

The Antioxidant Ability and Extraction Yield of Beta Carotene

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Abstract. Beta carotene (β -Carotene), one of the most abundant carotenoids in fruit and vegetables, is an essential source of antioxidants. The antioxidation ability of β -Carotene has been through in vitro and in vivo tests, which proved that the compound could prevent the oxidation of singlet and triplet oxygen, oil, and fats. Since β -Carotene is unstable and prone to oxidation when exposed to high temperature and high light intensity, various extraction methods have been tested and applied to get to the maximised yield and purity of the extract. Among all the extraction methods, enzyme-assisted extraction (EAE) is the most efficient, with almost 100% extraction yield. The need for antioxidants by the public promoted the production of β -Carotene nutraceuticals and cosmetics. This essay compared the efficacy of different extraction methods and bioactivity of β -Carotene in different research papers and concluded the extraction method with the highest efficiency.

Keywords: β -carotene, peroxy radical scavenging, extraction methods.

1. Introduction

The focus on developing and applying pharmaceutical products has been increasingly emphasized throughout the development of modern technology. Antioxidation has been considered one of the trendiest nutraceutical effects demanded by customers. The popular commercialized antioxidants include vitamin C, vitamin E, flavonoids, lycopene, etcetera. Among them, β -Carotene belonging to the carotenoid family is frequently talked about because of its antioxidation ability and because it naturally exists in many vegetables.

As the most abundant carotenoid in the carrot, β -Carotene is often advertised as the direct extraction from the natural source, carrot. The advertisement is owing to a marketing preference for natural food chemicals over synthetic ones. For implementation as the direct antioxidant nutraceuticals, β -Carotene was initially added to food as a food colorant, which is still in use today to attract customers with the need for antioxidants.

Since β -Carotene is an unstable compound, the extraction methods and bioactivity after entering the body are important factors in measuring the nutraceutical's economic efficacy and effectiveness. Several methods have been applied to optimize the extraction yield and purity. This article will compare the commonly used methods and identify the one with maximum yield. Except for the extraction yield, the bioactivity of β -Carotene is also measured by several different measurements. We compared the pros and cons for each of them, chose the most commonly used measurement, and demonstrated its application in different experiments.

The bioactivity of β -Carotene has been verified by *in vivo* test. The antioxidation ability of β -Carotene acts differently, corresponding to the concentration of the compounds and their effective sites. Overall, β -Carotene is an effective antioxidation nutraceutical acting on humans to bring about various beneficial effects across tissues and organs.

2. Sources of β -Carotene

β -Carotene is widely found in green, yellow and red fruits and vegetables and is the most abundant carotenoid. β -Carotene acts as a pigment responsible for the red and orange colouration of fruits and vegetables [1]. Table 1 shows some β -Carotene-rich food sources.

Table 1. β -Carotene contents in different sources [2]

Description	Food group	β Carotene contents ($\mu\text{g}/100\text{ g}$)
Perilla seed oil	Oils and fats	58.56
Sesame seed, black sesame, dried	Nuts and seeds	41.27
Green tea, leaves, dried	Teas	13,671.85
Young barley, powder	Vegetables	17,293.95
Apricot, raw	Fruits	2,280.35

3. β -Carotene Categories

Beta-Carotene is an isoprenoid compound composed of 40 carbons with two C20 geranyl-geranyl diphosphate molecules linking with each other, as shown in figure 1. The natural β -Carotenes are mainly in the trans form, which is highly unstable and prone to oxidation when exposed to oxygen, light and high temperature [1]. As a member of the carotenoid family, which contains more than 600 different carotenoids isolated and identified from the natural resources, β -Carotene is one of the most well-studied and most abundant in the natural resources, while the unstable chemical property of β -Carotene makes the extraction process and extraction yield challenging to maintain [1].

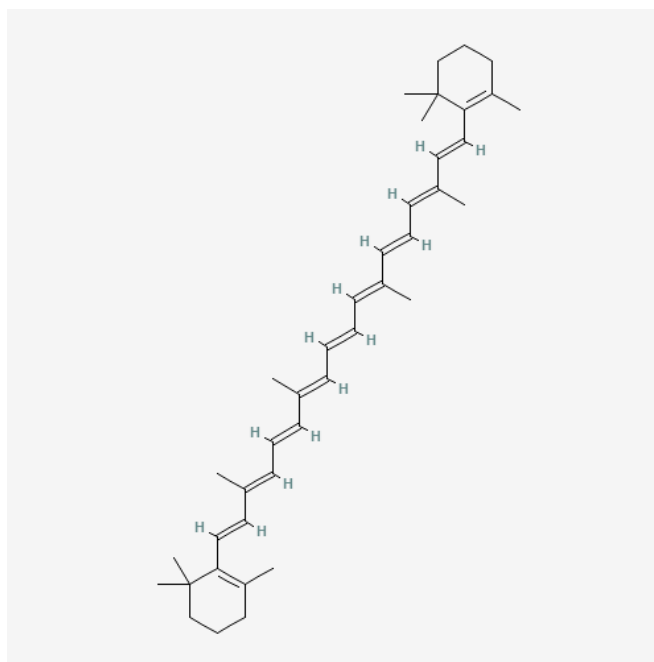


Figure 1. The 2D structure of β -Carotene.

