



## Increase in stability and change in supramolecular structure of $\beta$ -carotene through encapsulation into polylactic acid nanoparticles

Lan Cao-Hoang<sup>a,b,\*</sup>, Rémi Fougère<sup>a</sup>, Yves Waché<sup>a,\*\*</sup>

<sup>a</sup>Laboratoire de Génie des Procédés Microbiologiques et Alimentaires, AgroSup Dijon, Université de Bourgogne, 1 Esplanade Erasme, F-21000 DIJON, France

<sup>b</sup>Laboratory of Post-harvest Technology, School of Biological and Food Technology, Hanoi University of Science and Technology, 1 Dai Co Viet Road, Hanoi, Viet Nam

### ARTICLE INFO

#### Article history:

Received 10 November 2009

Received in revised form 19 April 2010

Accepted 24 May 2010

#### Keywords:

$\beta$ -Carotene

Oxidation

Oxidation products

Prooxidant

Encapsulation

Poly(lactic acid)

Xanthine oxidase

Supramolecular aggregation

Spectrophotometry

### ABSTRACT

$\beta$ -Carotene (BC) exhibits controversial antioxidant properties as it may act also as a prooxidant. Its stability toward oxidation depends on its dispersion form and can be increased through encapsulation. In this study, oxidation of BC from synthetic and natural origins was investigated after dispersion in Tween micelles or poly lactic acid (PLA) particles. Two oxidation systems were used: autooxidation and oxidation by xanthine oxidase-generated-reactive oxygen species. Results showed that synthetic BC formed nanometric negatively-charged particles in both Tween micelle and PLA systems, whereas the natural BC sample used was shown to be already pre-oxidised, forming micrometric-uncharged aggregates in Tween micelles and nanosize PLA particles. Samples also displayed different type of supramolecular aggregation (H or J), as shown in their UV–vis spectra, which were related to their particle size and their origin. Natural BC-loaded Tween micelles displayed high absorption in the range 350–450 nm (absorption of oxidation products) and rapid autodegradation rate which could reflect a high prooxidant activity. Interestingly, these properties were both significantly reduced with PLA encapsulation. Furthermore, the degradation rate and the oxidation product apparition of the two BC forms could be related to the supramolecular structure adopted by BC during dispersion.

© 2010 Elsevier Ltd. All rights reserved.

### 1. Introduction

The beneficial effect of carotenoids to health is widely recognised. The contribution of such compounds can be attributed to several properties.  $\beta$ -Carotene (BC) for instance, one of the most important carotenoid in human nutrition, is a major precursor of vitamin A and other retinoids but it exhibits also radical scavenging and antioxidant properties (2003). Some authors (Bertram & Bortkiewicz, 1995; Shklar & Schwartz, 1993; Toma, Losardo, Vincent, & Palumbo, 1995) have shown anticarcinogenic effects and these observations resulted in the test of this compound as a preventive medicine for certain types of cancers. Surprisingly, some intervention trials testing the effects of BC in human found a higher relative risk for lung cancer in smokers who were given BC (Blumberg, 1994; Omenn et al., 1996). Amongst the hypotheses that were proposed to explain these unexpected results, one deals with the high reactivity of BC which results in the possible shift

from antioxidant to prooxidant properties. This effect has been observed with increasing concentrations of BC (Lankin, Tikhaze, Konvalova, & Kozachenko, 1999; Palozza, 2005) but it also depends on the physicochemical environment (Palozza, Serini, Di Nicuolo, Piccioni, & Calviello, 2003; Young & Lowe, 2001). In tissues where the oxygen pressure is low, antioxidant properties predominate (Burton & Ingold, 1984) but the environment of BC can increase its prooxidant properties through the generation of oxidation and cleavage products. Beside autooxidation, reactive oxygen species (ROS) can be generated in the organism through the activity of oxidases (NADPH oxidases, xanthine oxidase, lipoxygenase, acyl-CoA oxidases etc.) (Nauseef, 2008; Puddu, Puddu, Cravero, Rosati, & Muscari, 2008).

