Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 264–268
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Preparation and stability of the inclusion complex of astaxanthin with hydroxypropyl-β-cyclodextrin

Chao Yuan, Zhengyu Jin *, Xueming Xu, Haining Zhuang, Wangyang Shen

School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, Wuxi 214122, China

Received 5 April 2007; received in revised form 26 June 2007; accepted 19 July 2007

Abstract

The inclusion complex of astaxanthin (ASX) with hydroxypropyl-β-cyclodextrin (HP-β-CD) was prepared. Infrared spectroscopy (IR) proved the formation of the inclusion complex. The water solubility of the inclusion complex was >1.0 mg/ml, which is much better than that of ASX. The solid state thermal behaviour of the inclusion complex was investigated by thermogravimetric/differential thermal analysis (TG/DTA). The starting decomposition temperature of ASX was enhanced to about 290 °C. The stability of the inclusion complex in solution was also tested. Forming of the inclusion complex greatly enhanced the stability of ASX against light and oxygen. Furthermore, the release of ASX from the inclusion complex was controlled.

Keywords: Astaxanthin; Hydroxypropyl-β-cyclodextrin; Inclusion complex; Infrared spectroscopy; Thermal analysis; Stability

1. Introduction

Astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione, ASX) is a high value carotenoid pigment with strong antioxidant properties, which has widespread applications in nutraceutical, cosmetic, food and feed industries (Guerin, Huntley, & Olaizola, 2003; Lorenz & Cysewski, 2000). It is naturally synthesised by a few species of microorganisms such as *Haematococcus pluvialis* and *Phaffia rhodozyma* (Dong & Zhao, 2004). ASX cannot be synthesised by animals and must be acquired from the diet. In many aquatic animals, in which it is found, ASX has several essential biological functions including protection against oxidation and UV light effects; immune response; pigmentation; communication and reproduction behaviour (Naguib, 2000; Shimidzu, Goto, & Miki, 1996).

ASX 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 anticanxor activity, in both humans and rodents (Krinsky & Johnson, 2005; Krinsky & Yeum, 2003; Stahl & Sies, 2005). It has reported that ASX can be significantly more effective than β-carotene and lutein at preventing UV light photooxidation of lipids (Santocono, Zurria, Berrettini, Fedeli, & Falcioni, 2006; Shimidzu et al., 1996). The antioxidant activity of ASX has been demonstrated in several studies. In some cases, ASX 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 ASX are belived 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 (Guerin et al., 2003).

ASX is a highly unsaturated molecule, it can easily be decomposed by light and oxygen, which can cause the loss of its antioxidant properties. Furthermore, the poor aqueous

* Corresponding author. Tel.: +86 510 85913299; fax: +86 510 85919189.
E-mail address: zjin@jiangnan.edu.cn (Z. Jin).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.07.051

solubility of ASX limits its use as an aqueous phase antioxidant.

Cyclodextrins (CDs) have been used extensively as additives to increase the solubility of poorly water-soluble organic compounds, by the formation of an inclusion complex between the host cyclodextrin molecules and the guest molecules. 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 guests water solubility, bioavailability and stability, they can also regulate the release of the guest molecules (Del Valle, 2004; Duchêne & Wouessidjewe, 1990; Szejtli, 1998). Hydroxylpropyl-β-cyclodextrin (HP-β-CD), a hydroxyalkyl derivative, is an alternative to parent CDs, with improved water solubility property and may be slightly more toxicologically benign (Sarah & Robert, 2005; Uekama, Hirayama, & Irie, 1998). As the first approved CD derivative by FDA, HP-β-CD has wide applications in food, pharmaceuticals and agriculture etc. (Szente & Szejtli, 1999).

Several studies have been performed on the reaction between CDs and carotenoids. 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. Mele, Mendichi, and Selva (1998) have prepared the water-soluble complexes of the dietary carotenoid, lycopene with α-CD and β-CD. Bixin complexation with α-CD, using both column percolation and sonication was investigated by Lyng, Passos, and Fontana (2005). Another group (Chen, Chen, Guo, Li, & Li, 2007) has prepared the inclusion complex of ASX with β-CD. However, no reports were found about the ASX/ HP-β-CD complex.

