We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Effects of Z-Isomerization on the Bioavailability and Functionality of Carotenoids: A Review

Masaki Honda, Hayato Maeda, Tetsuya Fukaya and Motonobu Goto

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.78309>

Abstract

Carotenoids, the most common fat-soluble plant pigments in nature, are beneficial to human health due to their strong antioxidant activities and abilities to prevent various diseases. Carotenoids have many geometrical isomers forms caused by *E/Z*-isomerization at arbitrary sites within the multiple conjugated double bonds. Several studies have addressed that the bioavailability as well as the antioxidant, anticancer, and antiatherosclerotic activities of carotenoids varies among the isomers. In addition, those variations differ among carotenoids: *Z*-isomerization resulted in “positive” or “negative” effect for carotenoids bioavailability and functionality, for example, *Z*-isomers of lycopene are more bioavailable than the all-*E*-isomer, whereas the opposite is observed for β -carotene. Thus, to efficiently promote the beneficial effects of carotenoids by ingestion, it is important to have a good understanding of the impact of *E/Z*-isomerization on the corresponding functional changes. The objective of this contribution is to review the effects of carotenoid *Z*-isomerization on bioavailability and functionality and describe their differences among carotenoids.

Keywords: lycopene, β -carotene, astaxanthin, *E/Z*-isomer, bioavailability, antioxidant activity

1. Introduction

Carotenoids are the most common lipid-soluble pigments responsible for the colors of plants, animals, and microorganisms, and over 1100 different types of carotenoids have been characterized so far [1, 2]. Carotenoids can be divided into the following two groups: (1) carotenes,

which are nonoxygenated molecules such as lycopene and β -carotene; (2) xanthophylls, which are oxygen-containing molecules such as astaxanthin and fucoxanthin (**Figure 1**) [3]. The daily consumption of carotenoid-rich foods would be beneficial for human health because of their high antioxidant, anticancer, and antiatherosclerotic activities [4–6]. Because carotenoids contain numerous conjugated double bonds, many kinds of geometrical isomers are theoretically possible (**Figure 1C, E and F**). In general, carotenoids in plants occur predominantly in the (all-*E*)-configuration, whereas the *Z*-isomers are present in the human body and processed foods in considerable quantity, for example, over 50% of total lycopene is present as the *Z*-isomers in serum and tissues [7–9]. Data from several studies have shown that the *Z*-isomerization of carotenoids induced changes in important properties, such as the bioavailability, antioxidant activity, and anticancer activity [10–13]. However, these outcomes vary depending on the type of carotenoid: there were cases where the beneficial effects of carotenoids increased or reduced by the *Z*-isomerization [10–15]. For example, *Z*-isomers of lycopene and astaxanthin have higher bioavailability than the all-*E*-isomers [12, 16], whereas *Z*-isomers of β -carotene have lower bioavailability than the all-*E*-isomers [14]. Furthermore, the results may depend on the evaluation method used. For instance, when the antioxidant activity of β -carotene was evaluated based on oxidation of the low-density lipoprotein (LDL), the all-*E*-isomer showed higher antioxidant activity than the 9*Z*-isomer [17], whereas the 9*Z*-isomer showed higher antioxidant activity when evaluated based on antiperoxidative activity [18]. Moreover, the beneficial effects of carotenoids differ between the *Z*-isomers. For example, when the antioxidant activity of fucoxanthin was evaluated in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity assay, the order of activity was 13*Z*-isomer $\approx$ 13'*Z*-isomer > all-*E*-isomer > 9'*Z*-isomer [19]. The above findings indicate that a good understanding of the effects of *E/Z*-isomerization on functional changes is important for increasing the beneficial effects of carotenoid ingestion and for the industrial processing of carotenoids. The objective of this chapter is to highlight the impact of *E/Z*-isomerization of carotenoids on their bioavailabilities, antioxidant activities,

Figure 1. Chemical structures of (A) (all-*E*)-lycopene, (B) (all-*E*)- β -carotene, (C) (all-*E*)-astaxanthin, (D) (all-*E*)-fucoxanthin, (E) (9*Z*)-astaxanthin, and (F) (13*Z*)-astaxanthin.

and inhibitory effects against diseases, such as atherogenesis and cancer. Furthermore, aspects of the change factor of the carotenoid bioavailability and functionality, modification of the physicochemical properties of carotenoids by *E/Z*-isomerization, and *Z*-isomerization methods used for carotenoids are also discussed in this chapter.

2. Effect of Z-isomerization of carotenoids on their bioavailabilities and functionalities

The effects of *Z*-isomerization on the bioavailability and functionality of eight carotenoids have been investigated thus far, including: (1) the bioavailability and antioxidant activity of lycopene; (2) the antioxidant activity of α -carotene; (3) the bioavailability and antioxidant, antiatherogenic, and antiatherosclerotic activities of β -carotene; (4) the bioavailability and antioxidant activity of astaxanthin; (5) the antioxidant and pro-apoptotic activities of canthaxanthin; (6) the antioxidant and anticancer activities of fucoxanthin; (7) the bioavailability and antioxidant activity of lutein; and (8) the antioxidant activity of zeaxanthin. The changes caused by *Z*-isomerization varied according to the parental carotenoid molecules tested and the evaluation method employed. The findings are described in detail below.

2.1. Lycopene

Lycopene is an acyclic carotene ($C_{40}H_{56}$) that is principally responsible for the bright-red color found abundantly in vegetables and fruits such as tomatoes, guava, and watermelons [3, 9]. Lycopene shows an especially strong antioxidant activity among carotenoids [6] and can significantly reduce the risks for arteriosclerosis, atherogenesis, and many types of cancer (such as prostate and esophageal cancer) [4, 5]. Therefore, in recent years, the use of lycopene in health foods and supplements, and as a natural functional pigment has attracted attention. It is well documented that the bioavailability and antioxidant activity of lycopene are changed by *Z*-isomerization. Most previous findings have demonstrated that the *Z*-isomerization of lycopene results in “positive” health effects.

Data from both *in vitro* and *in vivo* tests have suggested that *Z*-isomers of lycopene are more bioavailable than the all-*E*-isomer. Testing conducted using a diffusion model [20], bile acid micelles [21, 22], human intestinal Caco-2 cells [23], and lymph-cannulated ferrets [21, 22] has provided strong evidence supporting the higher bioavailability of the *Z*-isomers. Moreover, in humans, the ingestion of foods rich in lycopene *Z*-isomers resulted in a measurable increase in blood lycopene concentrations compared to a sample abundant in the (all-*E*)-isomer [12, 24–27]. For example, Cooperstone et al. [12] investigated the effects of ingesting red tomato juice, which mainly contained (all-*E*)-lycopene (90% all-*E*-isomer) and *tangerine* tomato juice, which mainly contained *Z*-isomers of lycopene (94% *Z*-isomers), on plasma lycopene concentrations. Lycopene from the *tangerine* tomato juice showed approximately 8.5-fold greater bioavailability than lycopene from the red tomato juice. Unlu et al. [25] reported that when comparing two tomato sauces—one rich in all-*E*-lycopene (95% all-*E*-isomer) and the other rich in (*Z*)-lycopene (45% *Z*-isomers)—that the *Z*-isomer-rich tomato sauce was approximately

1.5 times more bioavailable than the all-*E*-isomer-rich sauce. In general, the uptake of carotenoids into intestinal mucosal cells is aided by the formation of bile acid micelles [21, 22, 24, 27]. Thus, it is believed that because lycopene *Z*-isomers are more soluble in bile acid micelles than the all-*E*-isomer, they are preferentially incorporated into enterocytes and efficiently form chylomicrons [21, 22]. Indeed, very recently, several reports showed that the solubility of lycopene in oils, organic solvents, and supercritical CO₂ (SC-CO₂) was significantly improved by *Z*-isomerization [28–32]. However, Richelle et al. [33] showed by human oral-dosing tests that the (9*Z*)- and (13*Z*)-isomers were less efficiently absorbed than the 5*Z*- and all-*E* isomers or were converted into 5*Z*- and all-*E* isomers.