Another specificity of BC is related to its physicochemical properties. This compound is highly hydrophobic and difficult to disperse (Cao-Hoang & Waché, 2009). Even in relatively lipophilic environments, only a small proportion of the molecules stands in the monomolecular form (Wisniewska, Widomska, & Subczynski, 2006) and BC is more encountered under aggregated forms (Köhn et al., 2008). Two main aggregation states have been observed called H- and J-aggregates. They possess different spectrophotometric spectra exhibiting shifts in the maximal absorption wavelengths and characteristic peaks (Auweter et al., 1999). The supramolecular organisation of BC is likely to affect its properties toward ROS. It could therefore be possible to work on the BC

\* Corresponding author at: Laboratoire de Génie des Procédés Microbiologiques et Alimentaires, AgroSup Dijon, Université de Bourgogne, 1 Esplanade Erasme, F-21000 DIJON, France. Tel.: +33(0) 3 80 39 66 80; fax: +33 (0) 3 80 39 66 41.

\*\* Corresponding author. Tel.: +33(0) 3 80 39 66 80; fax: +33 (0) 3 80 39 66 41.

E-mail addresses: [hoanglan\\_cao@yahoo.fr](mailto:hoanglan_cao@yahoo.fr), [lanch-ibft@mail.hut.edu.vn](mailto:lanch-ibft@mail.hut.edu.vn) (L. Cao-Hoang), [ywache@u-bourgogne.fr](mailto:ywache@u-bourgogne.fr) (Y. Waché).

dispersion to modify the oxidability and oxidation product generation. Recently, attempts have been made to increase the solubility and the stability of BC through various strategies, such as incorporation into aqueous phase containing micelles (Tyssandier, Lyan, & Borel, 2001; Waché, Bosser-De Ratuld, & Belin, 2006), microencapsulation into tapioca starch and maltodextrin (Loksuwan, 2006), embedding into starch-coated gelatin and sucrose beads (Cretu et al., 2008), and formulation of BC nanoparticles (Anantachoke et al., 2006; Chu, Ichikawa, Kanafusa, & Nakajima, 2008; Gutiérrez et al., 2008; Palozza, Muzzalupo, Trombino, Valdannini, & Picci, 2006; Tan & Nakajima, 2005; Yuan, Gao, Mao, & Zhao, 2008). Encapsulation is of great interest as it offers promising means of improving the bioavailability and the uptake of different compounds, especially poorly soluble substances such as BC and numerous other compounds that are widely used in food industry. Furthermore, the formation of a complex with the matrix protects the encapsulated molecule, thus increasing its stability (Polyakov et al., 2008; Tan & Nakajima, 2005).

In the dispersion systems of BC, stability to oxidation is usually tested through autooxidation and with synthetic BC but in the organism, BC is likely to be submitted to stresses caused by enzyme-generated ROS. Moreover, if natural BC is used to supplement diets, it might include other BC-related forms that have not been separated or that have been generated by extraction. Indeed, as carotenoids are well embedded into the raw material, most extraction processes use temperature or acid to disrupt cells and facilitate extraction (Haigh, 1994; Nonomura, 1987; Rose, Phillips & Sanderson, 1995; Wijffels, Hejazi, Holwerda, & Tramper, 2003). This results in isomerization and oxidation of carotenoids depending on the temperature, solvent, presence of acid, light etc. (Calvo, Dado, & Santa-María, 2007; Xianquan, Shi, Kakuda, & Yueming, 2005).

Therefore, the present study aims at investigating the oxidation of encapsulated  $\beta$ -carotene depending on the reactivity of the environment and on the preoxidation state of BC before encapsulation. Synthetic and natural BC were submitted to autooxidation or oxidation by xanthine oxidase-generated ROS after encapsulation in polylactic acid (PLA) particles, a biodegradable polymer derived from renewable resources which is recognised as safe (GRAS) and is of great interest in food applications because of its potentially useful physical and mechanical characteristics (Jin & Zhang, 2008). As BC is not dispersible in aqueous environment, the degradation of BC in PLA particles was compared with the classical dispersion in Tween micelles as proposed previously by Ben-Aziz, Grossman, Ascarelli, and Budowski (1971). The UV-vis spectrum of both synthetic and natural BC sources was compared together to determine the BC degradation prior to encapsulation as well as the supramolecular structure of BC molecules. Particle size and the aqueous dispersibility of the BC samples were also characterised. Finally, the stability of BC samples against oxidation was evaluated through changes in UV-vis spectrum during oxidation and evaluation of the degradation rate of the samples.

