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Storage stability and antioxidant activity of complex of astaxanthin with hydroxypropyl-β-cyclodextrin

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ABSTRACT

Storage stability of astaxanthin/hydroxypropyl-β-cyclodextrin (HPCD) inclusion complex was evaluated and which was compared with native astaxanthin. The storage stability of astaxanthin was enhanced after included in HPCD under 4 °C and 25 °C storage conditions. Antioxidant activity of astaxanthin/HPCD inclusion complex was also assayed using ascorbic acid as a control sample. The reducing power and DPPH radical scavenging activity of native astaxanthin were lower than ascorbic acid, while which of the complex were higher at low concentration for the good water solubility. The hydroxyl radical scavenging activities of astaxanthin and astaxanthin/HPCD complex far outclassed that of ascorbic acid, and the activity of the complex was a little lower than that of the native astaxanthin.

1. Introduction

Carotenoids are intensely colored liposoluble pigments synthesized by plants and micro-organisms and are present in many foods, particularly fruit, vegetables, and fish (El-Agamey et al., 2004). Carotenoids are distributed widely in nature and most carotenoids have a long chain of conjugated polyenes, which endow them with the characteristic yellow to red color and antioxidant activities, such as oxygen radical scavenging and singlet oxygen quenching (Elliott, 2005; Stahl & Sies, 2005). Furthermore, individual carotenoids have characteristic biological activities, including immune enhancement and anti-carcinogenesis (Chew & Park, 2004; Jaswir, Noviendri, Hasrini, & Octavianti, 2011). Of the 600 or so carotenoids identified to date, only about two dozen are found in human blood and tissues. Astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione), another high-value carotenoid, is included in them (El-Agamey et al., 2004).

Astaxanthin (Fig. 1a) is one of xanthophyll carotenoid pigments found in aquatic animals such as shrimps, crabs, salmon, and many other organisms (Shahidi, Metusalach, & Brown, 1998). The utility of astaxanthin as a pigmentation source in the aquaculture industry has been well documented (Lorenz & Cysewski, 2000). Moreover, astaxanthin has several key biological functions in fish. It serves as a precursor of vitamin A, it can associated with reproduction and embryo development and also with protecting cells against oxidative damage (Christiansen, Lie, & Torrissen, 1994; Nakano, Tosa, & Takeuchi, 1995). Recently, in human nutrition, astaxanthin has been gaining widespread popularity as a nutraceutical for the prevention and treatment of various diseases, as well as for general enhancement of immune responses (Guerin, Huntley, & Olaizola, 2003). It has reported that astaxanthin is more effective than β-carotene and lutein at preventing UV light photooxidation of lipids (Santocono, Zurria, Berrettini, Fedeli, & Falcioni, 2006) and has up to tens of folds stronger antioxidant activity than vitamin E and β-carotene (Kurashige, Okimasu, Inoue, & Utsumi, 1990; Miki, 1991).

As most carotenoids, astaxanthin is a highly unsaturated molecule and thus, can easily be degraded by heat, light and oxygen during the manufacture and storage (Christophersen, Jun, Jørgensen, & Skibsted, 1991). In addition, limited water solubility of astaxanthin has lowered its bioavailability and hampered its applications (Anarjan, Mirhosseini, Baharin, & Tan, 2010). Recent years, several studies have been performed to improve the solubility or stability of astaxanthin such as microencapsulation into chitosan matrix (Higuera-Ciapara, Felix-Valenzuela, Goycoolea, & Argüelles-Monal, 2004), encapsulation into polymeric nanospheres (Tachaprutinun, Udomsup, Luadthong, & Wanichwecharungruang, 2009), incorporation into emulsions (Ribeiro, Rico, Badolato, & Schubert, 2005), complexation with β-cyclodextrin (Chen, Chen, Guo, Li, & Li, 2007) and sulfobutyl ether β-cyclodextrin (Samuel, Sean, & Gerold, 2003). In our previous research, astaxanthin was