Several previous reports have shown that lycopene *Z*-isomers have higher antioxidant activity than the all-*E* isomer and that the relative activities of the isomers varied depending on the assay method [10, 11]. Böhm et al. [10] compared the antioxidant activity of (all-*E*)-lycopene with four unknown *Z*-isomers by measuring their abilities to reduce radical cations of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (TEAC assay), and each *Z*-isomer showed higher antioxidant activity than the all-*E*-isomer. Müller et al. [11] evaluated the antioxidant activities of (all-*E*)-, (5*Z*)-, (9*Z*)-, (13*Z*)-, and (7*Z*,9*Z*,7'*Z*,9'*Z*)-lycopene using four different *in vitro* assay, namely the TEAC assay, the ferric-reducing antioxidant power (FRAP) assay, the peroxyl radical-scavenging capacity (PSC) assay, and the heme-induced peroxidation of linoleic acid in mildly acidic emulsions mimicking postprandial lipid oxidation in the gastric compartment (MbFe^{III}-LP) assay. No significant changes were observed among the isomers in the TEAC and FRAP assay. However, the lycopene *Z*-isomers showed higher antioxidant activities than the all-*E* isomer in the PSC assay (9*Z*-isomer > 5*Z*-isomer ≈ 7*Z*,9*Z*,7'*Z*,9'*Z*-isomer > 13*Z*-isomer > all-*E*-isomer) and in the MbFe^{III}-LP assay (5*Z*-isomer > all-*E*-isomer ≈ 9*Z*-isomer ≈ 13*Z*-isomer ≈ 7*Z*,9*Z*,7'*Z*,9'*Z*-isomer). In TEAC assay, Böhm et al. [10] found that *Z*-isomers of lycopene had higher antioxidant activity, but Müller et al. [11] concluded that no significant differences occurred among the isomers. These discrepant findings may be explained by the fact that different concentrations of the isomers were used in each study [10, 11].

Based on the above findings, *Z*-isomerization effectively promotes the beneficial effects of lycopene. Among the *Z*-isomers, (5*Z*)-lycopene would have the highest bioavailability [33] and antioxidant activity [11]. Furthermore, the 5*Z*-isomer has the highest stability of the *Z*-isomers [34–36]. Therefore, regarding lycopene, it is very important to increase the 5*Z*-isomer level and its ingestion. As the *Z*-isomerization method to increase (5*Z*)-lycopene efficiently, heating in some alkyl halides [37] and some kinds of oils such as sesame oil [9, 38], light irradiation with photosensitizers [39], and catalytic treatment [20, 40] were effective. Moreover, to our best knowledge, because the effect of ingesting *Z*-isomer-rich lycopene on inhibiting the development of diseases such as atherogenesis and cancer has not been clarified, further research in that field is expected in the future.

2.2. β-Carotene

β-carotene is a cyclic carotene (C₄₀H₅₆) that is found abundantly in vegetables and fruits, and provides vegetables such as carrots and pumpkins with a deep orange-yellow color [3, 4]. As with other carotenoids, β-carotene has a high antioxidant capacity [6] and preventive effect

against various diseases such as cancer and atherogenesis [4, 17]. Furthermore, β -carotene is very important as a retinol precursor, with a high conversion rate [3, 4]. It is also well documented that the bioavailability and antioxidant activity of β -carotene as well as its antiatherogenic activity are changed by Z-isomerization. Most previous studies have shown that the Z-isomerization results in “negative” effect for bioavailability. In contrast, β -carotene Z-isomerization has shown both “positive” and “negative” effects on antioxidant activity, depending on the evaluation method, and “positive” effects have been shown in terms of antiatherogenic activity.

Data from several *in vitro* and *in vivo* tests have indicated that Z-isomers of β -carotene are less bioavailable than the all-*E*-isomer. For example, *in vivo* tests using Caco-2 cells, HSC-T6 cells, and liver microsomes [14], as well as *in vivo* tests using ferrets [41] and gerbils [42] have shown this phenomenon. In humans, the intake of *Dunaliella salina* and *Dunaliella bardawil* rich in (9Z)- β -carotene showed lower β -carotene bioavailability than foods rich in the all-*E*-isomer [43–48]. The effects of the Z-isomer content on the bioavailability were opposite between lycopene and β -carotene. Generally, after carotenoids are extracted from the food matrix and incorporated into mixed micelles, bioaccessible carotenoids can be internalized by enterocytes [21, 22, 24, 27]. The main absorption site of carotenoids is in the duodenum, and several proteins that are temporarily present at the apical membrane mediate selectivity in terms of carotenoid uptake [27, 49–51]. *In vitro* experiments with Caco-2 cells showed that carotenoid transport decreased in the following order: β -carotene $\approx$ α -carotene (50% inhibition) > β -cryptoxanthin $\approx$ lycopene (20% inhibition) > lutein: zeaxanthin (1:1) (7% inhibition) [49]. Because carotenoid Z-isomers have higher solubility than the all-*E*-isomers [28–32], they can incorporate into bile acid micelles more efficiency [21, 22]. Therefore, it is considered that Z-isomers of β -carotene have lower transport efficiency in Caco-2 cell than the all-*E*-isomers [13]. However, a few studies have suggested that Z-isomers of β -carotene have higher bioavailability than the all-*E*-isomers, as evaluated using human intestinal Caco-2 cells [52] and ferrets [53]. The use of different delivery systems with the cell model system and animal species might have caused discordant results [15].

Several studies have been conducted to compare the antioxidant activities of (all-*E*)- β -carotene and the Z-isomers, and the degree of antioxidant activity detected varied according to the assay method. Namely, the 9Z-isomer showed higher antioxidant activity than the all-*E*-isomer when evaluated in terms of the sensitivity to external oxidants [54], the antiperoxidative activity [18], and oral dose testing in rats [55]. However, the opposite results (or no significant differences) were observed when the antioxidant activities were evaluated by measuring the oxidation of LDL [17] or in TEAC assay [10, 56], and PSC assay [56, 57]. Rodrigues et al. [57] reported that β -carotene Z-isomers were less efficient as peroxyl radical scavengers than the corresponding all-*E*-isomers: the Z-isomers presented the values about 20% lower than that found for the all-*E*-isomer, and they addressed that the negative effect may be due to the decreasing of the orbital overlap. Based on the above findings, it is difficult to conclude whether antioxidant activity is enhanced by Z-isomerization of the all-*E*-isomer.

Moreover, as additional “positive” effects of β -carotene Z-isomers, it has been reported that the 9Z-isomer has higher antiatherogenic activity [58] and antiatherosclerotic activity

[59, 60] than the all-*E*-isomer. On the other hand, there are other “negative” effects. Namely, β -carotene is a very important retinol precursor with a high conversion rate. The (all-*E*)- and (9*Z*)- β -carotene can be metabolized respectively to (all-*E*)-retinoic acid and (9*Z*)-retinoic acid [61, 62], both of which are active in gene regulation [63, 64]. However, the rates of cleavage of β -carotene isomers to vitamin A and the composition of the respective isomer metabolites vary, that is, (all-*E*)- β -carotene was the preferred substrate for cleavage to vitamin A when compared with the *Z*-isomers [61, 65, 66].

Regarding β -carotene, considering that “positive” and “negative” effects are associated with *Z*-isomerization, it is considered important to use them properly depending on the situation. Besides, as the *Z*-isomerization method for (all-*E*)- β -carotene, heating [67, 68], light irradiation with photosensitizers [69], and catalytic treatment [70, 71] were well documented. Moreover, *Dunaliella salina* and *Dunaliella bardawil*, which contain a large amount of (9*Z*)- β -carotene, have been used as *Z*-isomer-rich materials [43–48].

2.3. Astaxanthin

Astaxanthin is a xanthophyll ($C_{40}H_{52}O_4$) that is principally responsible for the dark-red color in various microalgae and marine animals [1, 72]. Astaxanthin shows an especially strong antioxidant activity among carotenoids [6] and can significantly reduce the risk of cancer, eye disease, and cardiovascular disease [73, 74]. For instance, astaxanthin protected mice from carcinogenesis of the urinary bladder by reducing the incidence of chemically induced bladder carcinoma and further, astaxanthin supplementation in rats inhibited the stress-induced suppression of tumor-fighting natural killer cells [73]. In addition, astaxanthin is frequently used as an animal and fish feed additive to improve their body colors [75]. Data from several studies have demonstrated that the bioavailability and antioxidant activity of astaxanthin were changed by *Z*-isomerization.

In terms of the bioavailability, an *in vitro* test using a simulated digestion model and human intestinal Caco-2 cells [76] and human oral-dosing studies [16, 77] have shown that *Z*-isomers have higher bioavailability than the all-*E*-isomer. For example, Yang et al. [76] reported that (13*Z*)-astaxanthin showed higher bioaccessibility than (9*Z*)- and (all-*E*)-astaxanthins using an *in vitro*-digestion model, and (9*Z*)-astaxanthin exhibited higher cellular-transport efficiency than (all-*E*)- and (13*Z*)-astaxanthin in Caco-2 cell monolayers. However, oral-dosing studies in rainbow trout (*Oncorhynchus mykiss*) have shown a “negative” effect of astaxanthin *Z*-isomerization on bioavailability [78, 79]. These results suggest that the bioavailability of carotenoid isomers differs among species. Thus, future studies should seek to establish the biochemical basis for species-specific differences in the utilization of carotenoid isomers.

