak at about 302 °C originally in the β-CD was shifted to about 290 °C for inclusion complex, indicating an interaction between β-CD and soybean lecithin. All these results showed that soybean lecithin was wrapped into the cavity of β-CD.

3.4. XRD analysis

As shown in Fig. 5, β-CD exhibited many crystalline peaks between 5° and 60° (2θ = 9.1, 12.8, 23.1, 7.1, and 34.9°) (Fig. 5a), indicating that β-CD mainly existed in a crystalline form. Soybean lecithin showed a big broad peak, suggesting that it is the amorphous characteristics (Fig. 5b), the similar result was found by Formariz et al. (2008) When they recorded the XRD of soybean lecithin. Their physical mixture showed approximate superimposition of the individual patterns of β-CD and soybean lecithin (Fig. 5c). The β-CD/soybean lecithin inclusion complex exhibited distinctive peak patterns with relatively broad bands (Fig. 5d), indicating the structural changes of β-CD and soybean lecithin when they formed inclusion complex.

3.5. FT-IR analysis

As a common tool, FT-IR spectrum was used to confirm the formation of inclusion complex by investigating the variation of peak shape position and intensity (Szente, 1996; Zeng, Fang, & Ji, 2012). For the FT-IR spectrum of β-CD, a broad band with a transmittance peak at 3383 cm^{-1} represented the symmetric and asymmetric O—H stretching vibration due to the many intermolecular hydrogen bonds of β-CD, and another bond at 2925 cm^{-1} was assigned to C—H stretching vibration. The absorption band at 1647 cm^{-1} was due to H—O—H bending, the ones at 1158 cm^{-1} and 1028 cm^{-1} were respectively asymmetric C—O—C stretching vibration and symmetric C—O—C stretching vibration (Fig. 6a) (Serafini et al., 2012).

The FT-IR spectrum of soybean lecithin showed the prominent absorption bands at 2924 cm^{-1} (for C—H stretching vibration of methylene group), 1740 cm^{-1} (for C=O stretching vibration), 1463 cm^{-1} (for C—H stretching vibration of methyl group), 1234 cm^{-1} (for P=O stretching vibration), 1066 cm^{-1} (for P—O—C stretching vibration)

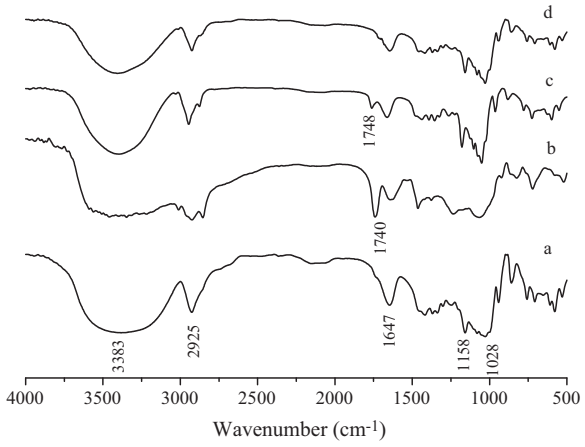

Fig. 6. FT-IR spectra. (a) β-CD; (b) soybean lecithin; (c) β-CD/soybean lecithin physical mixture and (d) β-CD/soybean lecithin inclusion complex.

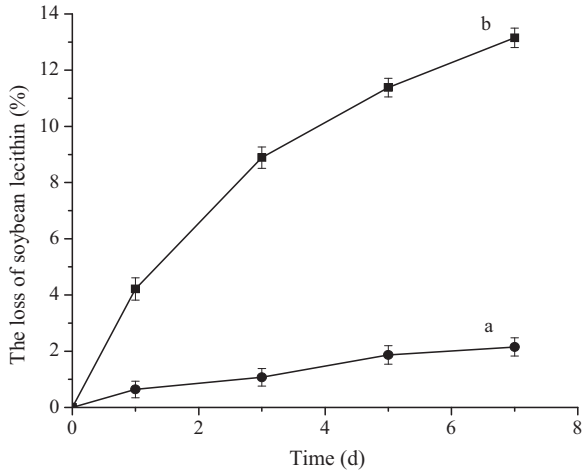

Fig. 7. The content loss of soybean lecithin within 7 days at 40 °C. (a) β-CD/soybean lecithin inclusion complex and (b) free soybean lecithin.

