Chapter 8

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

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Additional information is available at the end of the chapter

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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 ≈ 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,

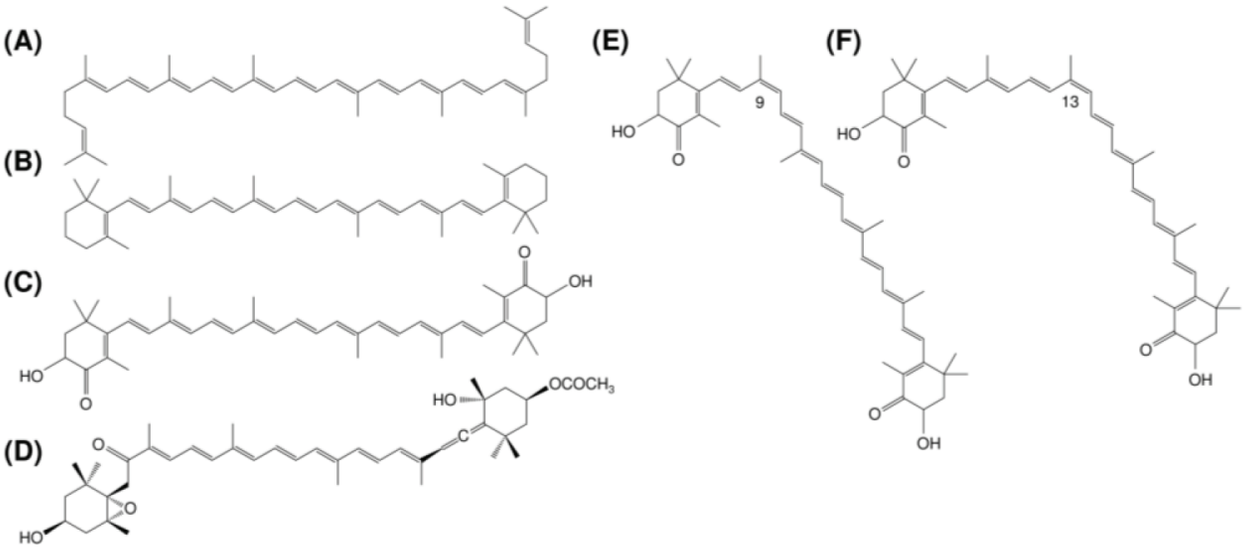

Figure 1. Chemical structures of (A) (all-*E*)-lycopene, (B) (all-*E*)-β-carotene, (C) (all-*E*)-astaxanthin, (D) (all-*E*)-fucoxanthin, (E) (9*Z*)-astaxanthin, and (F) (13*Z*)-astaxanthin.

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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, antiatherogenenic, 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 CO_2 (SC-CO_2) 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 ($C_{40}H_{56}$) 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 (9*Z*)-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 ≈ α-carotene (50% inhibition) > β-cryptoxanthin ≈ 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 9*Z*-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 9*Z*-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

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the antioxidant activity was 13*Z*-isomer > all-*E*-isomer > 9*Z*-isomer [81]. The results of these studies suggest that *Z*-isomers of astaxanthin, especially the 13*Z*-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 (9*Z*)-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 (9*Z*)-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 (9*Z*)-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: 13*Z*-isomer ≈ 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 ≈ 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 ≈ 9′*Z*-isomer > 9*Z*-isomer ≈ 13*Z*-isomer, whereas those for zeaxanthin were as follows: all-*E*-isomer ≈ 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-*E*-isomer, evaluated using a diffusion model	+	[20]
		Z-Isomers > all-*E*-isomer, evaluated using bile acid micelles and lymph-cannulated ferrets	+	[21, 22]
		Z-Isomers > all-*E*-isomer, evaluated in Caco-2 cells	+	[23]
		Z-Isomers > all-*E*-isomer, evaluated in human oral-dosing tests	+	[12, 24–26]
	Antioxidant activity	*Z*-Isomers > all-*E*-isomer, evaluated in TEAC assay	+	[10]
		Z-Isomers > all-*E*-isomer, evaluated in PSC and $MbFe^{III}$-LP assay	+	[11]
		All-*E*-isomer ≈ *Z*-isomers, evaluated in TEAC and FRAP assay	±	[11]
α-Carotene	Antioxidant activity	13′*Z*-Isomer > all-*E*-isomer ≈ 9′*Z*-isomer > 9*Z*-isomer ≈ 13*Z*-isomer, evaluated in TEAC assay	±	[10]
β-Carotene	Bioavailability/ bioaccessibility	All-*E*-isomer > *Z*-isomers, evaluated in Caco-2 cells, HSC-T6 cells, and rat liver microsomes	−	[14]
		All-*E*-isomer > *Z*-isomers, evaluated in ferret oral-dosing test	−	[41]
		All-*E*-isomer > *Z*-isomers, evaluated in gerbil oral-dosing test	−	[42]
		All-*E*-isomer > 9*Z*-isomer, evaluated in human oral-dosing tests	−	[43–48]
		9*Z*-Isomer > all-*E*-isomer, evaluated in Caco-2 cells	+	[52]
		9*Z*-Isomer > all-*E*-isomer, evaluated in the small intestines of ferrets	+	[53]
	Antioxidant activity	9*Z*-Isomer > all-*E*-isomer, evaluated by measuring the sensitivity to external oxidants	+	[54]
		9*Z*-Isomer > all-*E*-isomer, evaluated by determining the antiperoxidative activity	+	18
		9*Z*-Isomer > all-*E*-isomer, evaluated in rat oral-dosing tests	+	[55]
		All-*E*-isomer > 9*Z*-isomer, evaluated by measuring LDL oxidation	−	[17]
		All-*E*-isomer ≈ 9*Z*-isomer ≈ 13*Z*-isomer ≈ 15*Z*-isomer, evaluated in TEAC assay	−	[10]
		All-*E*-isomer ≈ 9*Z*-isomer ≈ 13*Z*-isomer > 15*Z*-isomer, evaluated in TEAC and PSC assay	−	[56,57]
	Atherogenesis activity	9*Z*-Isomer > all-*E*-isomer, evaluated in knockout mice	+	[58]
	Atherosclerosis activity	9*Z*-Isomer > all-*E*-isomer, evaluated in female LDLR−/− and apoE-deficient mice	+	[59, 60]