Although the antioxidant activity measured depends on the assay method employed, many studies have shown “positive” effects. Namely, assay that measure antioxidant enzyme activities, DPPH radical scavenging, oxygen radical-absorption capacity (ORAC), photochemiluminescence (PLC) and peroxidation have shown higher antioxidant activities of astaxanthin *Z*-isomers than detected for the all-*E*-isomer [76, 80, 81]. In contrast, when the antioxidant activity was evaluated by a cellular antioxidant activity (CAA) assay, the order of

the antioxidant activity was 13Z-isomer > all-*E*-isomer > 9Z-isomer [81]. The results of these studies suggest that Z-isomers of astaxanthin, especially the 13Z-isomer, have higher antioxidant activity than the all-*E*-isomer.

Most investigators have concluded that “positive” effects on the bioavailability and antioxidant activity occurred following astaxanthin Z-isomerization. Thus, the ingestion of astaxanthin Z-isomers could be effective in these terms. As with other carotenoids, Z-isomers of astaxanthin could be obtained by heating [81, 82] and catalytic treatment [76, 81, 83] of the all-*E*-isomer.

2.4. Canthaxanthin

Canthaxanthin is a xanthophyll ($C_{40}H_{52}O_2$) that is principally responsible for the orange-pink color found abundantly in egg yolk and various microbes such as *Bradyrhizobium* sp. and *Halobacterium* sp. [84, 85]. Canthaxanthin can significantly reduce the risk of cancer and neurodegenerative disorder [86, 87] and shows strong antioxidant activity [88], that is, canthaxanthin administration decreased mammary tumor volumes in mice [86] and exhibited antiinflammatory activities by increasing the activity of GPX and catalase, thereby reducing the production of IL-1, IL-6, and TNF- α [87]. Furthermore, canthaxanthin is widely used as feed for hens and fish to improve the egg yolk color and the body color, respectively [84, 89]. A few reports have shown the effect of canthaxanthin Z-isomerization on antioxidant activity and functionality. Venugopalan et al. [88] reported that (9Z)-canthaxanthin isolated from *Dietzia* sp. had higher antioxidant activity, as evaluated by performing DPPH radical-scavenging assay, superoxide radical-scavenging assay and fluorescence assay to detect reactive oxygen species generated in THP-1 cells. Moreover, the (9Z)-isomer exhibited higher pro-apoptotic activity than the all-*E*-isomer, which was evaluated in THP-1 macrophages [90]. The above literature indicates that Z-isomerization of canthaxanthin has “positive” effects. Canthaxanthin Z-isomerization can be achieved by heating and catalytic treatment [91, 92], and *Dietzia* sp. can serve as a source of (9Z)-canthaxanthin [88, 89].

2.5. Fucoxanthin

Fucoxanthin is an allenic xanthophyll ($C_{42}H_{58}O_6$) that is found abundantly in edible shellfish and brown seaweeds such as *Mactra chinensis* and *Undaria pinnatifida* [1, 93]. Fucoxanthin has high antioxidant capacity [94] and shows anticancer and antiangiogenic activities [95, 96]. For example, fucoxanthin remarkably reduced the viability of human colon cancer cell lines, such as Caco-2, HT-29, and DLD-1 cells [95]. In addition, fucoxanthin has antiobesity and antidiabetic effects [97–99], for example, administration of Wakame (*Undaria pinnatifida*) (which is rich in fucoxanthin) significantly suppressed body weight and white adipose tissue weight gain induced by the high fat diet in an obese murine model [98], which has attracted much attention recently in the food industry. The Z-isomerization of (all-*E*)-fucoxanthin can induce changes in the antioxidant and anticancer activities. Namely, Zhang et al. [19] reported that when the antioxidant activity of fucoxanthin isomers was evaluated by performing DPPH radical-scavenging and superoxide-detection assay, the following relative activities were observed: 13Z-isomer $\approx$ 13'Z-isomer > all-*E*-isomer > 9'Z-isomer. Evaluation by performing

2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and hydroxyl radical-scavenging assay revealed the following relative activities: 9'*Z*-isomer > all-*E*-isomer > 13*Z*-isomer $\approx$ 13'*Z*-isomer. Moreover, Kawee-ai et al. [100] showed that when the ratio of the *Z*-isomer of fucoxanthin increased by 2% from 11 to 13% by heating, the scavenging activities against DPPH, hydrogen peroxide, and superoxide anions, and the reducing power decreased by 21.0, 10.3, 16.0 and 19.7%, respectively. Hence, it is considered that the *Z*-isomerization of fucoxanthin negatively affects the antioxidant activity. However, Nakazawa et al. [13] demonstrated that *Z*-isomers of fucoxanthin had higher anticancer activity than the all-*E*-isomer, as evaluated by measuring the potent inhibitory effects on human promyelocytic leukemia cells (HL-60) and colon cancer cells (Caco-2). To the best of our knowledge, only fucoxanthin was investigated in terms of the effect of *Z*-isomerization on anticancer activity. Thus, it is expected that such investigation will extend to other carotenoids in the future. *Z*-isomerization of (all-*E*)-fucoxanthin has been achieved by heating and light irradiation [100, 101].

2.6. Lutein

Lutein is a xanthophyll ($C_{40}H_{56}O_2$) that is principally responsible for the yellow-orange color found abundantly in vegetables, for example, corn, carrots, kale, and peas, and in egg yolks [102]. Lutein has preventive effects against various diseases such as eye diseases and cardiovascular diseases [102–104]. In particular, several studies have addressed the role of lutein in reducing the risk of the two most common eye diseases in older people, that is, cataracts and macular degeneration [102–104]. Only a few reports have shown the effect of lutein *Z*-isomerization on bioavailability and antioxidant activity [15, 105]. *In vitro* tests using a digestion model have shown a higher bioaccessibility of *Z*-isomers of lutein than the all-*E*-isomer, and a Caco-2 cell monolayer model has shown a lower bioavailability. These results indicated that *Z*-isomers of lutein are more efficiently incorporated into bile acid micelles, but they have lower transport efficiency in enterocytes via the activities of carotenoid-transport proteins like β -carotene, as described above [15, 27, 49–51].

In terms of antioxidant activity, the *Z*-isomers, especially the 13'*Z*-isomer, have shown higher antioxidant activities than the all-*E*-isomer in FRAP, DPPH, and ORAC assay, but no significant differences in the activities of the isomers were observed in CAA assay [15]. Since few reports are available regarding the effects of *Z*-isomerization on lutein bioavailability and functionality, and no such studies have been conducted in humans, further studies are needed to clarify whether *Z*-isomerization shows “positive” or “negative” effects. Several studies have reported that (all-*E*)-lutein can be isomerized to the *Z*-isomers by heating [106, 107] and catalytic treatment [15].

2.7. Other carotenoids

The effects of *Z*-isomerization on the antioxidant activities of other carotenoids, such as α -carotene and zeaxanthin, were investigated by Böhm et al. [10] by performing TEAC assay. The following relative antioxidant activities of α -carotene stereoisomers were found: 13'*Z*-isomer > all-*E*-isomer $\approx$ 9'*Z*-isomer > 9*Z*-isomer $\approx$ 13*Z*-isomer, whereas those for zeaxanthin were as follows: all-*E*-isomer $\approx$ 13*Z*-isomer > 9*Z*-isomer. It is difficult to discern whether

Carotenoid	Evaluation	Overview of results	Effect*	Reference
Lycopene	Bioavailability/ bioaccessibility	Z-Isomers > all- <i>E</i> -isomer, evaluated using a diffusion model	+	[20]
		Z-Isomers > all- <i>E</i> -isomer, evaluated using bile acid micelles and lymph-cannulated ferrets	+	[21, 22]
		Z-Isomers > all- <i>E</i> -isomer, evaluated in Caco-2 cells	+	[23]
		Z-Isomers > all- <i>E</i> -isomer, evaluated in human oral-dosing tests	+	[12, 24–26]
	Antioxidant activity	Z-Isomers > all- <i>E</i> -isomer, evaluated in TEAC assay	+	[10]
		Z-Isomers > all- <i>E</i> -isomer, evaluated in PSC and MbFe ^{III} -LP assay	+	[11]
		All- <i>E</i> -isomer ≈ <i>Z</i> -isomers, evaluated in TEAC and FRAP assay	±	[11]
α-Carotene	Antioxidant activity	13' <i>Z</i> -Isomer > all- <i>E</i> -isomer ≈ 9' <i>Z</i> -isomer > 9 <i>Z</i> -isomer ≈ 13 <i>Z</i> -isomer, evaluated in TEAC assay	±	[10]
β-Carotene	Bioavailability/ bioaccessibility	All- <i>E</i> -isomer > <i>Z</i> -isomers, evaluated in Caco-2 cells, HSC-T6 cells, and rat liver microsomes	–	[14]
		All- <i>E</i> -isomer > <i>Z</i> -isomers, evaluated in ferret oral-dosing test	–	[41]
		All- <i>E</i> -isomer > <i>Z</i> -isomers, evaluated in gerbil oral-dosing test	–	[42]
		All- <i>E</i> -isomer > 9 <i>Z</i> -isomer, evaluated in human oral-dosing tests	–	[43–48]
	Antioxidant activity	9 <i>Z</i> -Isomer > all- <i>E</i> -isomer, evaluated in Caco-2 cells	+	[52]
		9 <i>Z</i> -Isomer > all- <i>E</i> -isomer, evaluated in the small intestines of ferrets	+	[53]
		9 <i>Z</i> -Isomer > all- <i>E</i> -isomer, evaluated by measuring the sensitivity to external oxidants	+	[54]
		9 <i>Z</i> -Isomer > all- <i>E</i> -isomer, evaluated by determining the antiperoxidative activity	+	18
		9 <i>Z</i> -Isomer > all- <i>E</i> -isomer, evaluated in rat oral-dosing tests	+	[55]
		All- <i>E</i> -isomer > 9 <i>Z</i> -isomer, evaluated by measuring LDL oxidation	–	[17]
		All- <i>E</i> -isomer ≈ 9 <i>Z</i> -isomer ≈ 13 <i>Z</i> -isomer ≈ 15 <i>Z</i> -isomer, evaluated in TEAC assay	–	[10]
		All- <i>E</i> -isomer ≈ 9 <i>Z</i> -isomer ≈ 13 <i>Z</i> -isomer > 15 <i>Z</i> -isomer, evaluated in TEAC and PSC assay	–	[56,57]
	Atherogenesis activity	9 <i>Z</i> -Isomer > all- <i>E</i> -isomer, evaluated in knockout mice	+	[58]
	Atherosclerosis activity	9 <i>Z</i> -Isomer > all- <i>E</i> -isomer, evaluated in female LDLR ^{–/–} and apoE-deficient mice	+	[59, 60]