(Fig. 6b) (Wang et al., 2006). The FT-IR spectrum of the physical mixture showed approximate superimposition of individual patterns of soybean lecithin and β-CD. The majority of soybean lecithin prominent absorption bands were covered by that of β-CD, except that the C=O stretching (1748 cm^{-1}) of soybean lecithin was visible in the FT-IR spectrum of physical mixture (Fig. 6c). The similar result was found by Hu et al. (2012) when they studied the FT-IR spectrum of the β-CD/vitamin C physical mixture. However, the FT-IR spectrum of inclusion complex was identical to β-CD but had no features similar to soybean lecithin (Fig. 6d). All the absorption bands of soybean lecithin were covered by that of β-CD in the FT-IR spectrum of inclusion complex. These results showed that soybean lecithin entered the cavity of β-CD and β-CD/soybean lecithin inclusion complex was formed.

3.6. Thermal stability analysis

As shown in Figs. 7 and 8, the content losses of soybean lecithin of inclusion complex and free soybean lecithin were recorded at 40, 80 °C during 7 days, respectively. Whether it was at 40 °C or 80 °C, the color of inclusion complex kept white within 7 days, while the color of free soybean lecithin changed from yellow to brown, which might be due to the oxidation of soybean lecithin. In addition, the content loss of soybean lecithin was always more than that of soybean lecithin in inclusion complex whether it was at 40 or 80 °C.

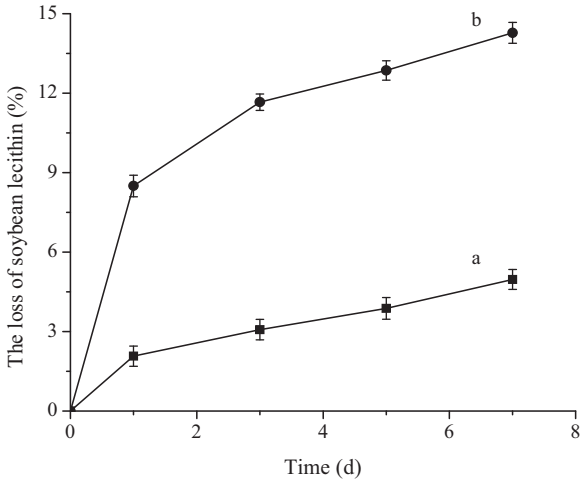

Fig. 8. The content loss of soybean lecithin within 7 days at 80 °C. (a) β-CD/soybean lecithin inclusion complex and (b) free soybean lecithin.

The reason was the stability of soybean lecithin was enhanced when the β-CD/soybean lecithin inclusion complex was formed. Therefore, the thermal stability of soybean lecithin in inclusion complex was promoted obviously compared with free soybean lecithin.

4. Conclusions

The results of this work clearly demonstrated that soybean lecithin could be complexed with β-CD to form inclusion complex. The optimum conditions for preparing the β-CD/soybean lecithin inclusion complex were concluded as follows: reaction time 2 h, the molar ratio of β-CD/soybean lecithin 2:1, reaction temperature 60 °C. The results of UV–vis, DSC, XRD and FT-IR suggested that the β-CD/soybean lecithin inclusion complex was formed and the inclusion complex had different physicochemical characteristics from free soybean lecithin. Thermal stability experiment confirmed that the thermal stability of soybean lecithin in inclusion complex was promoted significantly. The β-CD/soybean lecithin inclusion complex improved the physicochemical properties of soybean lecithin to a great extent, which can make soybean lecithin have better application in instant milk powder, bread, medicine, cosmetics, and coatings etc.

Acknowledgments

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