Carbohydrate Polymers 89 (2012) 492–496

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Inclusion complex of astaxanthin with hydroxypropyl-β-cyclodextrin: UV, FTIR, ^{1}H NMR and molecular modeling studies

Chao Yuan [a,*], Zhengyu Jin [b,**], Xueming Xu [b]

[a] School of Biotechnology and Food, Anyang Institute of Technology, Anyang, Henan 455000, PR China
[b] School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, PR China

ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form 2 March 2012
Accepted 10 March 2012
Available online 20 March 2012

Keywords:
Astaxanthin
Hydroxypropyl-β-cyclodextrin
Inclusion complex
Structure
Molecular modeling

ABSTRACT

The structure and complex mode of the inclusion complex of astaxanthin with hydroxypropyl-β-cyclodextrin (HPCD) were investigated by UV, FTIR, ^{1}H NMR and molecular modeling test. UV, FTIR and ^{1}H NMR results indicated that the hexatomic ring of the astaxanthin molecules were partly included into the HPCD cavities. The implementation of molecular modeling test confirmed that the complexation could reduce the energy of the system and the complex of 2:1 host–guest stoichiometry had the lowest ΔE value, −30.57 kcal/mol, two hexatomic ring ends of one astaxanthin molecule inserted into two HPCD cavities respectively, and that should be the most predominant configuration.

1. Introduction

Astaxanthin is the main carotenoid pigment naturally synthesized in some plants, algae and bacteria, and is amassed in aquatic animals including salmon, trout, red seabream, shrimp, lobster and fish eggs and birds such as flamingoes and quails through food chains in nature (Guerin, Huntley, & Olaizola, 2003; Lia, Daling, Jianfeng, Songdong, & Guangce, 2011). Astaxanthin cannot be synthesized by animals and must be acquired from the diet. It is commercially available either from chemical synthesis or natural resources such as microalgae, yeast and crustacean byproducts (Higuera-Ciapara, Félix-Valenzuela, & Goycoolea, 2006). Astaxanthin is closely related to other well-known carotenoids, such as β-carotene, zeaxanthin and lutein, thus they share many of the metabolic and physiological functions attributed to carotenoids. Carotenoids have been found to provide several common biological functions, such as photoprotection, antioxidant effects including singlet oxygen quenching, immunomodulatory and anticancer activity, in both humans and rodents (Krinsky & Johnson, 2005). It has reported that astaxanthin can be significantly more effective than β-carotene and lutein at preventing UV light photooxidation of lipids (Santocono, Zurria, Berrettini, Fedeli, & Falcioni, 2006) and has up to several folds stronger free radical antioxidant activity than vitamin E and β-carotene (Kurashige, Okimasu, Inoue, & Utsumi, 1990; Miki, 1991). The antioxidant properties of astaxanthin are believed to play a key role in several other properties such as photoprotectant, protection against age-related diseases or the promotion of the immune response, liver function and eye health. Due to the special function properties, astaxanthin has widespread applications in nutraceutical, cosmetic, food and feed industries (Guerin et al., 2003; Lorenz & Cysewski, 2000).

However, astaxanthin can easily be decomposed by light and oxygen, which can cause the loss of antioxidant properties in processing and store. Furthermore, it cannot be readily absorbed by the human body because of its poor bioavailability. The low bioavailability of these kinds of functional lipids is because of their poor water solubility (Anarjan, Mirhosseini, Baharin, & Tan, 2010).

Cyclodextrins and its derivatives have been used extensively as the host to increase the solubility of poor water soluble organic guest molecules by formation of inclusion complexes (Duchêne, Wouessidjewe, & Ponchel, 1999; Martin Del Valle, 2004). The resulting noncovalent inclusions or host–guest complexes are of current scientific and technological interest for their peculiar physical, chemical and biological properties. Such noncovalent associations can actually improve the guest water solubility, bioavailability and stability; they can also regulate the release of the guest molecules (Loftsson & Duchêne, 2007; Szente & Szejtli, 1999).