Carotenoid	Evaluation	Overview of results	Effect*	Reference
Astaxanthin	Bioavailability/ bioaccessibility	Z-Isomers > all- <i>E</i> -isomer, evaluated using a digestion model and Caco-2 cells	+	[76]
		13Z-Isomer > all- <i>E</i> -isomer, 9Z-isomer, evaluated in human oral-dosing test	+	[77]
		Z-Isomers > all- <i>E</i> -isomer, evaluated in human oral-dosing test	+	[16]
		All- <i>E</i> -isomer > Z-isomers, evaluated in rainbow trout (<i>Oncorhynchus mykiss</i>) oral-dosing tests	–	[78, 79]
	Antioxidant activity	Z-Isomers > all- <i>E</i> -isomer, evaluated in antioxidant enzyme-activity assay	+	[76]
		Z-Isomers > all- <i>E</i> -isomer, evaluated in DPPH and lipid-peroxidation assay	+	[80]
		Z-Isomers > all- <i>E</i> -isomer, evaluated in DPPH, ORAC, and PLC assay	+	[81]
Canthaxanthin	Antioxidant activity	13Z-Isomer > all- <i>E</i> -isomer > 9Z-isomer, evaluated in CAA assay	±	[81]
		9Z-Isomer > all- <i>E</i> -isomer, evaluated in DPPH, superoxide radical-scavenging, and fluorescence assay	+	[88]
	Pro-apoptotic activity	9Z-Isomer > all- <i>E</i> -isomer, evaluated in THP-1 macrophages	+	[90]
Fucoxanthin	Antioxidant activity	13Z-Isomer ≈ 13'Z-isomer > all- <i>E</i> -isomer > 9'Z-isomer, evaluated in DPPH and superoxide-detection assay	±	[19]
		9'Z-Isomer > all- <i>E</i> -isomer > 13Z-isomer ≈ 13'Z-isomer, evaluated in ABTS and hydroxyl radical-scavenging assay	±	[19]
		Z-Isomers > all- <i>E</i> -isomer, evaluated in DPPH, hydrogen peroxide-scavenging, superoxide anion, and reducing-power assay	–	[100]
	Anticancer activity	Z-Isomers > all- <i>E</i> -isomer, evaluated in HL-60 cells and Caco-2 cells	+	[13]
Lutein	Bioavailability/ bioaccessibility	Z-Isomers > all- <i>E</i> -isomer, evaluated using a digestion model	+	[15]
		All- <i>E</i> -isomer > Z-isomers, evaluated in Caco-2 cells	–	[15]
		13Z-Isomer > all- <i>E</i> -isomer, evaluated using a digestion model	+	[105]
	Antioxidant activity	Z-Isomers > all- <i>E</i> -isomer, evaluated in FRAP assay	+	[15]
		13'Z-Isomer > all- <i>E</i> -isomer ≈ 9Z-isomer, evaluated in DPPH and ORAC assay	+	[15]
		All- <i>E</i> -isomer ≈ Z-isomers, evaluated in CAA assay	±	[15]
Zeaxanthin	Antioxidant activity	All- <i>E</i> -isomer ≈ 13Z-isomer > 9Z-isomers, evaluated in TEAC assay	–	[10]

*Expected effect of carotenoid Z-isomerization on humans: +, “positive” effect; –, “negative” effect; ±, no change or indetermine.

Table 1. Summary of the effects of Z-isomerization of different carotenoids on the bioavailability and functionality.

Z-isomers of both carotenoids have higher antioxidant activity than the all-*E*-isomer based on the TEAC assay results alone; thus, further evaluations by multiple testing methods are necessary.

To the best of our knowledge, the effect of Z-isomerization of other important carotenoids such as capsanthin and β -cryptoxanthin (which have large markets and high functionalities) on the bioavailability and functionality has not been reported. Among the over 1100 reported carotenoids found in nature, only the eight carotenoids mentioned above have been characterized in terms of the effects of Z-isomerization, as summarized in **Table 1**. Thus, further progress in this research area is expected in the future.

3. Changes in the physicochemical properties of carotenoids by Z-isomerization

Changes in the bioavailability and functionality of carotenoids after Z-isomerization should have strong correlations with changes in their physicochemical properties. Several reports have shown that the Z-isomerization of carotenoids can induce changes in various properties such as the stability, solubility, and crystallinity. Some computational approaches using a Gaussian program have revealed that the Z-isomerization of carotenoids affected the Gibbs free energy [34, 35, 108, 109], that is, the relative stability of all-*E*- and mono-Z-isomers were in the following order: all-*E*-isomer $\approx$ 5Z-isomer $>$ 9Z-isomer $>$ 13Z-isomer $>$ 15Z-isomer $>$ 7Z-isomer $\approx$ 11Z-isomer for lycopene [34, 35, 108]; all-*E*-isomer $>$ 9Z-isomer $>$ 13Z-isomer $>$ 15Z-isomer $>$ 7Z-isomer $\approx$ 11Z-isomer for β -carotene [109]. Thus, (all-*E*)-carotenoids should be more stable than the Z-isomers, which was confirmed experimentally by Murakami et al. [36] using lycopene isomers. Changes in the Gibbs free energy, stability, of carotenoids following Z-isomerization would affect their antioxidant activities. In addition, there is limited experimental evidence that the Z-isomers of carotenoids such as lycopene, β -carotene, and astaxanthin have higher solubility in vegetable oil, organic solvents and SC-CO₂ than the all-*E*-isomer [28–32, 110, 111], for example, the solubility of lycopene Z-isomers in ethanol was over 4000 times higher than that of the all-*E*-isomer [29]. These properties should affect the bioavailability of carotenoids. Namely, Z-isomerization of carotenoids could enhance uptake into bile acid micelles due to an increased solubility; thus, the bioavailability of lycopene and astaxanthin was improved [20–22]. On the other hand, regarding β -carotene and lutein, whose Z-isomers showed lower bioavailability [15, 41–48, 105], the uptake into bile acid micelles could potentially be improved by Z-isomerization, but they might have lower transport efficiency in enterocytes due to the activities of several carotenoids transport proteins, which are temporarily present at the apical membrane [27, 49–51]. *In vitro* tests of lutein support this hypothesis, that is, the Z-isomers showed higher bioaccessibility than the all-*E*-isomers in a digestion model [15, 105], whereas the opposite result was obtained in Caco-2 cells [15]. It has been predicted that Z-isomers of lycopene and astaxanthin can be efficiently internalized via carotenoid transporters, based on the results of testing conducted using Caco-2 cells [23, 76]. The abovementioned theory is strongly supported by the observations that, in human blood, over 50% of total lycopene exists in the Z-form, but only 5% of total β -carotene exists in the Z-form [112]. To attain a better understanding of the underlying mechanisms, further study

on the uptake process of (Z)-carotenoids in enterocytes by carotenoid transport proteins is necessary. Furthermore, the crystallinity of carotenoids was changed by Z-isomerization: although (all-*E*)-carotenoids existed in a crystalline state, the Z-isomers were in an amorphous state, which was confirmed by optical observations, differential scanning calorimetry, powder X-ray diffraction, and scanning electron microscopy analyses [20, 28, 29, 113]. The change in crystallinity resulting from Z-isomerization may also influence changes in carotenoid bioavailability and functionality.