The object of this study was to develop a new, water-soluble formulation of ASX using HP-β-CD as solubiliser. The stability of the resultant complex against heat and light were also investigated.

2. Materials and methods

2.1. Chemicals

ASX (>98%) was purchased from Sigma (Shanghai, China). HP-β-CD (>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 deionised.

2.2. Preparation of the inclusion complex of ASX with HP-β-CD

2 ml ASX in dichloromethane (1 mg/ml) was added to 200 mg HP-β-CD dissolved in 8 ml methanol. The mixture was sealed under a nitrogen atmosphere. Then it was subjected to an ultrasonic environment for 5 min to thoroughly blend the mixture. 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 lyophilised (Labconco Freeze Dry System/Freezone 4.5, Labconco, Kansas City, MO, USA).

2.3. ASX quantitation in organic extracts of the inclusion complex

ASX (98%, 2.8 mg) was dissolved into acetone/*n*-hexane (50 ml). 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml solution was taken out and made up to 25 ml with methanol, respectively. These samples were analysed by a UNICO 2100 UV/Vis spectroscopy (Unico, Shanghai, China), monitoring the absorbance at 480 nm. Then the regression equation was made between the absorbance (Y) and the concentrations (X) of ASX. The inclusion complex of ASX with HP-β-CD (10.0 mg) was dissolved into acetone/*n*-hexane (25 ml). The solution was subjected to an ultrasonic environment for 10 min and this was repeated three times. Then, the solution was analysed by UV/Vis spectroscopy.

2.4. Fourier transform infrared spectroscopy (FTIR)

FTIR was conducted using a Nicolet 5DXC IR Spectrometer (Nicolet, Madison, WI, USA). The diffuse reflectance technique was utilised 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.

2.5. Thermogravimetric/differential thermal analysis (TG/DTA)

The TG/DTA system TGA/SDTA851 (Mettler Toledo, Surich, Switzerland) was adjusted to operate at the following conditions: dynamic atmosphere of helium (99.99%) at 20 ml/min and heating rate of 20 °C/min from 50 to 900 °C, with sample mass of about 10 mg.

2.6. The stability of the inclusion complex

The inclusion complex was divided into three groups and stored at 4, 25 and 50 °C in incubators with a fluorescent lamp opening (15 W). The inclusion complex was dissolved in water (2.65 μg/ml), ASX was dissolved in acetone/*n*-hexane (1:1, v/v, 3.28 μg/ml). The absorbance of the samples was measured at 480 nm. Each test was repeated at least three times.

3. Results and discussion

Mixing ASX with HP-β-CD, an orange, clear water solution was achieved. The solution was freeze–dried, a pink solid state sample was obtained. The weight of the sample was 179 mg. The total recovery rate was 88.6%.

The results of UV/Vis spectroscopy showed that the concentrations (X) and the absorbance (Y) of ASX had good linear relationship, when the concentration of ASX was <6.70 μg/ml. The regression equation was as follows:

$$Y = 0.1243X + 0.008 \quad R^2 = 0.9993 \tag{1}$$

As the absorbance of the ASX/HP-β-CD inclusion complex was 0.264, according to the regression equation (Eq. (1)), the concentration of ASX in the disposed inclusion complex was 2.06 μg/ml. Therefore, the solvent extraction did remove just 0.52% w/w of ASX from the inclusion complex. The inclusion rate was obtained by the quotient of ASX in the inclusion complex divided by the amount of ASX used in the preparation. According to the data tested in this experiment, the rate of inclusion was 46.5%. Dispersing the inclusion complex into water, more than 400 mg was dissolved into 2 ml water. The aqueous solubility of ASX was highly enhanced (>1.0 mg/ml). The result was similar to that of other carotenoids/CD complexes (Pfitzner et al., 2000). Compared with the ASX/β-CD inclusion complex which has been prepared before (Chen et al., 2007), the aqueous solubility was greatly enhanced, due to the high solubility of HP-β-CD. However, the quantity of ASX in the ASX/HP-β-CD complex was lower than that of the ASX/β-CD complex. Probably the HP substituents concentrated at the edge of the cavity of the CDs made it more difficult for the ASX molecules to enter. Furthermore, Mele et al. (1998) have reported that the carotenoid could not be extracted completely from the inclusion complex. This might also be a reason.