Recent years, several studies which focused on the reaction between cyclodextrins and carotenoids have been carried out.

* Corresponding author. Tel.: +86 372 2909876; fax: +86 372 2909900.
** Corresponding author. Tel.: +86 510 85913299; fax: +86 510 85913299.
E-mail addresses: wheat1997@yahoo.com (C. Yuan), jinlab2008@yahoo.com (Z. Jin).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2012.03.033

Pfitzner, Francz and Biesalski (2000) have developed a physiological, water-soluble complex of carotenoids (zeaxanthin, lutein, lycopene and β-carotene) with methyl-β-cyclodextrin for the purpose of cell supplementation. The stability of the different carotenoid/methyl-β-cyclodextrin complex solutions under cell culture conditions was found higher than uncomplex carotenoids. A water-soluble complexes of the dietary carotenoid, lycopene with α-cyclodextrin and β-cyclodextrin have been prepared and characterized (Mele, Mendichi, Selva, Molnar, & Toth, 2002). Bixin was investigated to complexation with α-cyclodextrin using both column percolation and sonication by Lyng, Passos and Fontana (2005). Another group has prepared the inclusion complex of astaxanthin with β-cyclodextrin (Chen, Chen, Guo, Li, & Li, 2007). Those works are mainly focused on the formation and properties of the inclusion complexes. The molecular structure should have important relationship with the character of the inclusion complexes, however, there are little reports were found about the molecular structure of the carotenoids/cyclodextrins complex.

In our former work, the inclusion complex which formed between astaxanthin and hydroxypropyl-β-cyclodextrin (HPCD) has been successfully prepared. The water solubility and stability of astaxanthin was obviously increased. Furthermore, the release of astaxanthin from the inclusion complex was controlled (Yuan, Jin, Xu, Zhuang, & Shen, 2008).

The object of this study was to further investigate the molecular structure of the astaxanthin/HPCD complex by UV, FTIR and ^{1}H NMR methods, and find some new information between the structure and character.

2. Materials and methods

2.1. Chemicals

Astaxanthin (purity > 98%) was purchased from Sigma–Aldrich Co. LLC. (Shanghai, China). HPCD (purity > 99%, DS = 5.5) purchased from Wako Pure Chemical Industries, Ltd. (Chuoku, Osaka, Japan). All other reagents were of analytical grade. The water used was double distilled and deionized.

2.2. Preparation of the inclusion complex of astaxanthin with HPCD

The preparation method of the inclusion complex was according to the former study (Yuan et al., 2008). 2 ml astaxanthin in dichloromethane (1 mg/ml) was added to 200 mg HPCD dissolved in 8 ml methanol. 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 24 h at 35 °C and 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. Characterization of inclusion complex

UV spectra of the inclusion complex, pure astaxanthin and HPCD in methanol solution were obtained by UNICO 2100 UV–vis spectroscopy (Unico, Shanghai, China). The scans were registered from 200 to 800 nm.

FTIR was conducted using a Nicolet 5DXC IR Spectrometer (Nicolet, Madison, WI, USA). The diffuse reflectance technique was utilized in the mid-IR (400–4000 cm^{-1}) spectral region. The procedure consisted of grinding the sample together with KBr (about 200–400 mg) into a fine powder, placing the powder into the sampling cup, smoothing the powder, and compressing the powder bed into the holder using a compression gauge. The sample was placed in the light path and the spectrum was obtained.

^{1}H NMR spectra were recorded at 25 °C using a Bruker AM-400 NMR spectrometer (Germany) at 500 MHz. HPCD, astaxanthin and the astaxanthin/HPCD complex were dissolved in DMSO solution and tested, respectively.