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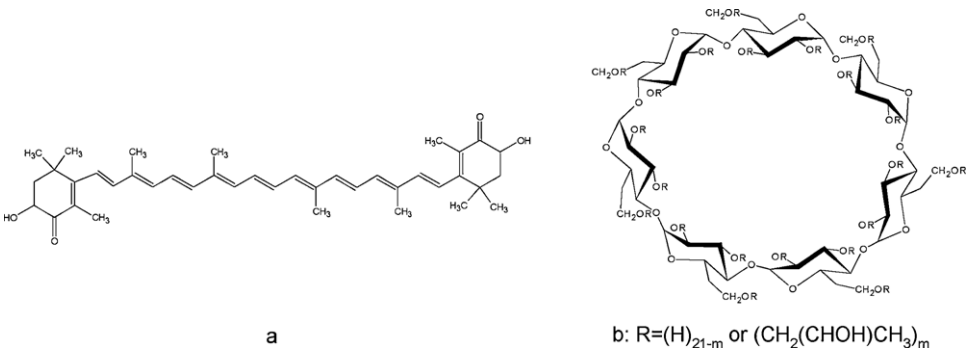

Fig. 1. Chemical structure of astaxanthin (a) and HPCD (b).

successfully embedded in hydroxypropyl-β-cyclodextrin (HPCD, Fig. 1b) and the water solubility and stability of astaxanthin were obviously increased (Yuan, Jin, Xu, Zhuang, & Shen, 2008).

The information about effect of complexation on stability and antioxidant activity of astaxanthin is very scarce. Therefore, the aim of the following study was to evaluate storage stability of astaxanthin/HPCD complex under different storage temperatures, and study its antioxidant activity by three different methods: (1) ferric ion reducing antioxidant power assay, (2) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay, and (3) hydroxyl radicals scavenging activity assay.

2. Materials and methods

2.1. Chemicals

Astaxanthin (purity > 98%) was purchased from Sigma–Aldrich Co. LLC (Shanghai, China). HP-β-CD (purity > 99%, DS = 5.5) purchased from Wako Pure Chemical Industries Ltd. (Chuoku, Osaka, Japan). Astaxanthin/HPCD inclusion complex was prepared in our own lab. All other reagents were of analytical grade. The water used was double distilled and deionized.

2.2. Preparation of astaxanthin/HPCD inclusion complex

10 mg astaxanthin was dissolved in 10 mL dichloromethane and added to 40 mL HPCD methanol solution (25 mg/mL). The mixture was sealed under a nitrogen atmosphere. Then it was put under ultrasonic environment for 5 min to make the mixture blended thoroughly. The purple suspension was stirred for 48 h at 35 °C in the dark, and then dried in a vacuum concentrator. The dried residue was redissolved in water and filtered under vacuum. The orange filtrate was frozen and then lyophilized (Labconco Freeze Dry System/Freezone 4.5, Labconco, Kansas City, MO, USA).

2.3. Storage stability

The inclusion complex was dissolved in water (2.65 μg/mL). Astaxanthin was dissolved in acetone/*n*-hexane (1:1, v/v, 3.28 μg/mL). The samples were put into amber glass bottle and blanketed with N_2. Then, the samples were divided into two groups and stored at 4 °C and 25 °C in dark incubators. Absorbance of the samples was measured at 480 nm every 24 h. Each test was repeated at least three times.

2.4. Antioxidant activity

2.4.1. Reducing power

Reducing power was determined according to the method described by Siddhuraju and Becker (Siddhuraju & Becker, 2003). 0.5 mL of sample solution (astaxanthin and astaxanthin/HPCD inclusion complex, ascorbic acid was employed as a control sample) at various concentrations were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1.0%, w/v). The mixture was incubated at 50 °C for 20 min, and then cooled rapidly. 2.5 mL of trichloroacetic acid (10.0%, w/v) was added to the mixture, which was then centrifuged at 4000 rpm for 10 min. 2.5 mL of upper layer of the solution was taken out and mixed with 2.5 mL of deionized water and 0.5 mL of ferric chloride (0.1%, w/v), the mixture was stood for 10 min, then the absorbance was measured at 700 nm by a 2100 UV–VIS spectroscopy (Unico, Shanghai, China).