Fig. 1 shows the IR spectrums of ASX, HP-β-CD, the inclusion complex and the physical mixture of ASX and HP-β-CD at the same molar rate as the inclusion complex. ASX showed a very strong absorption band at 1654 cm^{-1} for the C=O stretching vibration. 1552 cm^{-1} was assigned to the stretching vibration of C=C in the aromatic ring. 974 cm^{-1} was for absorption band of C–H in C, C conjugate system. The IR spectrums of the inclusion complex and the physical mixture of ASX and HP-β-CD are similar to that of HP-β-CD, due to the low quantity of ASX in the system. However, several variations were found in the spectra. The absorption band at 1654 and 1552 cm^{-1} also showed in the physical mixture of ASX and HP-β-CD. Different from this, the absorption band at 1654 cm^{-1} disappeared or shifted to low wavenumbers in the ASX/ HP-β-CD inclusion complex, indicating that the C=O stretching vibration was restricted after formation of the inclusion complex. The signal at 1552 cm^{-1} was greatly weakened, indicating that a majority of the aromatic ring of ASX was included by HP-β-CD but maybe in a few of the ASX only one aromatic ring of the two was included. The result of the IR indicated the inclusion complex of ASX with HP-β-CD was obtained.

The TG and DTA curves of HP-β-CD, ASX and their inclusion complex are illustrated in Figs. 2–4, respectively. It can be clearly seen that the TG and DTA curves of HP-β-CD and ASX only have one step or one peak, indicating that the two samples were pure compound. Along with the increase of the temperature, HP-β-CD began to decompose

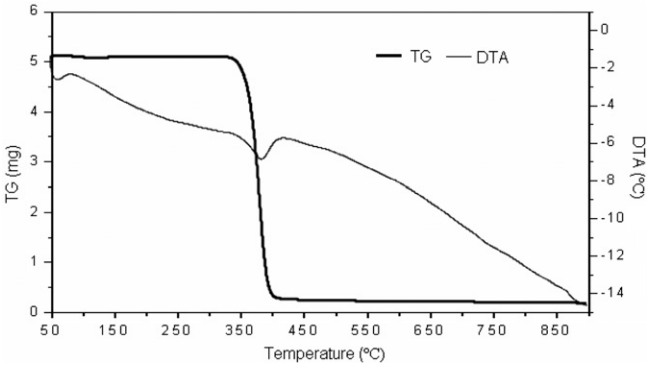

Fig. 2. The TG/DTA curves of HP-β-CD.

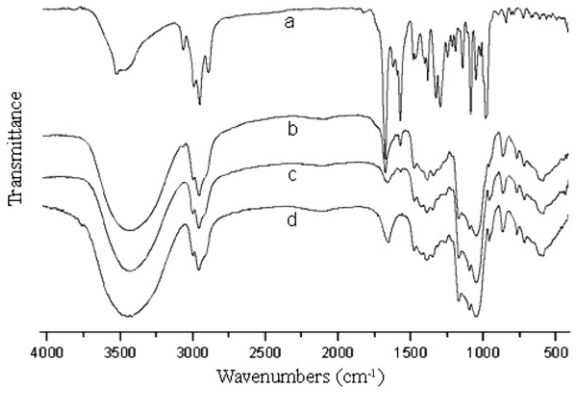

Fig. 1. The IR spectra of ASX (a), HP-β-CD (d) and their physical mixture (b) and inclusion complex (c).