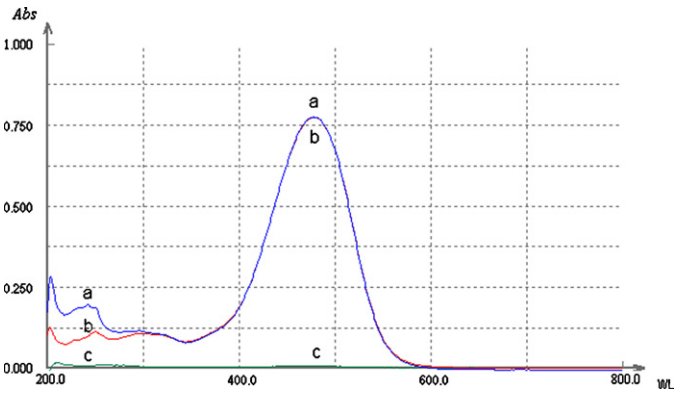

Fig. 1. The UV absorption spectra of astaxanthin (b), HPCD (c) and their inclusion complex (a).

2.4. Molecular modeling

The molecular modeling was carried out by Hyperchem 8.0 software. The molecular mechanics MM2 force field method was used for molecular modeling calculations. The initial structures of astaxanthin and HPCD were constructed with the help of Hyperchem 8.0 and optimized with PM3. The modeling was performed by docking the optimized structure of the astaxanthin molecule into the HPCD cavity and allowing for full-geometry optimization. Modeling calculations were performed in two steps. Firstly, for one HPCD and one astaxanthin (1:1), the hexatomic ring of astaxanthin was stepwise inserted into the cavity of HPCD. In the second step, for two HPCD and one astaxanthin, the optimized 1:1 complex above was docked into the second HPCD cavity from the other end. All modes were minimized using a conjugate gradient optimization procedure until a root mean square (RMS) value of 0.01 kcal/(mol Å) was obtained (Wen, Liu, & Zhu, 2005). ΔE of the minimum energy mode was calculated according to Eq. (1):

$$\Delta E = E_{\text{complex}} - (E_{\text{host}} + E_{\text{guest}}) \quad (1)$$

The calculation energy of HPCD, astaxanthin and the inclusion complex molecules were E_{host}, E_{guest} and E_{complex} (kcal/mol), respectively.

3. Results and discussion

3.1. UV/VIS spectra

The UV/VIS spectra of astaxanthin/HPCD complex, astaxanthin and HPCD were recorded respectively, according to the procedure of UV spectra. Fig. 1 showed that HPCD had no absorption in the range 225–800 nm and had slight absorption at about 210 nm. Astaxanthin had 4 absorption peaks in the scanned range, thereinto, the highest absorption peak was at 478 nm which was K band absorption generated by $\pi \rightarrow \pi^*$ transition of the large conjugated system of the whole molecule. The absorption peak at 203 nm was K band absorption of unsaturated hydroxyketone, it was generated by $\pi \rightarrow \pi^*$ transition of the C=C and C=O conjugated system in the hexatomic ring of astaxanthin molecule. 250 nm was K band absorption of the whole hexatomic ring. The weak absorption peak at 295 nm was R band absorption generated by $n \rightarrow \pi^*$ transition

Table 1
Variation of ^{1}H chemical shift (δ/ppm) of HPCD before and after forming complex with astaxanthin (DMSO, 300 K).

HPCD	H-1	H-2	H-3	H-4	H-5	H-6	OCH_2	CH_3	OH
δ_{free}	4.833	3.441	3.751	3.412	3.478	3.615	3.327	1.025	2.501
$\delta_{complex}$	4.833	3.439	3.754	3.395	3.465	3.615	3.301	1.025	2.500
$\Delta\delta$	0.000	−0.002	0.003	−0.007	−0.013	0.000	−0.026	0.000	−0.001

of the carbonyl group and it is the characteristic peak of carbonyl compounds. There were several changes on the UV/VIS spectra of astaxanthin/HPCD complex compared with that of astaxanthin. The intensity of K band absorption peak at 203 nm increased greatly. The peak at 250 nm increased too, and blue-shifted. The R band absorption peak at 295 nm was slightly affected by the formation of complex. Nevertheless, the peak at 478 nm was not visibly affected. Those changes mainly caused by the host–guest interaction of the astaxanthin/HPCD complex for HPCD had no absorption in the scanned range. It could be speculated that HPCD formed complex with the hexatomic ring at the end of the astaxanthin molecule which contained a carbonyl group from the change of absorption peaks. The hexatomic ring entered into the lyophobic cavity of HPCD formed a new hydrogen bond and leaded the change of density of electron cloud. Therefore, the absorption peaks of the complex changed from that of astaxanthin.