2.4.2. DPPH radical scavenging activity

The 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging activity of astaxanthin/HPCD inclusion complex was measured using the method of Gülçin, Küfrevioğlu, Oktay, and Büyükokuroğlu (2004) with some modifications. 0.1 mM solution of DPPH in ethanol was prepared. Then, 2 mL of this solution was added to 2 mL of sample solutions at different concentrations. The mixture was shaken and allowed to stand at 25 °C for 30 min. The absorbance was measured at 517 nm in 2100 UV–VIS spectroscopy. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH scavenging effect (K_D) was calculated using the following equation:

$$K_D\ (\%) = \frac{A_c - (A_i - A_j)}{A_c} \times 100 \quad (1)$$

where A_c was the absorbance of the blank control, A_i was the absorbance in the presence of the samples and A_j was the absorbance of the samples alone.

2.4.3. Hydroxyl radical scavenging activity

Hydroxyl radical was generated by Fenton system to test the hydroxyl radical scavenging activity of the inclusion complex (Valentão et al., 2002). 1.5 mL phenanthroline (5 mM) was mixed with 4.0 mL phosphate buffer (0.75 M, pH 7.4), 1.0 mL ferrous sulfate (7.5 mM) and 2.5 mL sample solution was added and shaken, then, 1.0 mL hydrogen peroxide (1.0%, w/v) was added to the mixtures. After incubation at 37 °C for 1 h, the absorbance of the resulting solution (A_x) was measured at 536 nm. According to the above procedure, the sample solution was replaced by deionized water, the absorbance (A_s) was measured, finally, both the sample solution and hydrogen peroxide were replaced by the same volume of deionized water, and the absorbance (A_0) was obtained. The hydroxyl radical scavenging effect (K_H) was calculated using the following equation:

$$K_H\ (\%) = \frac{A_0 - A_x}{A_0} \times 100 \quad (2)$$

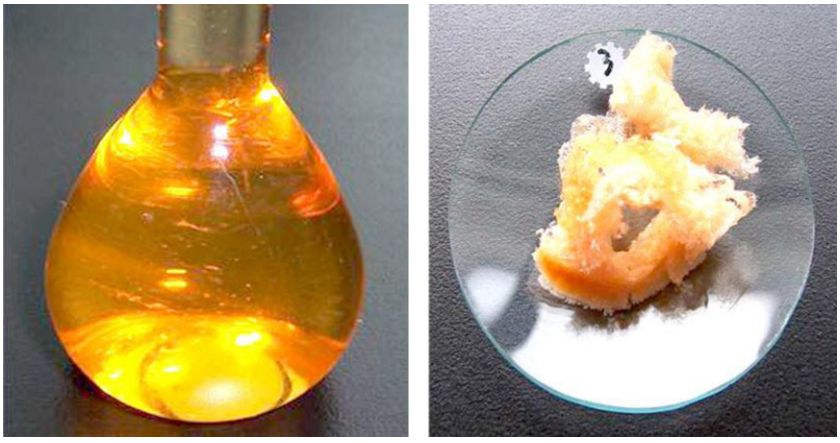

Fig. 2. Aqueous solution and freeze dried sample of astaxanthin/HPCD complex.

3. Results and discussion

3.1. Storage stability of the astaxanthin/HPCD inclusion complex

According to our previous research (Yuan et al., 2008), mixing astaxanthin with HPCD, an orange, clear water solution was achieved. Then, the solution was freeze-dried, a pink solid state sample was obtained. Fig. 2 shows the aqueous solution and freeze-dried sample of astaxanthin/HPCD complex. The aqueous solubility of astaxanthin was highly enhanced (>1.0 mg/mL) after complexation. The result is similar to that of other carotenoids/cyclodextrin complexes (Pfitzner, Francz, & Biesalski, 2000). Astaxanthin should show a good antioxidant activity under this concentration. Moreover, the complex also has fine tinctorial power for food or cosmetic products.

However, good antioxidant activity and tinctorial power are depended on the storage stability of astaxanthin. Figs. 3 and 4 show the storage stabilities of astaxanthin and the complex at 4 °C and 25 °C respectively. It can be found that the absorbance of astaxanthin and the complex reduced slowly within test periods. The stability of the complex was higher than that of native astaxanthin. After 28 days test, 6.09% of native astaxanthin decomposed, meanwhile, 4.59% of astaxanthin in the complex lost, at 4 °C. When stored at 25 °C, 8.58% of native astaxanthin and 7.20% of astaxanthin in the complex decomposed, respectively. The data is slightly higher than that of 4 °C. Overall, both native and astaxanthin in the complex showed high stability under storage condition. This result was agreed with former report (Rao, Sarada, & Ravishankar, 2007). Complexation was helpful to improve stability of astaxanthin under storage condition.