## 2. Materials and methods

### 2.1. Chemicals

Synthetic  $\beta$ -carotene (purity >97%) was obtained from Fluka (Sigma-Aldrich, St. Quentin Fallavier, France).  $\beta$ -Carotene extracted from natural raw material (called natural  $\beta$ -carotene) was obtained from a Chinese retailer without information on the extraction method. The impact of the process on the carotene quality had thus to be evaluated prior to other investigations. Chloroform, acetone, acetonitrile, petroleum ether, Tween 80, xanthine oxidase (XO) (grade III from buttermilk) and poly(lactic) acid

(PLA,  $M_w$  60,000) were purchased from Sigma (Sigma-Aldrich). All other chemicals used were of analytical grade.

### 2.2. Thin layer chromatography(TLC) analysis and characterisation of spectral fine structure by UV-spectrometry

Both sources of BC were dissolved in acetonitrile before separation on silica gel TLC plates (SiLG, Merck, Germany). The mobile phase used was 5% v/v acetone in petroleum ether. The development time was approximately 16 min. After development, the plates were air dried for 3 min, and the pigments were visualised as yellow bands on a white background. The formed bands were scrapped off then dissolved in acetonitrile for the UV-vis spectrum measurement using UV-absorption spectroscopy (V-570 UV/VIS/NIR, JASCO Corporation, Tokyo, Japan). Spectra were recorded in the range of 300–550 nm at data intervals of 0.1 nm, an average time of 0.1 s and a scan rate of 100 nm/min.

The spectral fine structure was characterised by the large value of %III/II, which is the ratio of the height of the longest-wavelength absorption peak, designated III, and that of the middle absorption peak, designated II, relative to the trough between them (Rowan, 1989). This value can be equal to 0 if the peak III is present only as a shoulder. The *Z*-isomers were identified by  $\lambda_{\text{max}}$  lower than those of the all-*E*-carotenoids and by the presence of the “*cis*” peak at about 142 nm below the longest-wavelength absorption maximum of the all-*E*-form (Rowan, 1989). The location of the *Z*-double bond was indicated by the  $\%A_B/A_{II}$ , which is the ratio of the height of the “*cis*” peak, designated  $A_B$ , and that of the middle main absorption peak, designated  $A_{II}$  (Britton, 1995). This ratio is an indicator of the intensity of the “*cis*” peak, which is greater as the *Z*-double bond is closer to the centre of the molecule, being equal to 10, 45 and 56 for (9*Z*), (13*Z*) and (15*Z*)- $\beta$ -carotene, respectively (Mercadante, Steck, & Pfander, 1998).

### 2.3. Dispersion of $\beta$ -carotene by different methods

In order to increase the solubility of  $\beta$ -carotene in water solutions, powder of synthetic or natural  $\beta$ -carotene was encapsulated into Tween micelles or polylactic acid (PLA) particles. Without this step,  $\beta$ -carotene would be insoluble and could only be suspended as particles in the aqueous phase, forming readily instable aggregates in solutions.

#### 2.3.1. Incorporation of $\beta$ -carotene into Tween 80 micelles

Synthetic and natural BC were incorporated into Tween 80 micelles (named Syn-Tw80 and Nat-Tw80, respectively) according to the method of Ben-Aziz et al. (1971) with modification as proposed by Bosser and Belin (1994). 500 mg of  $\beta$ -carotene were first dispersed with Tween 80 (10% w/w). The mixture was homogenised for 3 min at room temperature with an IKA Ultra-Turax T25 System (IMLAB, Lille, France) set to 24,000 rpm before solubilisation in 500 ml of chloroform under moderate magnetic stirring. Chloroform was then removed from the emulsion by rotary evaporation under reduced pressure. The residue was dissolved in EDTA 0.25%, filtered and diluted in phosphate buffer (PBS, pH 8, 50 mM) to the desired concentration.

#### 2.3.2. Encapsulation of $\beta$ -carotene into PLA nanoparticles

Encapsulation of synthetic and natural BC into nanoparticles of PLA (named Syn-PLA and Nat-PLA, respectively) was performed using the water-in-oil solvent displacement method. Briefly,  $\beta$ -carotene (50 mg) and Tween 80 (10% w/w) were completely dissolved in 300 ml of chloroform. 25 mg of PLA was then added to this organic phase. The mixture was stirred at room temperature for 10 min before addition of 100 ml of PBS. Finally, the organic phase

Download English Version:

<https://daneshyari.com/en/article/1187625>

Download Persian Version:

<https://daneshyari.com/article/1187625>

[Daneshyari.com](https://daneshyari.com)