3.2. FTIR spectra studies

The variation of the shape, shift, and intensity of the IR absorption peaks of the guest or host can provide enough information for the occurrence of the inclusion. Fig. 2 showed the IR spectra of astaxanthin, HPCD and their inclusion complex. The IR spectrum of astaxanthin showed its characteristic bands in agreement with the previously report (Chen et al., 2007). There was a very strong absorption band at 1654 cm^{-1} for C=O stretching vibration. Absorption band at 1552 cm^{-1} was denoted for stretching vibration of C=C in the hexatomic ring. 974 cm^{-1} was for absorption band of C—H in C, C conjugate system. The IR spectrums of the inclusion complex are similar to that of HPCD, due to the low quantity of astaxanthin in the system. However, several variations were found in the spectra. The absorption band at 1654 cm^{-1} were disappeared or shifted to low wavenumbers in the astaxanthin/HPCD inclusion complex, indicating that the C=O stretching vibration was restricted after the formation of inclusion complex. 1552 cm^{-1} was greatly weakened, indicating that a majority of hexatomic ring of astaxanthin was included by HPCD, but maybe a few of astaxanthin only one hexatomic ring of the two was included.

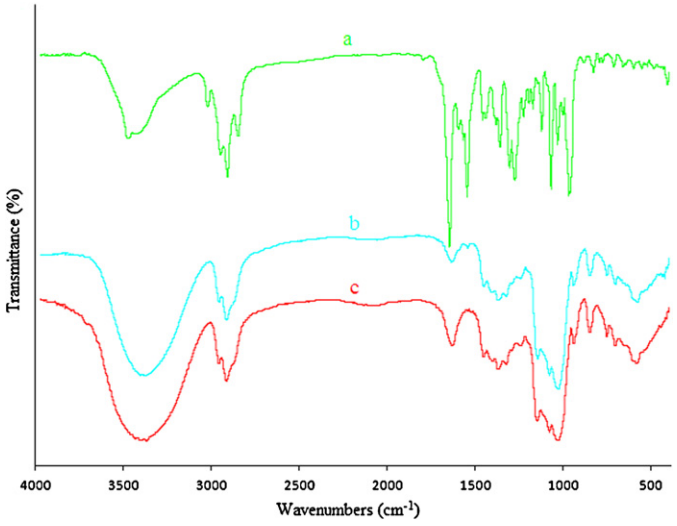

Fig. 2. The IR spectra of astaxanthin (a), HPCD (b) and their inclusion complex (c).

3.3. ^{1}H NMR spectra

^{1}H NMR spectra are one of the most direct evidence for the formation of the inclusion complex (Hamdi, Abderrahim, & Meganem, 2010). If a guest molecule is incorporated into the HPCD cavity, the screening constants of the HPCD protons inside the cavity (H-3 and H-5) should be sensitive to the changed environment, but that of the outside protons (H-1, H-2, and H-4) should not. This should result in chemical shift changes of the inside protons (Polyakov, Leshina, Konovalova, Hand, & Kispert, 2004). The ^{1}H chemical shifts of HPCD were determined through the ^{1}H NMR spectra of HPCD, astaxanthin and their complex and the results showed in Table 1. The ^{1}H chemical shifts of free HPCD were agreed with former report (Ge et al., 2011). ^{1}H NMR spectra of the complex showed the proton peaks both of HPCD and astaxanthin; furthermore, several ^{1}H chemical shifts of HPCD were changed. It confirmed that the complex was formed. Table 1 showed that chemical shift of outside protons H-1 and H-6 had no variation before and after forming complex, $\Delta\delta$ of the inside protons H-3 and H-5 were 0.003 ppm and −0.013 ppm, respectively. When the complex was formed, the hexatomic ring of astaxanthin should enter the cavity of HPCD, electron cloud was dense at the H-5 proton located near the terminal of the hexatomic ring where electron cloud was dense made it to be shielded and upfield shift, while the H-3 proton presented downfield shift owning to the deshielding zone of the hexatomic ring which were generated by Van der Waals force. The relative position between HPCD and the hexatomic ring of astaxanthin was shown in Fig. 3.