Carotenoid	Evaluation	Overview of results	Effect*	Reference
Astaxanthin	Bioavailability/ bioaccessibility	*Z*-Isomers > all-*E*-isomer, evaluated using a digestion model and Caco-2 cells	+	[76]
		13*Z*-Isomer > all-*E*-isomer, 9*Z*-isomer, evaluated in human oral-dosing test	+	[77]
		Z-Isomers > all-*E*-isomer, evaluated in human oral-dosing test	+	[16]
		All-*E*-isomer > *Z*-isomers, evaluated in rainbow trout (*Oncorhynchus mykiss*) oral-dosing tests	−	[78, 79]
	Antioxidant activity	*Z*-Isomers > all-*E*-isomer, evaluated in antioxidant enzyme-activity assay	+	[76]
		Z-Isomers > all-*E*-isomer, evaluated in DPPH and lipid-peroxidation assay	+	[80]
		Z-Isomers > all-*E*-isomer, evaluated in DPPH, ORAC, and PLC assay	+	[81]
		13*Z*-Isomer > all-*E*-isomer >9*Z*-isomer, evaluated in CAA assay	±	[81]
Canthaxanthin	Antioxidant activity	9*Z*-Isomer > all-*E*-isomer, evaluated in DPPH, superoxide radical-scavenging, and fluorescence assay	+	[88]
	Pro-apoptotic activity	9*Z*-Isomer > all-*E*-isomer, evaluated in THP-1 macrophages	+	[90]
Fucoxanthin	Antioxidant activity	13*Z*-Isomer ≈ 13′*Z*-isomer > all-*E*-isomer > 9′*Z*-isomer, evaluated in DPPH and superoxide-detection assay	±	[19]
		9′*Z*-Isomer > all-*E*-isomer > 13*Z*-isomer ≈ 13′*Z*-isomer, evaluated in ABTS and hydroxyl radical-scavenging assay	±	[19]
		Z-Isomers > all-*E*-isomer, evaluated in DPPH, hydrogen peroxide-scavenging, superoxide anion, and reducing-power assay	−	[100]
	Anticancer activity	*Z*-Isomers > all-*E*-isomer, evaluated in HL-60 cells and Caco-2 cells	+	[13]
Lutein	Bioavailability/ bioaccessibility	*Z*-Isomers > all-*E*-isomer, evaluated using a digestion model	+	[15]
		All-*E*-isomer > *Z*-isomers, evaluated in Caco-2 cells	−	[15]
		13*Z*-Isomer > all-*E*-isomer, evaluated using a digestion model	+	[105]
	Antioxidant activity	*Z*-Isomers > all-*E*-isomer, evaluated in FRAP assay	+	[15]
		13′*Z*-Isomer > all-*E*-isomer ≈ 9*Z*-isomer, evaluated in DPPH and ORAC assay	+	[15]
		All-*E*-isomer ≈ *Z*-isomers, evaluated in CAA assay	±	[15]
Zeaxanthin	Antioxidant activity	All-*E*-isomer ≈ 13*Z*-isomer > 9*Z*-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 ≈ 5*Z*-isomer > 9*Z*-isomer > 13*Z*-isomer > 15*Z*-isomer > 7*Z*-isomer ≈ 11*Z*-isomer for lycopene [34, 35, 108]; all-*E*-isomer > 9*Z*-isomer > 13*Z*-isomer > 15*Z*-isomer > 7*Z*-isomer ≈ 11*Z*-isome 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-CO_2 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.

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