4. Conclusions

Z-Isomerization of carotenoids can cause changes in the bioavailability, antioxidant activity, and other functionalities (such as anticancer and antiatherogenic activities), and it may result in “positive” or “negative” effects, which vary according to the type of carotenoid. Although more than 1100 carotenoids are found in nature, only the eight carotenoids discussed above have been investigated in terms of these effects. Thus, further progress in this research area is expected. Furthermore, most investigations have focused on the effects of Z-isomerization of carotenoids on the bioavailability and antioxidant activity, but the Z-isomerization has been shown to enhance the anticancer and antiatherogenic activities of β -carotene and fucoxanthin. Since these data provide important evidence for the roles of carotenoid Z-isomerization in human health, examination of other carotenoids is expected in the future.

Author details

Masaki Honda^{1*}, Hayato Maeda², Tetsuya Fukaya³ and Motonobu Goto⁴

*Address all correspondence to: honda@meijo-u.ac.jp

1 Faculty of Science and Technology, Meijo University, Nagoya, Japan

2 Faculty of Agriculture and Life Science, Hirosaki University, Aomori, Japan

3 Innovation Division, Kagome Company, Limited, Nishitomiya, Nasushiobara, Japan

4 Department of Materials Process Engineering, Nagoya University, Nagoya, Japan

References

- [1] Maoka T. Carotenoids in marine animals. *Marine Drugs*. 2011;**9**:278-293. DOI: 10.3390/md9020278
- [2] Yabuzaki J. Carotenoids database: Structures, chemical fingerprints and distribution among organisms. *Database*. 2017;**2017**. DOI: 1-11. DOI: 10.1093/database/bax004
- [3] Amorim-Carrilho KT, Cepeda A, Fente C, Regal P. Review of methods for analysis of carotenoids. *TrAC Trends in Analytical Chemistry*. 2014;**56**:49-73. DOI: 10.1016/j.trac.2013.12.011

- [4] Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*. 2005;**26**:459-516. DOI: 10.1016/j.mam.2005.10.001
- [5] Xu XR, Zou ZY, Huang YM, Xiao X, Ma L, Lin XM. Serum carotenoids in relation to risk factors for development of atherosclerosis. *Clinical Biochemistry*. 2012;**45**:1357-1361. DOI: 10.1016/j.clinbiochem.2012.07.101
- [6] Ouchi A, Aizawa K, Iwasaki Y, Inakuma T, Terao J, Nagaoka S, Mukai K. Kinetic study of the quenching reaction of singlet oxygen by carotenoids and food extracts in solution. Development of a singlet oxygen absorption capacity (SOAC) assay method. *Journal of Agricultural and Food Chemistry*. 2010;**58**:9967-9978. DOI: 10.1021/jf101947a
- [7] Krinsky NI, Russett MD, Handelman GJ, Snodderly DM. Structural and geometrical isomers of carotenoids in human plasma. *The Journal of Nutrition*. 1990;**120**:1654-1662. DOI: 10.1093/jn/120.12.1654
- [8] Schierle J, Bretzel W, Bühler I, Faccin N, Hess D, Steiner K, Schüep W. Content and isomeric ratio of lycopene in food and human blood plasma. *Food Chemistry*. 1997;**59**:459-465. DOI: 10.1016/S0308-8146(96)00177-X
- [9] Honda M, Murakami K, Watanabe Y, Higashiura T, Fukaya T, Wahyudiono, Kanda H, Goto M. The E/Z isomer ratio of lycopene in foods and effect of heating with edible oils and fats on isomerization of (all-E)-lycopene. *European Journal of Lipid Science and Technology*. 2017;**119**:1600389(1-9). DOI: 10.1002/ejlt.201600389
- [10] Böhm V, Puspitasari-Nienaber NL, Ferruzzi MG, Schwartz SJ. Trolox equivalent antioxidant capacity of different geometrical isomers of α -carotene, β -carotene, lycopene, and zeaxanthin. *Journal of Agricultural and Food Chemistry*. 2002;**50**:221-226. DOI: 10.1021/jf010888q
- [11] Müller L, Goupy P, Fröhlich K, Dangles O, Caris-Veyrat C, Böhm V. Comparative study on antioxidant activity of lycopene (Z)-isomers in different assay. *Journal of Agricultural and Food Chemistry*. 2011;**59**:4504-4511. DOI: 10.1021/jf1045969
- [12] Cooperstone JL, Ralston RA, Riedl KM, Haufe TC, Schweiggert RM, King SA, Timmers CD, Francis DM, Lesinski GB, Clinton SK, Schwartz SJ. Enhanced bioavailability of lycopene when consumed as *cis*-isomers from *tangerine* compared to red tomato juice, a randomized, cross-over clinical trial. *Molecular Nutrition & Food Research*. 2015;**59**:658-669. DOI: 10.1002/mnfr.201400658
- [13] Nakazawa Y, Sashima T, Hosokawa M, Miyashita K. Comparative evaluation of growth inhibitory effect of stereoisomers of fucoxanthin in human cancer cell lines. *Journal of Functional Foods*. 2009;**1**:88-97. DOI: 10.1016/j.jff.2008.09.015
- [14] During A, Hussain MM, Morel DW, Harrison EH. Carotenoid uptake and secretion by CaCo-2 cells: β -carotene isomer selectivity and carotenoid interactions. *The Journal of Lipid Research*. 2002;**43**:1086-1095. DOI: 10.1194/jlr.M200068-JLR200
- [15] Yang C, Fischer M, Kirby C, Liu R, Zhu H, Zhang H, Chen Y, Sun Y, Zhang L, Tsao R. Bioaccessibility, cellular uptake and transport of luteins and assessment of their antioxidant activities. *Food Chemistry*. 2018;**249**:66-76. DOI: 10.1016/j.foodchem.2017.12.055

- [16] Coral-Hinostroza GN, Ytrestøyl T, Ruyter B, Bjerkeng B. Plasma appearance of unesterified astaxanthin geometrical *E/Z* and optical *R/S* isomers in men given single doses of a mixture of optical 3 and 3'*R/S* isomers of astaxanthin fatty acyl diesters. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 2004;**139**:99-110. DOI: 10.1016/j.cca.2004.09.011
- [17] Lavy A, Amotz AB, Aviram M. Preferential inhibition of LDL oxidation by the all-*trans* isomer of β -carotene in comparison with 9-*cis* β -carotene. *Clinical Chemistry and Laboratory Medicine*. 1993;**31**:83-90. DOI: 10.1515/cclm.1993.31.2.83
- [18] Levin G, Mokady S. Antioxidant activity of 9-*cis* compared to all-*trans* β -carotene in vitro. *Free Radical Biology and Medicine*. 1994;**17**:77-82. DOI: 10.1016/0891-5849(94)90009-4
- [19] Zhang Y, Fang H, Xie Q, Sun J, Liu R, Hong Z, Yi R, Wu H. Comparative evaluation of the radical-scavenging activities of fucoxanthin and its stereoisomers. *Molecules*. 2014;**19**:2100-2113. DOI: 10.3390/molecules19022100
- [20] Sun Q, Yang C, Li J, Raza H, Zhang L. Lycopene: Heterogeneous catalytic *E/Z* isomerization and *in vitro* bioaccessibility assessment using a diffusion model. *Journal of Food Science*. 2016;**81**:C2381-C2389. DOI: 10.1111/1750-3841.13419
- [21] Boileau AC, Merchen NR, Wasson K, Atkinson CA, Erdman JW Jr. *Cis*-lycopene is more bioavailable than *trans*-lycopene in vitro and in vivo in lymph-cannulated ferrets. *The Journal of Nutrition*. 1999;**129**:1176-1181. DOI: 10.1093/jn/129.6.1176
- [22] Boileau TWM, Boileau AC, Erdman JW Jr. Bioavailability of all-*trans* and *cis*-isomers of lycopene. *Experimental Biology and Medicine*. 2002;**227**:914-919. DOI: 10.1177/153537020222701012
- [23] Failla ML, Chitchumroonchokchai C, Ishida BK. In vitro micellarization and intestinal cell uptake of *cis* isomers of lycopene exceed those of all-*trans* lycopene. *The Journal of Nutrition*. 2008;**138**:482-486. DOI: 10.1093/jn/138.3.482
- [24] Stahl W, Sies H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *The Journal of Nutrition*. 1992;**122**:2161-2166. DOI: 10.1093/jn/122.11.2161
- [25] Unlu NZ, Bohn T, Francis DM, Nagaraja HN, Clinton SK, Schwartz SJ. Lycopene from heat-induced *cis*-isomer-rich tomato sauce is more bioavailable than from all-*trans*-rich tomato sauce in human subjects. *British Journal of Nutrition*. 2007;**98**(2007):140-146. DOI: 10.1017/S0007114507685201
- [26] Burri BJ, Burri BJ, Chapman MH, Neidlinger TR, Seo JS, Ishida BK. Tangerine tomatoes increase total and tetra-*cis*-lycopene isomer concentrations more than red tomatoes in healthy adult humans. *International Journal of Food Sciences and Nutrition*. 2009;**60**:1-16. DOI: 10.1080/09637480701782084
- [27] Desmarchelier C, Borel P. Overview of carotenoid bioavailability determinants: From dietary factors to host genetic variations. *Trends in Food Science & Technology*. 2017;**69**:270-280. DOI: 10.1016/j.tifs.2017.03.002