3.2. Antioxidant activity of the inclusion complex

The antioxidant activity of putative antioxidants have been attributed to various mechanisms, main of which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (El-Agamey et al., 2004; Oktay, Gülçin, & Küfrevioğlu, 2003). Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants work. Of these, reducing power, DPPH assay, active oxygen species such as H_2O_2, $O_2^{\bullet -}$ and $OH^{\bullet}$ quenching assays are most commonly used for the evaluation of antioxidant activities of functional materials (Roy et al., 2011; You et al., 2012).

3.2.1. Reducing power

Reducing ability to convert Fe^{3+} to Fe^{2+} is an indirect evidence for the antioxidant activity of an extract or a compound (Roy et al., 2011). In the reducing power assay, the antioxidants, i.e. the reducing species present in the solution causes the reduction of the

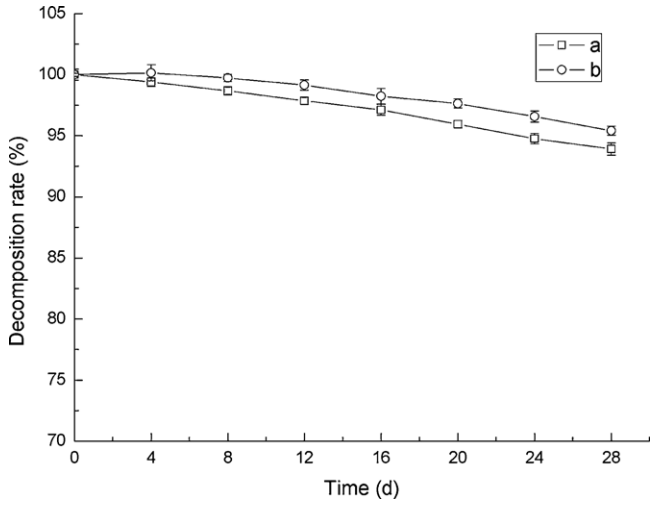

Fig. 3. Storage stability of astaxanthin (a) and astaxanthin/HPCD (b) complex at 4 °C.

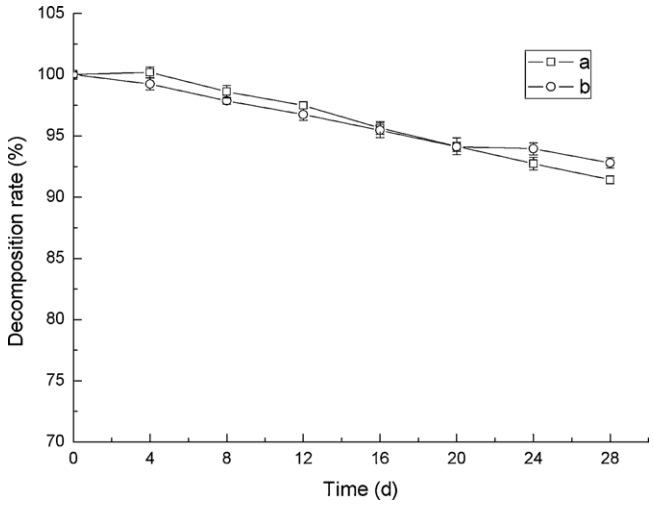

Fig. 4. Storage stability of astaxanthin (a) and astaxanthin/HPCD (b) complex at 25 °C.

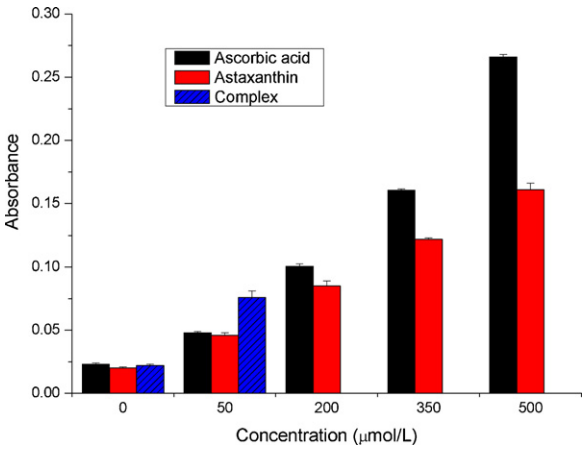

Fig. 5. Reduce power of ascorbic acid, astaxanthin and astaxanthin/HPCD complex.