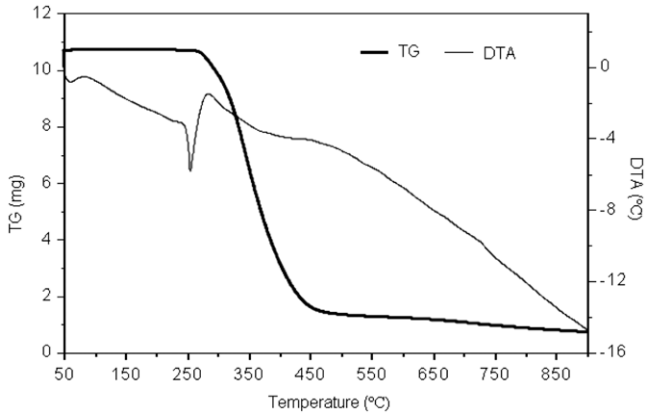

Fig. 3. The TG/DTA curves of ASX.

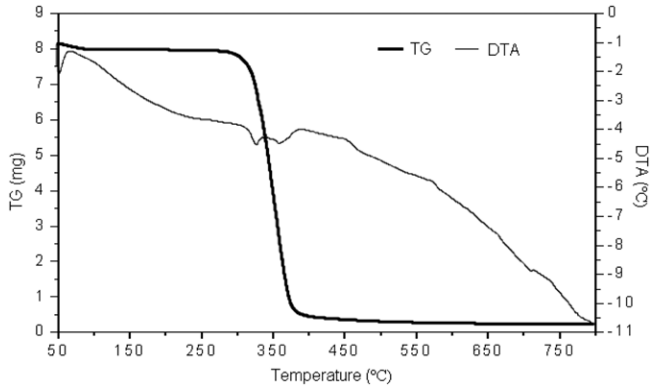

Fig. 4. The TG/DTA curves of HP-β-CD.

at about 330 °C and this ended at about 400 °C. The decomposing peak value, which was obtained from the DTA curve, is 365 °C. The fusion and degradation of HP-β-CD were completed in one step. Different from this, the DTA curve of ASX has an obvious endothermic peak where the weight of ASX has not decreased, suggesting that ASX melted at about 234 °C. Then ASX began degradation at about 250 °C, ending at about 450 °C. The degrading endothermic peak at the DTA curve of ASX was wide and unconspicuous, the peak value was about 360 °C. In Fig. 4, the TG curve was also one step, partly proving the formation of the complex. The starting temperature for mass loss was about 290 °C and ended at about 390 °C. At the DTA curve, there are two endothermic peak at 324 and 356 °C, respectively. The latter should be the decomposition peak of ASX, with 324 °C being the peak for HP-β-CD. The fill of ASX in the cavity of HP-β-CD made it degrade at a lower temperature. Furthermore, the DTA curve shows the disappearance of the melting peak of ASX. A similar result has been reported by Giordano, Novak, and Moyano (2001). The starting decomposition temperature of ASX increased by about 40 °C, due to

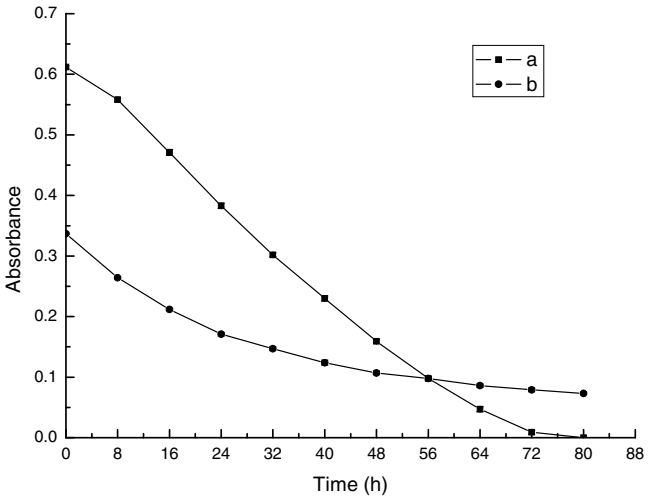

Fig. 6. The absorbance of ASX (a) and The TG/DTA curves of HP-β-CD (b) at 25 °C.

the protection of HP-β-CD. The decomposition process should be that along with the increase of the temperature, HP-β-CD decomposed first and exposed ASX to the heat, ASX melted and degraded almost at the same time under so high temperature.