- [28] Murakami K, Honda M, Wahyudiono, Kanda H, Goto M. Thermal isomerization of (all-*E*)-lycopene and separation of the *Z*-isomers by using a low boiling solvent: Dimethyl ether. *Separation Science and Technology*. 2017;**52**:2573-2582. DOI: 10.1080/01496395.2017.1374412
- [29] Murakami K, Honda M, Takemura R, Fukaya T, Kubota M, Wahyudiono, Kanda H, Goto M. The thermal *Z*-isomerization-induced change in solubility and physical properties of (all-*E*)-lycopene. *Biochemical and Biophysical Research Communications*. 2017;**491**:317-322. DOI: 10.1016/j.bbrc.2017.07.103
- [30] Honda M, Watanabe Y, Murakami K, Takemura R, Fukaya T, Wahyudiono, Kanda H, Goto M. Thermal isomerization pre-treatment to improve lycopene extraction from tomato pulp. *LWT-Food Science and Technology*. 2017;**86**:69-75. DOI: 10.1016/j.lwt.2017.07.046
- [31] Honda M, Watanabe Y, Murakami K, Hoang NN, Wahyudiono, Kanda H, Goto M. Enhanced lycopene extraction from gac (*Momordica cochinchinensis* Spreng.) by the *Z*-isomerization induced with microwave irradiation pre-treatment. *European Journal of Lipid Science and Technology*. 2018;**120**:1700293(1-8). DOI: 10.1002/ejlt.201700293
- [32] Watanabe Y, Honda M, Higashiura T, Fukaya T, Machmudah S, Wahyudiono, Kanda H, Goto M. Rapid and selective concentration of lycopene *Z*-isomers from tomato pulp by supercritical CO₂ with CO-solvents. *Solvent Extraction Research and Development, Japan*. 2018;**25**:47-57
- [33] Richelle M, Lambelet P, Rytz A, Tavazzi I, Mermoud AF, Juhel C, Borel P, Bortlik K. The proportion of lycopene isomers in human plasma is modulated by lycopene isomer profile in the meal but not by lycopene preparation. *British Journal of Nutrition*. 2012;**107**:1482-1488. DOI: 10.1017/S0007114511004569
- [34] Takehara M, Kuwa T, Inoue Y, Kitamura C, Honda M. Isolation and characterization of (15*Z*)-lycopene thermally generated from a natural source. *Biochemical and Biophysical Research Communications*. 2015;**467**:58-62. DOI: 10.1016/j.bbrc.2015.09.122
- [35] Honda M, Kudo T, Kuwa T, Higashiura T, Fukaya T, Inoue Y, Kitamura C, Takehara M. Isolation and spectral characterization of thermally generated multi-*Z*-isomers of lycopene and the theoretically preferred pathway to di-*Z*-isomers. *Bioscience, Biotechnology, and Biochemistry*. 2017;**81**:365-371. DOI: 10.1080/09168451.2016.1249454
- [36] Murakami K, Honda M, Takemura R, Fukaya T, Wahyudiono, Kanda H, Goto M. Effect of thermal treatment and light irradiation on the stability of lycopene with high *Z*-isomers content. *Food Chemistry*. 2018;**250**:253-258. DOI: 10.1016/j.foodchem.2018.01.062
- [37] Honda M, Takahashi N, Kuwa T, Takehara M, Inoue Y, Kumagai T. Spectral characterisation of *Z*-isomers of lycopene formed during heat treatment and solvent effects on the *E/Z* isomerisation process. *Food Chemistry*. 2015;**171**:323-329. DOI: 10.1016/j.foodchem.2014.09.004
- [38] Honda M, Horiuchi I, Hiramatsu H, Inoue Y, Kitamura C, Fukaya T, Takehara M. Vegetable oil-mediated thermal isomerization of (all-*E*)-lycopene: Facile and efficient production of *Z*-isomers. *European Journal of Lipid Science and Technology*. 2016;**118**:1588-1592. DOI: 10.1002/ejlt.201500446

- [39] Honda M, Igami H, Kawana T, Hayashi K, Takehara M, Inoue Y, Kitamura C. Photo-sensitized *E/Z* isomerization of (all-*E*)-lycopene aiming at practical applications. *Journal of Agricultural and Food Chemistry*. 2014;**62**:11353-11356. DOI: 10.1021/jf504502t
- [40] Honda M, Kawana T, Takehara M, Inoue Y. Enhanced *E/Z* isomerization of (all-*E*)-lycopene by employing iron(III) chloride as a catalyst. *Journal of Food Science*. 2015;**80**:C1453-C1459. DOI: 10.1111/1750-3841.12916
- [41] Erdman JW, Thatcher AJ, Hofmann NE, Lederman JD, Block SS, Lee CM, Mokady S. All-*trans* β -carotene is absorbed preferentially to 9-*cis* β -carotene, but the latter accumulates in the tissues of domestic ferrets (*Mustela putorius puro*). *The Journal of Nutrition*. 1998;**128**:2009-2013. DOI: 10.1093/jn/128.11.2009
- [42] Deming DM, Teixeira SR, Erdman JW. All-*trans* β -carotene appears to be more bioavailable than 9-*cis* or 13-*cis* β -carotene in gerbils given single oral doses of each isomer. *The Journal of Nutrition*. 2002;**132**:2700-2708. DOI: 10.1093/jn/132.9.2700
- [43] Stahl W, Schwarz W, Sies H. Human serum concentrations of all-*trans* β - and α -carotene but not 9-*cis* β -carotene increase upon ingestion of a natural isomer mixture obtained from *Dunaliella salina* (Betatene). *The Journal of Nutrition*. 1993;**123**:847-851. DOI: 10.1093/jn/123.5.847
- [44] Stahl W, Schwarz W, von Laar J, Sies H. All-*trans* β -carotene preferentially accumulates in human chylomicrons and very low density lipoproteins compared with the 9-*cis* geometrical isomer. *The Journal of Nutrition*. 1995;**125**:2128-2133. DOI: 10.1093/jn/125.8.2128
- [45] Gaziano JM, Johnson EJ, Russell RM, Manson JE, Stampfer MJ, Ridker PM, Frei B, Hennekens CM, Krinsky NI. Discrimination in absorption or transport of β -carotene isomers after oral supplementation with either all-*trans*- or 9-*cis*- β -carotene. *The American Journal of Clinical Nutrition*. 1995;**61**:1248-1252. DOI: 10.1093/ajcn/61.6.1248
- [46] Johnson EJ, Qin J, Krinsky NI, Russell RM. β -Carotene isomers in human serum, breast milk and buccal mucosa cells after continuous oral doses of all-*trans* and 9-*cis* β -carotene. *The Journal of Nutrition*. 1997;**127**:1993-1999. DOI: 10.1093/jn/127.10.1993
- [47] Johnson EJ, Krinsky NI, Russell RM. Serum response of all-*trans* and 9-*cis* isomers of β -carotene in humans. *Journal of the American College of Nutrition*. 1996;**15**:620-624. DOI: 10.1080/07315724.1996.10718639
- [48] Tamai H, Morinobu T, Murata T, Manago M, Mino M. 9-*cis* β -carotene in human plasma and blood cells after ingestion of β -carotene. *Lipids*. 1995;**30**:493-498. DOI: 10.1007/BF02537022
- [49] During A, Dawson HD, Harrison EH. Carotenoid transport is decreased and expression of the lipid transporters SR-BI, NPC1L1, and ABCA1 is downregulated in Caco-2 cells treated with ezetimibe. *The Journal of Nutrition*. 2005;**135**:2305-2312. DOI: 10.1093/jn/135.10.2305
- [50] Kotake-Nara E. Intestinal absorption of carotenoid. *Journal of Lipid Nutrition*. 2012;**21**:35-43. DOI: 10.4010/jln.21.35
- [51] Yonekura L, Nagao A. Intestinal absorption of dietary carotenoids. *Molecular Nutrition & Food Research*. 2007;**51**:107-115. DOI: 10.1002/mnfr.200600145