3.4. Molecular modeling studies

In recent years, widespread use has been made of computer aided molecular modeling to rapidly and simply obtain a three dimensional image of the most likely structure of the inclusion complex (Anguiano-Igea, Otero-Espinar, Vila-Jato, & Blanco-Méndez, 1997; Wen et al., 2005). Calculations by PM3 method were performed in order to obtain some global information about the geometry of the host–guest complexes and to find the intermolecular interaction in HPCD and astaxanthin inclusion complexation. ΔE of the complexation was calculated for the minimum energy mode according to Eq. (1) and the data of $E_{complex}$, $E_{host} + E_{guest}$, ΔE were listed in Table 2. For one HPCD molecule and one astaxanthin molecule, the ΔE values of the model a and model b were −9.39 and −23.29 kcal/mol respectively, while for the 2:1 stoichiometric ratio of the complex (model c), the ΔE value was −30.57 kcal/mol. It was indicated that the complex of 2:1 host–guest stoichiometry was the most predominant configuration of the three modes, both hexatomic ring ends of one astaxanthin molecule, respectively, inserted into two HPCD cavities due to hydrophobic interactions and hydrogen bonds. The three modes of astaxanthin/HPCD complexes with minimum energies were shown in Fig. 3.

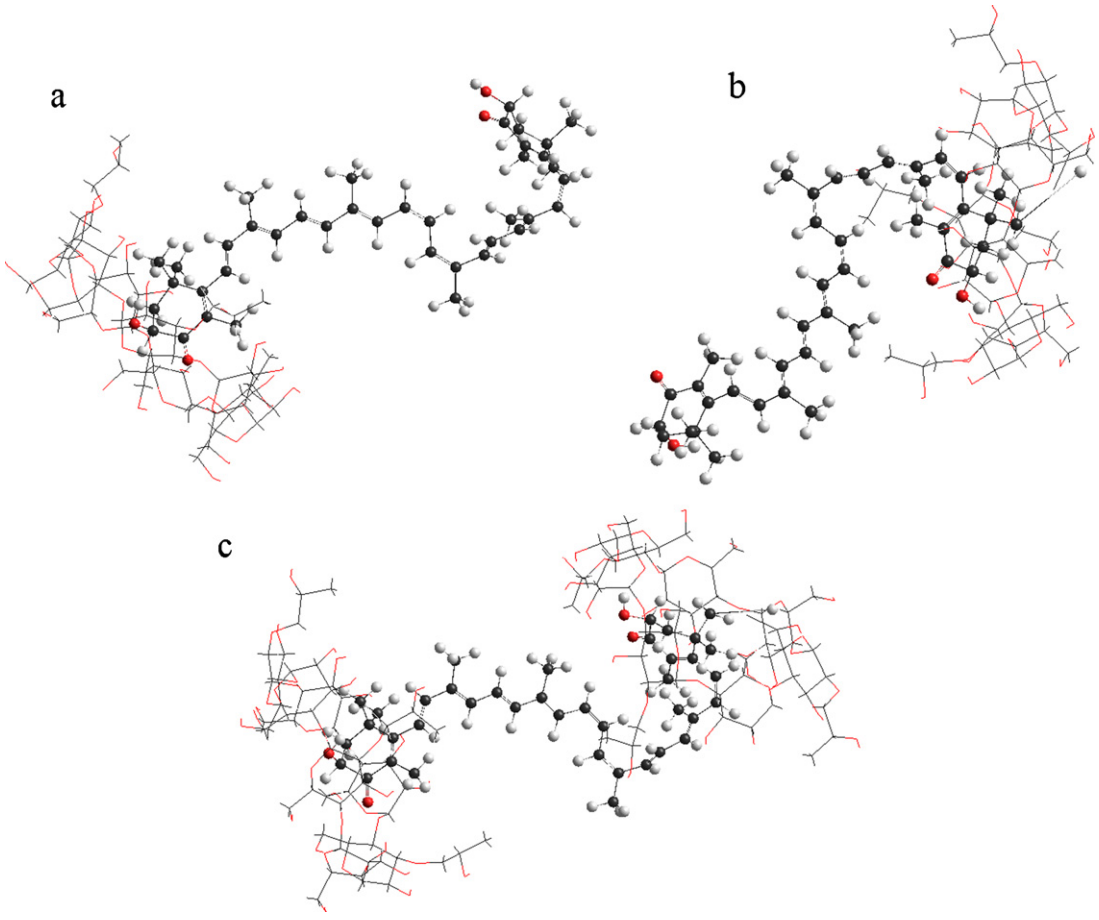

Fig. 3. Energy-minimized modes obtained by PM3 calculations for astaxanthin/HPCD complex.

Table 2
The interaction energy between astaxanthin and HPCD calculated with PM3.

Models	HPCD:astaxanthin	$E_{complex}$ (kcal/mol)	$E_{host} + E_{guest}$ (kcal/mol)	ΔE (kcal/mol)
a	1:1	−27011.60	−27002.21	−9.39
b	1:1	−27025.50	−27002.21	−23.29
c	1:2	−44173.65	−44143.08	−30.57

4. Conclusions