Figs. 5–7 show the stability of ASX and the inclusion complex under oxygen and light at 4, 25 and 50 °C, respectively. The absorbance of ASX reduced rapidly at the test. Furthermore, the absorbance of ASX at higher temperatures was reduced more rapidly than that at lower temperatures. At 4 °C, ASX reached the degradation percentage of 68.5% in 120 h. At 25 and 50 °C, ASX degraded completely within 80 and 32 h. Those results agree with that of Chen et al. (2007). The absorbance of the inclusion complex reduced over time. The reduction speed was much less than that of pure ASX and gradually decreased. The reason was probably that the equilibrium of Eq. (2) was

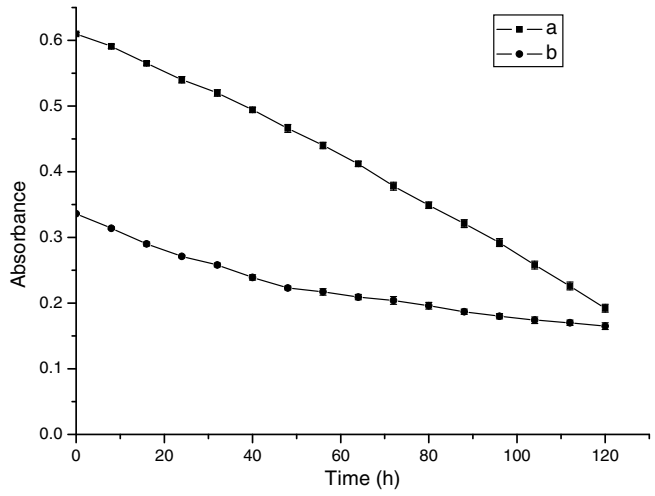

Fig. 5. The absorbance of ASX (a) and The TG/DTA curves of HP-β-CD (b) at 4 °C.

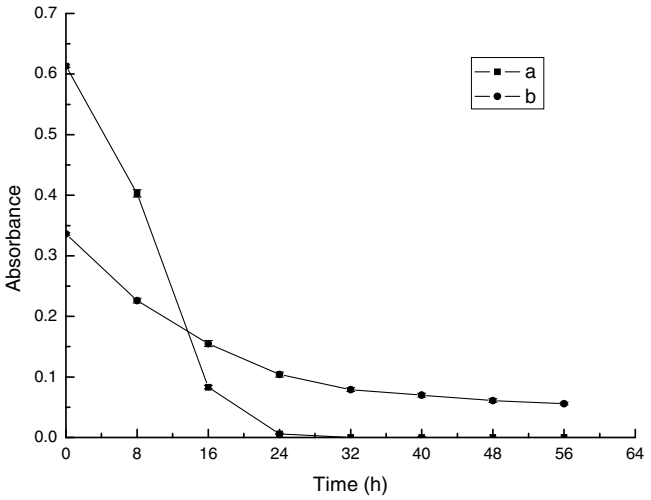

Fig. 7. The absorbance of ASX (a) and The TG/DTA curves of HP-β-CD (b) at 50 °C.

sloped to the synthesis, due to the decrease of ASX. This result indicates that the inclusion of ASX into HP-β-CD protected the guest from damage caused by light and oxygen and controlled the release of ASX.

$$\mathrm{ASX} + \mathrm{CD} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{CD} - \mathrm{ASX} \quad (2)$$

4. Conclusions

ASX is the main carotenoid with several essential biological functions, including protection against oxidation of unsaturated fatty acids, protection against UV light effects, immune response, pigmentation, and reproductive behaviour etc. Therefore, it has important applications in the nutraceutical, cosmetics, food and feed industries. However, free ASX is sensitive to oxygen and light, so it is important to stabilise the ASX.