- [52] Ferruzzi MG, Lumpkin JL, Schwartz SJ, Failla M. Digestive stability, micellarization, and uptake of β -carotene isomers by Caco-2 human intestinal cells. *Journal of Agricultural and Food Chemistry*. 2006;**54**:2780-2785. DOI: 10.1021/jf0530603
- [53] Hébuterne X, Wang XD, Johnson EJ, Krinsky NI, Russell RM. Intestinal absorption and metabolism of 9-*cis*- β -carotene in vivo: Biosynthesis of 9-*cis*-retinoic acid. *The Journal of Lipid Research*. 1995;**36**:1264-1273
- [54] Jiménez C, Pick U. Differential reactivity of β -carotene isomers from *Dunaliella bardawil* toward oxygen radicals. *Plant Physiology*. 1993;**101**:385-390. DOI: 10.1104/pp.101.2.385
- [55] Levin G, Yeshurun M, Mokady S. *In vivo* antiperoxidative effect of 9-*cis* β -carotene compared with that of the all-*trans* isomer. *Nutrition and Cancer*. 1997;**27**:293-297. DOI: 10.1080/01635589709514540
- [56] Mueller L, Boehm V. Antioxidant activity of β -carotene compounds in different *in vitro* assay. *Molecules*. 2011;**16**:1055-1069. DOI: 10.3390/molecules16021055
- [57] Rodrigues E, Mariutti LR, Chisté RC, Mercadante AZ. Development of a novel micro-assay for evaluation of peroxy radical scavenger capacity: Application to carotenoids and structure–activity relationship. *Food Chemistry*. 2012;**135**:2103-2111. DOI: 10.1016/j.foodchem.2012.06.074
- [58] Harari A, Harats D, Marko D, Cohen H, Barshack I, Kamari Y, Gonen A, Gerber Y, Ben-Amotz A, Shaish A. A 9-*cis* β -carotene–enriched diet inhibits atherogenesis and fatty liver formation in LDL receptor knockout mice. *The Journal of Nutrition*. 2008;**138**:1923-1930. DOI: 10.1093/jn/138.10.1923
- [59] Relevy NZ, Rühl R, Harari A, Grosskopf I, Barshack I, Ben-Amotz A, Nir U, Gottlieb H, Kamari Y, Harats D, Shaish A. 9-*cis* β -Carotene inhibits atherosclerosis development in female LDLR–/– mice. *Functional Foods in Health & Disease*. 2015;**5**:67-79
- [60] Harari A, Abecassis R, Relevi N, Levi Z, Ben-Amotz A, Kamari Y, Harats A, Shaish, A. Prevention of atherosclerosis progression by 9-*cis*- β -carotene rich alga *Dunaliella* in apoE-deficient mice. *BioMed Research International*. 2013;**2013**:169517(1-7). DOI: 10.1155/2013/169517
- [61] Nagao A, Olson JA. Enzymatic formation of 9-*cis*, 13-*cis*, and all-*trans* retinals from isomers of β -carotene. *FASEB Journal*. 1994;**8**:968-973. DOI: 10.1096/fasebj.8.12.8088462
- [62] Wang XD, Krinsky NI, Benotti PN, Russell RM. Biosynthesis of 9-*cis*-retinoic acid from 9-*cis*- β -carotene in human intestinal mucosa in vitro. *Archives of Biochemistry and Biophysics*. 1994;**313**:150-155. DOI: 10.1006/abbi.1994.1371
- [63] Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eichele G, Evans RM, Thaller C. 9-*Cis* retinoic acid is a high affinity ligand for the retinoid X receptor. *Cell*. 1992;**68**:397-406. DOI: 10.1016/0092-8674(92)90479-V
- [64] Levin AA, Sturzenbecker LJ, Kazmer S, Bosakowski T, Huselton C, Allenby G, Speck J, Kratzeisen CL, Rosenberger M, Lovey E, Grippo JF. 9-*Cis* retinoic acid stereoisomer binds and activates the nuclear receptor RXR α . *Nature*. 1992;**355**:359-361. DOI: 10.1038/355359a0

- [65] Minguez-Mosquera MI, Hornero-Mendez D, Perez-Galvez A. Carotenoids and provitamin A in functional foods. In: Hurst WJ, editor. *Methods of Analysis for Functional Foods and Nutraceuticals*. Oxford: Taylor & Francis; 2002. pp. 101-157. DOI: 10.1201/9781420014679.ch3
- [66] Schieber A, Carle R. Occurrence of carotenoid *cis*-isomers in food: Technological, analytical, and nutritional implications. *Trends in Food Science & Technology*. 2005;**16**:416-422. DOI: 10.1016/j.tifs.2005.03.018
- [67] Lemmens L, De Vleeschouwer K, Moelants KR, Colle IJ, Van Loey AM, Hendrickx ME. β -Carotene isomerization kinetics during thermal treatments of carrot puree. *Journal of Agricultural and Food Chemistry*. 2010;**58**:6816-6824. DOI: 10.1021/jf100449t
- [68] Knockaert G, Pulissey SK, Lemmens L, Van Buggenhout S, Hendrickx M, Van Loey A. Carrot β -carotene degradation and isomerization kinetics during thermal processing in the presence of oil. *Journal of Agricultural and Food Chemistry*. 2012;**60**:10312-10319. DOI: 10.1021/jf3025776
- [69] Kuki M, Koyama Y, Nagae H. Triplet-sensitized and thermal isomerization of all-trans, 7-cis, 9-cis, 13-cis and 15-cis isomers of β -carotene: Configurational dependence of the quantum yield of isomerization via the T_1 state. *The Journal of Physical Chemistry*. 1991;**95**:7171-7180. DOI: 10.1021/j100172a016
- [70] Gao Y, Kispert LD. Reaction of carotenoids and ferric chloride: Equilibria, isomerization, and products. *The Journal of Physical Chemistry B*. 2003;**107**:5333-5338. DOI: 10.1021/jp034063q
- [71] Gao Y, Kispert LD, Konovalova TA, Lawrence JN. Isomerization of carotenoids in the presence of MCM-41 molecular sieves: EPR and HPLC studies. *The Journal of Physical Chemistry B*. 2004;**108**:9456-9462. DOI: 10.1021/jp036091e
- [72] Yuan JP, Peng J, Yin K, Wang JH. Potential health-promoting effects of astaxanthin: A high-value carotenoid mostly from microalgae. *Molecular Nutrition & Food Research*. 2011;**55**:150-165. DOI: 10.1002/mnfr.201000414
- [73] Guerin M, Huntley ME, Olaizola M. *Haematococcus* astaxanthin: Applications for human health and nutrition. *Trends in Biotechnology*. 2003;**21**:210-216. DOI: 10.1016/S0167-7799(03)00078-7
- [74] Pashkow FJ, Watumull DG, Campbell CL. Astaxanthin: A novel potential treatment for oxidative stress and inflammation in cardiovascular disease. *American Journal of Cardiology*. 2008;**101**:S58-S68. DOI: 10.1016/j.amjcard.2008.02.010
- [75] Ambati RR, Phang SM, Ravi S, Aswathanarayana RG. Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications—A review. *Marine Drugs*. 2014;**12**:128-152. DOI: 10.3390/md12010128
- [76] Yang C, Zhang H, Liu R, Zhu H, Zhang L, Tsao R. Bioaccessibility, cellular uptake, and transport of astaxanthin isomers and their antioxidative effects in human intestinal epithelial Caco-2 cells. *Journal of Agricultural and Food Chemistry*. 2017;**65**:10223-10232. DOI: 10.1021/acs.jafc.7b04254