In present work, the inclusion complexes of astaxanthin with HPCD were prepared and the structures of the complexes were investigated by UV, FTIR and ^{1}H NMR. The experimental results showed that the mode of the complex was the hexatomic ring part of the astaxanthin molecules was included into the HPCD cavities. Furthermore, the implementation of molecular modeling test confirmed that the complex of 2:1 host–guest stoichiometry had the lowest ΔE value, two hexatomic ring ends of one astaxanthin molecule inserted into two HPCD cavities, respectively and it should be the most predominant configuration.

Acknowledgments

This work was supported by National Science & Technology Pillar Program during the Eleventh Five-year Plan Period (Grant no. 2006BAD27B06). We are grateful to Prof. Yibing Tan for providing technical assistance.

References

Anarjan, N., Mirhosseini, H., Baharin, B. S., & Tan, C. P. (2010). Effect of processing conditions on physicochemical properties of astaxanthin nanodispersions. *Food Chemistry*, *123*(2), 477–483.

Anguiano-Igea, S., Otero-Espinar, F. J., Vila-Jato, J. L., & Blanco-Méndez, J. (1997). Interaction of clofibrate with cyclodextrin in solution: Phase solubility, ^{1}H NMR and molecular modelling studies. *European Journal of Pharmaceutical Sciences*, *5*(4), 215–221.

Chen, X., Chen, R., Guo, Z., Li, C., & Li, P. (2007). The preparation and stability of the inclusion complex of astaxanthin with β-cyclodextrin. *Food Chemistry*, *101*(4), 1580–1584.

Duchêne, D., Wouessidjewe, D., & Ponchel, G. (1999). Cyclodextrins and carrier systems. *Journal of Controlled Release*, *62*(1–2), 263–268.

Ge, X., He, J., Qi, F., Yang, Y., Huang, Z., Lu, R., & Huang, L. (2011). Inclusion complexation of chloropropham with β-cyclodextrin: Preparation, characterization and molecular modeling. *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy*, *81*(1), 397–403.