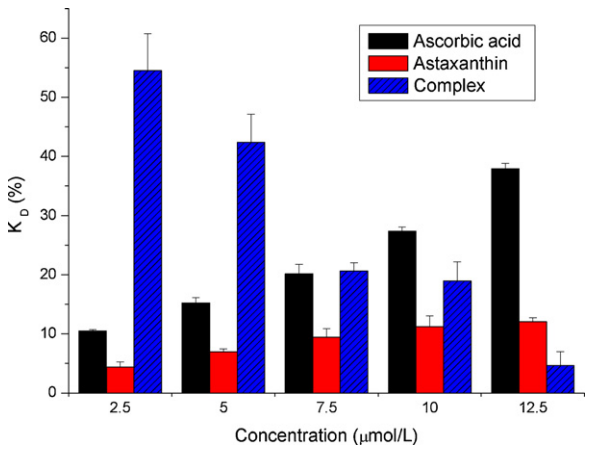

Fig. 6. DPPH radical scavenging activity of ascorbic acid, astaxanthin and astaxanthin/HPCD complex.

Fe^{3+}/ferricyanide complex to form Fe^{2+} ions. This reaction was monitored spectrophotometrically by recording the absorbance of the reaction mixture at 700 nm (Barros et al., 2008). According to the mentioned principle, the higher absorbance value at 700 nm is gained, the stronger reducing power of the assayed sample shows. Reducing power characteristics of ascorbic acid, astaxanthin and astaxanthin/HPCD complex are shown in Fig. 5. Reducing powers of the three samples were all enhanced along with the increase of concentration among the tested range. The reducing power of ascorbic acid was stronger than astaxanthin under the same concentration. It has reported that astaxanthin was poorly soluble and assembled in water (Köpsel et al., 2005). The assembled astaxanthin molecules could not fully close and react with the Fe^{3+}/ferricyanide ions in the test. Nevertheless, the complexation made that astaxanthin was completely dissolved in water. Hence, the reducing power of the complex was stronger than that of native astaxanthin. Due to the limits of solubility and sample quantity, the complex was only tested at the concentration of 50 μmol/L.

3.2.2. DPPH radical scavenging activity

The DPPH antioxidant assay is based on the characteristic of DPPH a stable free radical which centered on nitrogen atom, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the strong absorbance at 517 nm, it is, a visible deep purple color. When DPPH accepts an electron donated by an antioxidant, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance (Ara & Nur, 2009). DPPH radical scavenging activity of astaxanthin and astaxanthin/HPCD complex were evaluated and compared with that of the ascorbic acid, a control compound (Fig. 6). Scavenging activities of ascorbic acid and astaxanthin were enhanced with the increase of concentration among the evaluated range. The scavenging effect of astaxanthin was agreed with the previous reports (Chen, Wang, Ma, Zheng, & Li, 2007; Liu & Osawa, 2007). However, the scavenging activity of astaxanthin was weaker than ascorbic acid (about a half) under the same concentration. The DPPH radical scavenging activity of the complex degraded with the increase of concentration. Nevertheless, it also has a considerable scavenging effect at lower concentration, scavenging rate reached 54.49% at 2.5 μmol/L, and the result was higher than that of ascorbic acid and astaxanthin, 10.50% and 4.43%, respectively. When the concentration was raised to 7.5 μmol/L, scavenging activity of the complex was equal to ascorbic acid, and then became lower. It indicates that the complexation was helpful to enhance the antioxidant activity of astaxanthin in aqueous solution. However, at a high concentration, the increase of HPCD made that the release of astaxanthin became difficult from the complex and the activity obviously decreased. Rao et al. (2007) reported that astaxanthin exhibited about 50% of antioxidant activity at the 20 ppm level of carotenoid by using the DPPH method and hydroxy radical scavenging activity and which clearly indicated that astaxanthin is stable in its ester form in almost all the edible oils at room temperature rather than in aqueous form. Those result coincides with our present study.

