Food Chemistry 177 (2015) 339–345

Contents lists available at ScienceDirect

Food Chemistry

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

Enhanced biological activity of carotenoids stabilized by phenyl groups

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ARTICLE INFO

Article history:
Received 25 October 2014
Received in revised form 9 January 2015
Accepted 9 January 2015
Available online 17 January 2015

Keywords:
Carotenoid
Phenyl group
Antioxidant
ROS
C. elegans

ABSTRACT

Carotenoids are lipid soluble food ingredients with multifunction including antioxidant and anticancer activities. However, carotenoids are destructively oxidized upon reaction with radicals resulting in toxic effects on biological systems. Two synthetic carotenoids (BAS and BTS) containing the aromatic phenyl groups with a *para*-substituent (OMe and Me, respectively) at C-13 and C-13′ position were prepared in order to overcome a structural instability of carotenoid. Both BAS and BTS exerted stronger radical scavenging activity than β-carotene in DPPH and ABTS assays. In particular, BTS significantly reduced in vivo ROS (reactive oxygen species) levels and improved body growth and reproduction of *Caenorhabditis elegans*. BTS has a great potential for the advanced and modified carotenoid material with stability leading to enhanced bioavailability.

1. Introduction

Reactive oxygen species (ROS) are by-products of chemical reactions in living cells, which is influenced by environmental stress such as UV or heat exposure (Simon, Haj-Yehia, & Levi-Schaffer, 2000). ROS induce an oxidative stress in cells, which leads to damages of lipid, DNA and protein. Two strategies for avoiding oxidative stress are adopted in cells. One is the detoxification by the catalytic enzymes (e.g. SOD and catalase) or antioxidants (e.g. tocopherol, carotenoids and ascorbic acid) (Wolkow, Kimura, Lee, & Ruvkun, 2000). The other is to repair oxidation damages through insulin/insulin-like growth factor-1 (Ins/IGF-1) signaling, mitogen-activated protein kinase (MAPK) activation and apoptosis (Matsuo & Kaneko, 2000; Yazaki, Yoshikoshi, Oshiro, & Yanase, 2011).

Carotenoids, natural pigments found in photosynthetic organisms, act as vitamin A precursors and efficient antioxidants (Naves & Moreno, 1998) and were known to prevent the cancers, cardiovascular disease and macular degeneration (Castenmiller & West, 1998; Edge, McGarvey, & Truscott, 1997). Despite those effects, there are impediments for the application of carotenoids in food, nutraceutical or pharmaceutical industry (Yuan, Gao, Zhao, & Mao, 2008). First, carotenoids easily form crystal structure because they are not dissolved in water, but only in oils at room temperature (Edge et al., 1997; Ribeiro & Cruz, 2005). Second, they are not sufficient to quench singlet oxygen in complicated biological system (Weishaupt, Gomer, & Dougherty, 1976). Finally, carotenoids are too sensitive to oxidation and degradation in response to oxygen, heat and light (Nonier, Gaulejac, Vivas, Vivas, & Vitry, 2004; Qian, Decker, Xiao, & McClements, 2012).

Chemical structure determines its stability as well as chemical and/or biological reactivity of the compound. Previous studies have reported that antioxidant activity of carotenoids was originated either from the functional groups or the conjugated polyene. For example, the effectiveness of antioxidation tends to vary depending on the polyene length of carotenoids. Carotene without functional groups other than carbon and hydrogen shows better antioxidant activity than xanthophyll with oxo and hydroxyl functional groups at the terminal rings (Mortensen & Skibsted, 1997). In previous study, Maeng et al. (2010) devised stabilized carotenoids based on the fact that vitamin E with the aromatic phenyl group has a protective effect on carotenoids. The terminal cyclohexene rings of β-carotene were replaced by 4-methylthiobenzene rings, and two more phenyl groups were attached at C-13 and C-13′ position in the series of the stabilized carotenoids (Kim et al., 2011; Maeng et al., 2010). It was envisioned that these stabilized carotenoids would exhibit improved biological activity such as antioxidation. Two unnatural carotenoids were selected in our study of antioxidation based on the electronic nature of the substituent groups. One has 4-methoxyphenyl (anisyl) groups at C-13

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http://dx.doi.org/10.1016/j.foodchem.2015.01.062
0308-8146/

and C-13′ position (abbreviated as BAS). The other has 4-methylphenyl (tolyl) groups at C-13 and C-13′ position (abbreviated as BTS). Chemical structures of these two carotenoids and β-carotene used in this study were shown in Fig. 1. No previous study has investigated the effect of these carotenoids on antioxidant activity and stability.