In the present work, the inclusion complex of ASX with HP-β-CD was prepared successfully. IR spectroscopy proved the formation of the inclusion complex. The total recovery rate was 88.6%. The water solubility of the inclusion complex was >1.0 mg/ml, which is much better than that of ASX. The results of TG/DTA investigation indicated that the starting decomposition temperature of ASX was enhanced about 40 °C, after forming inclusion complex with HP-β-CD in solid state. Besides, the stability of the inclusion complex was tested in solution. Forming of the inclusion complex greatly enhanced the stability of ASX against light and oxygen. Furthermore, the release of ASX from the inclusion complex was controlled.

References

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*, 1580–1584.

Del Valle, E. M. M. (2004). Cyclodextrins and their uses: A review. *Process Biochemistry, 39*, 1033–1046.

Dong, Q. L., & Zhao, X. M. (2004). In situ carbon dioxide fixation in the process of natural astaxanthin production by a mixed culture of *Haematococcus pluvialis* and *Phaffia rhodozyma*. *Catalysis Today, 98*, 537–544.

Duchêne, D., & Wouessidjewe, D. (1990). Pharmaceutical uses of cyclodextrins and derivatives. *Drug Development and Industrial Pharmacy, 16*, 2487–2499.

Giordano, F., Novak, C., & Moyano, J. R. (2001). Thermal analysis of cyclodextrins and their inclusion compounds. *Thermochimica Acta, 380*, 123–151.

Guerin, M., Huntley, M. E., & Olaizola, M. (2003). *Haematococcus astaxanthin*: Applications for human health and nutrition. *Trends in Biotechnology, 21*(5), 210–217.

Krinsky, N. I., & Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine, 26*, 459–516.

Krinsky, N. I., & Yeum, K. J. (2003). Carotenoid-radical interactions. *Biochemical and Biophysical Research Communications, 305*, 754–760.

Kurashige, M., Okimasu, E., Inoue, M., & Utsumi, K. (1990). Inhibition of oxidative injury of biological membranes by astaxanthin. *Physiological Chemistry and Physics and Medical NMR, 22*, 27–38.

Lorenz, R. T., & Cysewski, G. R. (2000). Commercial potential for *Haematococcus microalgae* as a natural source of astaxanthin. *Trends in Biotechnology, 18*, 160–166.

Lyng, S. M. O., Passos, M., & Fontana, J. D. (2005). Bixin and α-cyclodextrin inclusion complex and stability tests. *Process Biochemistry, 40*, 865–872.

Mele, A., Mendichi, R., & Selva, A. (1998). Non-covalent associations of cyclomaltooligosaccharides (cyclodextrins) with trans-β-carotene in water: Evidence for the formation of large aggregates by light scattering and NMR spectroscopy. *Carbohydrate Research, 310*, 261–267.

Miki, W. (1991). Biological functions and activities of carotenoids. *Pure and Applied Chemistry, 63*, 141–146.

Naguib, Y. M. A. (2000). Antioxidant acitivities of astaxanthin and related carotenoids. *Journal of Agriculture and Food Chemistry, 48*, 1150–1154.

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, 1474*, 163–168.

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*, 205–215.

Sarah, G., & Robert, C. S. (2005). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): A toxicology review. *Food and Chemical Toxicology, 43*, 1451–1459.

Shimidzu, N., Goto, M., & Miki, W. (1996). Carotenoids as singlet oxygen quenchers in marine organisms. *Fisheries Science, 62*, 134–137.

Stahl, W., & Sies, H. (2005). Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta, 1740*, 101–107.

Szejtli, J. (1998). Introduction and general overview of cyclodextrin chemistry. *Chemical Reviews, 98*, 1743–1753.

Szente, L., & Szejtli, J. (1999). Highly soluble cyclodextrin derivatives: Chemistry, properties, and trends in development. *Advanced Drug Delivery Reviews, 36*, 17–28.

Uekama, K., Hirayama, F., & Irie, T. (1998). Cyclodextrin drug carriersystem. *Chemical Reviews, 98*, 2045–2076.