- [77] Østerlie M, Bjerkeng B, Liaaen-Jensen S. Plasma appearance and distribution of astaxanthin *E/Z* and *R/S* isomers in plasma lipoproteins of men after single dose administration of astaxanthin. *The Journal of Nutritional Biochemistry*. 2000;**11**:482-490. DOI: 10.1016/S0955-2863(00)00104-2
- [78] Bjerkeng B, Følling M, Lagocki S, Storebakken T, Olli JJ, Alsted N. Bioavailability of all-*E*-astaxanthin and *Z*-isomers of astaxanthin in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 1997;**157**:63-82. DOI: 10.1016/S0044-8486(97)00146-4
- [79] Østerlie M, Bjerkeng B, Liaaen-Jensen S. Accumulation of astaxanthin all-*E*, 9*Z* and 13*Z* geometrical isomers and 3 and 3' *RS* optical isomers in rainbow trout (*Oncorhynchus mykiss*) is selective. *The Journal of Nutrition*. 1999;**129**:391-398. DOI: 10.1093/jn/129.2.391
- [80] Liu X, Osawa T. *Cis* astaxanthin and especially 9-*cis* astaxanthin exhibits a higher anti-oxidant activity *in vitro* compared to the all-*trans* isomer. *Biochemical and Biophysical Research Communications*. 2007;**357**:187-193. DOI: 10.1016/j.bbrc.2007.03.120
- [81] Yang C, Zhang L, Zhang H, Sun Q, Liu R, Li J, Wu L, Tsao R. Rapid and efficient conversion of all-*E*-astaxanthin to 9*Z*- and 13*Z*-isomers and assessment of their stability and antioxidant activities. *Journal of Agricultural and Food Chemistry*. 2017;**65**:818-826. DOI: 10.1021/acs.jafc.6b04962
- [82] Yuan JP, Chen F. Kinetics for the reversible isomerization reaction of *trans*-astaxanthin. *Food Chemistry*. 2011;**73**:131-137. DOI: 10.1016/S0308-8146(01)00107-8
- [83] Zhao L, Chen F, Zhao G, Wang Z, Liao X, Hu X. Isomerization of *trans*-astaxanthin induced by copper (II) ion in ethanol. *Journal of Agricultural and Food Chemistry*. 2005;**53**:9620-9623. DOI: 10.1021/jf0517750
- [84] Cho JH, Zhang ZF, Kim IH. Effects of canthaxanthin on egg production, egg quality, and egg yolk color in laying hens. *Journal of Agricultural Science*. 2012;**5**:269-274. DOI: 10.5539/jas.v5n1p269
- [85] Malik K, Tokkas J, Goyal S. Microbial pigments: A review. *International Journal of Microbial Resource Technology*. 2012;**1**:361-365
- [86] Chew BP, Park JS, Wong MW, Wong TS. A comparison of the anticancer activities of dietary β -carotene, canthaxanthin and astaxanthin in mice *in vivo*. *Anticancer Research*. 1999;**19**:1849-1853
- [87] Chan KC, Mong MC, Yin MC. Antioxidative and anti-inflammatory neuroprotective effects of astaxanthin and canthaxanthin in nerve growth factor differentiated PC12 cells. *Journal of Food Science*. 2009;**74**:H225-H231. DOI: 10.1111/j.1750-3841.2009.01274.x
- [88] Venugopalan V, Tripathi SK, Nahar P, Saradhi PP, Das RH, Gautam HK. Characterization of canthaxanthin isomers isolated from a new soil *Dietzia* sp. and their antioxidant activities. *Journal of Microbiology and Biotechnology*. 2013;**23**:237-245. DOI: 10.4014/jmb.1203.03032
- [89] Li M, Rahman MM, Wu B, Lin YC. Effects of dietary canthaxanthin on growth and body colour of blood parrot cichlid *Amphilophus citrinellus* $\times$ *Paraneotroplus synspilus*. *Aquaculture International*. 2017;**25**:705-713. DOI: 10.1007/s10499-016-0068-z

- [90] Venugopalan V, Verma N, Gautam HK, Saradhi PP, Das RH. 9-*cis*-Canthaxanthin exhibits higher pro-apoptotic activity than all-*trans*-canthaxanthin isomer in THP-1 macrophage cells. *Free Radical Research*. 2009;**43**:100-105. DOI: 10.1080/10715760802616668
- [91] Qiu D, Zhu WL, Tang CK, Shi LF, Gao HQ. Identification of the composition of isomeric canthaxanthin sample by NMR, HPLC, and mass spectrometry. *Food Analytical Methods*. 2014;**7**:597-605. DOI: 10.1007/s12161-013-9660-2
- [92] Sundquist AR, Hanusch M, Stahl W, Sies H. *Cis/trans* isomerization of carotenoids by the triplet carbonyl source 3-hydroxymethyl-3,4,4-trimethyl-1,2-dioxetane. *Photochemistry and Photobiology*. 1993;**57**:785-791. DOI: 10.1111/j.1751-1097.1993.tb09211.x
- [93] Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K. Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochemical and Biophysical Research Communications*. 2005;**332**:392-397. DOI: 10.1016/j.bbrc.2005.05.002
- [94] Sachindra NM, Sato E, Maeda H, Hosokawa M, Niwano Y, Kohno M, Miyashita K. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *Journal of Agricultural and Food Chemistry*. 2007;**55**:8516-8522. DOI: 10.1021/jf071848a
- [95] Hosokawa M, Kudo M, Maeda H, Kohno H, Tanaka T, Miyashita K. Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPAR γ ligand, troglitazone, on colon cancer cells. *Biochimica et Biophysica Acta (BBA)—General Subject*. 2004;**1675**:113-119. DOI: 10.1016/j.bbagen.2004.08.012
- [96] Sugawara T, Matsubara K, Akagi R, Mori M, Hirata T. Antiangiogenic activity of brown algae fucoxanthin and its deacetylated product, fucoxanthinol. *Journal of Agricultural and Food Chemistry*. 2006;**54**:9805-9810. DOI: 10.1021/jf062204q
- [97] Maeda H. Nutraceutical effects of fucoxanthin for obesity and diabetes therapy: A review. *Journal of Oleo Science*. 2015;**64**:125-132. DOI: 10.5650/jos.ess14226
- [98] Maeda H, Hosokawa M, Sashima T, Murakami-Funayama K, Miyashita K. Anti-obesity and anti-diabetic effects of fucoxanthin on diet-induced obesity conditions in a murine model. *Molecular Medicine Reports*. 2009;**2**:897-902. DOI: 10.3892/mmr_00000189
- [99] Maeda H, Kanno S, Kodate M, Hosokawa M, Miyashita K. Fucoxanthinol, metabolite of fucoxanthin, improves obesity-induced inflammation in adipocyte cells. *Marine Drugs*. 2015;**13**:4799-4813. DOI: 10.3390/md13084799
- [100] Kawee-ai A, Kuntiya A, Kim SM. Anticholinesterase and antioxidant activities of fucoxanthin purified from the microalga *Phaeodactylum tricornutum*. *Natural Product Communications*. 2013;**8**:1381-1386
- [101] Zhao D, Kim SM, Pan CH, Chung D. Effects of heating, aerial exposure and illumination on stability of fucoxanthin in canola oil. *Food Chemistry*. 2014;**145**:505-513. DOI: 10.1016/j.foodchem.2013.08.045
- [102] Abdel-Aal ESM, Akhtar H, Zaheer K, Ali R. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. *Nutrients*. 2013;**5**:1169-1185. DOI: 10.3390/nu5041169

- [103] Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. *The Journal of Nutrition*. 2002;**132**:518S-524S. DOI: 10.1093/jn/132.3.518S
- [104] Mozaffarieh M, Sacu S, Wedrich A. The role of the carotenoids, lutein and zeaxanthin, in protecting against age-related macular degeneration: A review based on controversial evidence. *Nutrition Journal*. 2003;**2**:1-8. DOI: 10.1186/1475-2891-2-20
- [105] Rodrigues DB, Mariutti LRB, Mercadante AZ. An *in vitro* digestion method adapted for carotenoids and carotenoid esters: Moving forward towards standardization. *Food & Function*. 2016;**7**:4992-5001. DOI: 10.1039/C6FO01293K
- [106] Subagio A, Morita N, Sawada S. Thermal isomerization of all-*trans*-lutein in a benzene solution. *Bioscience, Biotechnology, and Biochemistry*. 1998;**62**:2453-2456. DOI: 10.1271/bbb.62.2453
- [107] Updike AA, Schwartz SJ. Thermal processing of vegetables increases *cis* isomers of lutein and zeaxanthin. *Journal of Agricultural and Food Chemistry*. 2003;**51**:6184-6190. DOI: 10.1021/jf030350f
- [108] Chasse GA, Mak ML, Deretey E, Farkas I, Torday LL, Papp JG, Sarma DSR, Agarwal A, Chakravarthi S, Agarwal S, Rao AV. An ab initio computational study on selected lycopene isomers. *Journal of Molecular Structure: THEOCHEM*. 2001;**571**:27-37. DOI: 10.1016/S0166-1280(01)00424-9
- [109] Guo WH, Tu CY, Hu CH. Cis-trans isomerizations of β -carotene and lycopene: A theoretical study. *The Journal of Physical Chemistry B*. 2008;**112**:12158-12167. DOI: 10.1021/jp8019705
- [110] Gamlieli-Bonshtein I, Korin E, Cohen S. Selective separation of *cis-trans* geometrical isomers of β -carotene via CO₂ supercritical fluid extraction. *Biotechnology and Bioengineering*. 2002;**80**:169-174. DOI: 10.1002/bit.10357
- [111] Kaga K, Honda M, Adachi T, Honjo M, Wahyudiono, Kanda H, Goto M. Nanoparticle formation of PVP/astaxanthin inclusion complex by solution-enhanced dispersion by supercritical fluids (SEDS): Effect of PVP and astaxanthin Z-isomer content. *The Journal of Supercritical Fluids*. 2018;**36**:44-51. DOI: 10.1016/j.supflu.2018.02.008
- [112] Stahl W, Schwarz W, Sundquist AR, Sies H. *cis-trans* Isomers of lycopene and β -carotene in human serum and tissues. *Archives of Biochemistry and Biophysics*. 1992;**294**:173-177. DOI: 10.1016/0003-9861(92)90153-N
- [113] Hempel J, Schädle CN, Leptihn S, Carle R, Schweiggert RM. Structure related aggregation behavior of carotenoids and carotenoid esters. *Journal of Photochemistry and Photobiology A: Chemistry*. 2016;**317**:161-174. DOI: 10.1016/j.jphotochem.2015.10.024