Guerin, M., Huntley, M. E., & Olaizola, M. (2003). Haematococcus astaxanthin: Applications for human health and nutrition. *Trends in Biotechnology*, *21*(5), 210–216.

Hamdi, H., Abderrahim, R., & Meganem, F. (2010). Spectroscopic studies of inclusion complex of β-cyclodextrin and benzidine diammonium dipicrate. *Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy*, *75*(1), 32–36.

Higuera-Ciapara, I., Félix-Valenzuela, L., & Goycoolea, F. M. (2006). Astaxanthin: A review of its chemistry and applications. *Critical Reviews in Food Science and Nutrition*, *46*(2), 185–196.

Krinsky, N. I., & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, *26*(6), 459–516.
Kurashige, M., Okimasu, E., Inoue, M., & Utsumi, K. (1990). *Inhibition of oxidative injury of biological membranes by astaxanthin* (pp. 27–38).
Lia, J., Daling, Z., Jianfeng, N., Songdong, S., & Guangce, W. (2011). An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*. *Biotechnology Advances*, *29*(6), 568–574.
Loftsson, T., & Duchêne, D. (2007). Cyclodextrins and their pharmaceutical applications. *International Journal of Pharmaceutics*, *329*(1–2), 1–11.
Lorenz, R. T., & Cysewski, G. R. (2000). Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends in Biotechnology*, *18*(4), 160–167.
Lyng, S. M. O., Passos, M., & Fontana, J. D. (2005). Bixin and α-cyclodextrin inclusion complex and stability tests. *Process Biochemistry*, *40*(2), 865–872.
Martin Del Valle, E. M. (2004). Cyclodextrins and their uses: A review. *Process Biochemistry*, *39*(9), 1033–1046.
Mele, A., Mendichi, R., Selva, A., Molnar, P., & Toth, G. (2002). Non-covalent associations of cyclomaltooligosaccharides (cyclodextrins) with carotenoids in water. A study on the α- and β-cyclodextrin/ψ, ψ-carotene (lycopene) systems by light scattering, ionspray ionization and tandem mass spectrometry. *Carbohydrate Research*, *337*(12), 1129–1136.
Miki, W. (1991). Biological functions and activities of animal carotenoids. *Pure and Applied Chemistry*, *63*(1), 141–146.
Pfitzner, I., Francz, P. I., & Biesalski, H. K. (2000). Carotenoid:methyl-β-cyclodextrin formulations: An improved method for supplementation of cultured cells. *Biochimica et Biophysica Acta (BBA): General Subjects*, *1474*(2), 163–168.
Polyakov, N. E., Leshina, T. V., Konovalova, T. A., Hand, E. O., & Kispert, L. D. (2004). Inclusion complexes of carotenoids with cyclodextrins: 1 HNMR, EPR, and optical studies. *Free Radical Biology and Medicine*, *36*(7), 872–880.
Santocono, M., Zurria, M., Berrettini, M., Fedeli, D., & Falcioni, G. (2006). Influence of astaxanthin, zeaxanthin and lutein on DNA damage and repair in UVA-irradiated cells. *Journal of Photochemistry and Photobiology B: Biology*, *85*(3), 205–215.
Szente, L., & Szejtli, J. Z. (1999). Highly soluble cyclodextrin derivatives: Chemistry, properties, and trends in development. *Advanced Drug Delivery Reviews*, *36*(1), 17–28.
Wen, X., Liu, Z., & Zhu, T. (2005). Mass spectrometry and molecular modeling studies on the inclusion complexes between α, β-cyclodextrins and simvastatin. *Chemical Physics Letters*, *405*(1–3), 114–117.
Yuan, C., Jin, Z., Xu, X., Zhuang, H., & Shen, W. (2008). Preparation and stability of the inclusion complex of astaxanthin with hydroxypropyl-β-cyclodextrin. *Food Chemistry*, *109*(2), 264–268.