In this study, we monitored the radical scavenging activity of unnatural carotenoids with stabilized structure. The bioavailability of carotenoids was investigated through measuring the ROS levels in soil nematode *Caenorhabditis elegans*. To evaluate the in vivo safety, we examined the effects of these synthetic carotenoids on lifespan, body growth and reproduction in *C. elegans*.

2. Materials & methods

2.1. Chemicals

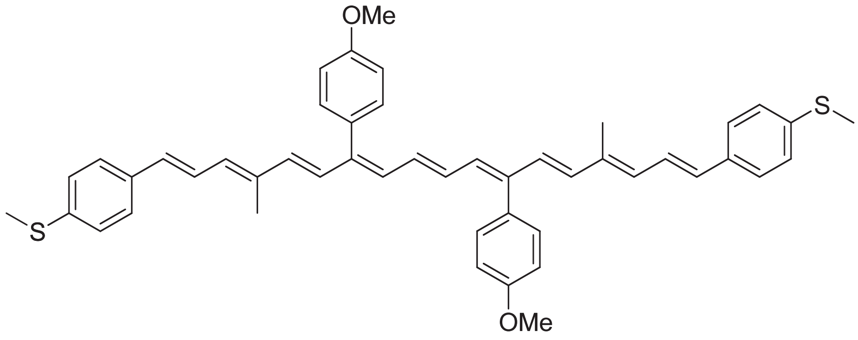
2,2’-Diphenylpicrylhydrazyl (DPPH), 2,2’-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) and 2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA) were purchased from Sigma–Aldrich Co. (St. Louis, USA). Standard β-carotene and dichloromethane from Wako Pure Chemical Co. (Tokyo, Japan) were used and ethanol was purchased from Samchun Pure Chemical Co. (Seoul, Korea).

2.2. *C. elegans* strains and maintenance

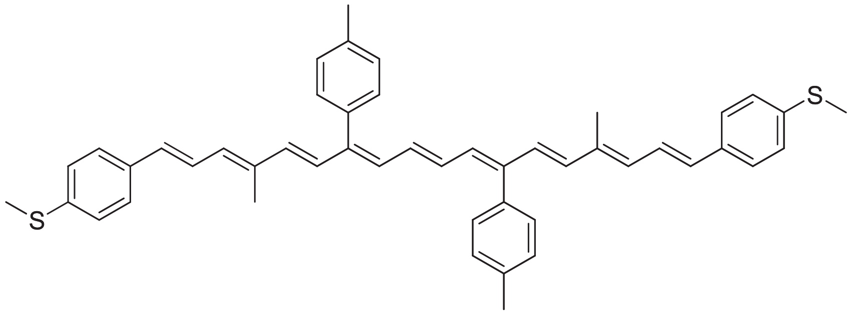
The nematode *C. elegans* wild type N2 (Bristol) were obtained from the *Caenorhabditis* Genome Center (CGC). Worms were maintained at 20 °C on Nematode Growth Media (NGM) agar as previously described (Brenner, 1974) and were fed *Escherichia coli* OP50 strain.

2.3. Synthesis of stabilized carotenoid

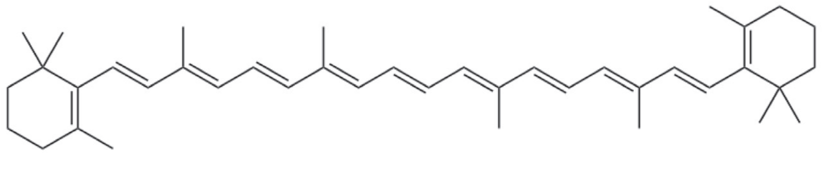
Two unnatural carotenoids, BAS and BTS, respectively, with anisyl and tolyl substituents at C-13 and C-13′ position were synthesized by the coupling of allylic sulfone subunit and dialdehyde subunit and the double elimination reaction as previously reported (Kim et al., 2011; Maeng et al., 2010).



BAS



BTS



β-carotene

Fig. 1. Structures of carotenoid compounds. New types of carotenoids have been prepared by coupling of allylic sulfone and dialdehyde subunits, followed by the double elimination reaction. Chemical structures of BAS with 13,13′-*para*-anisyl groups, BTS with 13,13′-*para*-tolyl groups, and β-carotene were represented.