3.2.3. Hydroxyl radical scavenging activity

The hydroxyl radical is the neutral form of the hydroxide ion. Hydroxyl radicals are highly reactive and consequently short-lived. The potentially reactive hydroxyl radicals can damage virtually

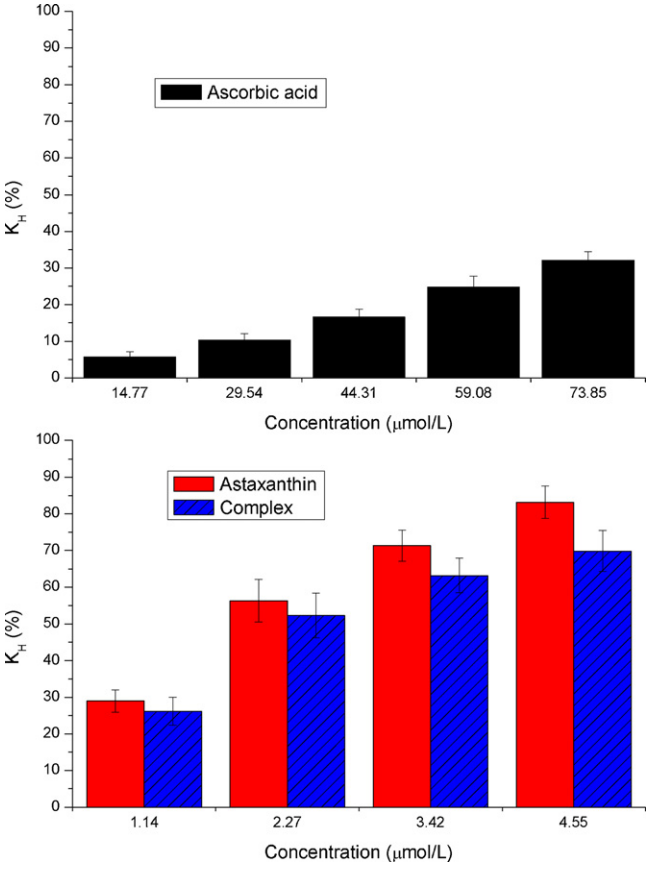

Fig. 7. Hydroxyl radical scavenging activity of ascorbic acid, astaxanthin and astaxanthin/HPCD complex.

all types of macromolecules: carbohydrates, nucleic acids, lipids and amino acids. This makes it a very dangerous compound to the organism (Reiter et al., 1995). Hydroxyl radical scavenging activity of astaxanthin and astaxanthin/HPCD complex were assayed and also compared with ascorbic acid (Fig. 7). The scavenging activity of the three compounds all showed a positive correlation with their concentrations. However, the scavenging effects of astaxanthin and astaxanthin/HPCD complex were about dozens of higher than ascorbic acid, ascorbic acid scavenged 32.13% of hydroxyl radicals at 73.86 μmol/L, while 83.16% of hydroxyl radicals were quenched by astaxanthin at 4.55 μmol/L. The result is in agreement with the report that astaxanthin has a higher antioxidant activity than α-tocopherol and β-carotene (Lorenz & Cysewski, 2000). The scavenging activity of the complex was close to astaxanthin; nevertheless, the former became lower at high concentrations. Maybe, the existence of HPCD hindered the reaction between astaxanthin and hydroxyl radical.

4. Conclusions

In this study, the storage stability and antioxidant activity of astaxanthin/HPCD complex were evaluated. Both native astaxanthin and the complex showed high stability under storage condition at 4 °C and 25 °C. Complexation improved the stability of astaxanthin under storage conditions. Both astaxanthin and astaxanthin/HPCD complex had high antioxidant activity in vitro. Compared with ascorbic acid, the reducing power and DPPH radical scavenging activity of astaxanthin were lower in the water due to the poor solubility and self-assembly; the complex was stronger at low concentration for solubility enhancing effect of HPCD. However, the activity decreased at high concentration for the difficult release of astaxanthin in the complex. Astaxanthin and the complex were about dozens of higher than ascorbic acid in the hydroxyl radical scavenging activity, and the complex was a little lower than native astaxanthin. In summary, complexation with HPCD could affect the antioxidant activity of astaxanthin; it should be improved at low concentration aqueous solution, but hindered at high due to the controlled-release effect. More detailed studies are underway in our laboratory to understand the storage stability and antioxidant potential of astaxanthin/HPCD complex in the food system.

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