4. β -Carotene as Vitamin A Precursor

The property and function of β -Carotene have been thoroughly studied. β -Carotene is a vitamin A precursor, which is cleaved into vitamin A after being absorbed by the enterocytes located in the gastro-intestinal tract [3]. Vitamin A is critical for human embryonic development and retinal health [4, 5]. A lack of vitamin A can lead to diseases like nyctalopia (night blindness) and xerophthalmia (lack of tears/abnormal dryness) [1]. β -Carotene also acts as a potential antioxidant quenching singlet molecular oxygen, thus protecting the skin from high UV light [6]. In addition, clinical studies have suggested alternative benefits of intaking β -Carotene to minimize risks of developing lung and skin cancer [7]. Alpha-carotene has the same property as β -carotene. However, the conversion efficacy of α -carotene into vitamin A is lower than that of β -Carotene [7].

The bioavailability of β -Carotene in carrots is restricted by the food matrix it is in [6]. The relative bioavailability of β -Carotene in the carrot is 19% comparing to 34% in the purified β -Carotene [8]. However, since β -Carotene is prone to thermal and chemical oxidation, isomerization, and

photosensitization when exposed to oxygen, light and high temperature, it is difficult to manufacture the compound at industrial level without particular modification [1, 9]. Because of the hydrophilic and the temperature-sensitive nature of the β -carotene, coacervation that requires no heat treatment can be a suitable approach to encapsulate the compound [1, 10].

5. Antioxidant Ability of β -Carotene

5.1. Anti-reactive oxygen species

β -Carotene is a singlet oxygen quencher that turns singlet oxygen into triplet oxygen, a generic term for the paramagnetic state of molecular oxygen that is less stable than the normal state of molecular oxygen [11]. Singlet oxygen is reactive oxygen, the excited state of ordinary oxygen [11]. It is continuously produced and buried in the body and plays a role in a variety of physiological and pathological processes. Singlet oxygen can attach to and oxidize proteins, lipids and nucleic acids [11].

The rate and response mechanism of free radical scavenging by β carotene is largely influenced by reactive oxygen species. As a polyunsaturated hydrocarbon, β carotene can effectively block chain radical reactions. The whole energy transfer mechanism is mainly $1O_2 + \beta$ carotene \rightarrow $3O_2 + \beta$ carotene. β carotene reacts with active allylic hydrogen atoms in the form of hydrocarbons [12], which consume free radicals and prevent further reactions from occurring. On the other hand, β carotene can react with peroxy radicals to form β carotene-peroxy radicals [13]. β carotene-peroxy radicals can react with unsaturated fatty acids in an amplification reaction, causing lipid peroxidation, thus exhibiting a pro-oxidative effect. The oxidation products obtained may have a pro-carcinogenic effect and cause damage to the body. The whole mechanism is mainly β carotene + R-OO' \rightarrow β carotene - OO'+LH \rightarrow β carotene + LOOH. This conversion generally occurs under high oxygen pressure conditions. At the same time, there is electron transfer between β -Carotene and different carotenoids, which can lead to interactions between carotenoid molecules in biological membranes. Moreover, carotenoids can show additive effects with VE and synergistic effects when VC is added. Therefore, the combination of β -Carotene with other antioxidants may be more effective in increasing the antioxidant levels in different tissues.

5.2. Anti-oxidation of Oils and Fats

Oxygen is required for the oxidation of oils and fats to occur, and β -Carotene is particularly sensitive to oxygen. When β -Carotene comes into contact with oxygen, it seizes the oxygen from the oil, and oxidation occurs rapidly to varying degrees, forming epoxides, vicinal epoxides, hydroxy carotene and ketones. The formation of free radicals in oils and fats is inhibited, which means that oxidation of oils and fats is avoided.

On the other hand, β carotene has a particularly strong absorption of UV light, filtering out shorter wavelengths such as UV and allowing only longer wavelengths to pass through it [14]. Photo-oxidation is faster than auto-oxidation. Short-wave light generates large amounts of oxygen radicals, which cause peroxidation of oils and fats. Since long wavelength light has little effect on the rate of oxidation of oils and fats, the β carotene in oils and fats has an inhibitory effect on the oxidation of oils and fats.

6. FRAP Assay Most Broadly Used

Ferric ion reducing antioxidant power (FRAP) assay is one of the most commonly used assays in measuring the antioxidant ability of polyphenols in food, beverages, and nutraceuticals [15]. The assay is also widely used in measuring the antioxidant ability of carotenoids [16]. The reducing power of the specimen is determined by the amount of Prussian blue produced. The antioxidant reduces the potassium ferricyanide and then uses the ferrous ions to produce Prussian blue, which has a maximum absorption peak at 700nm. The greater the absorbance value, the greater the reducing

power of the sample. The antioxidant converts ferric in the measuring solution into ferrous, which induces colour change [15]. The absorption value of the final product is measured to calculate the antioxidant ability which is represented as micromole Trolox equivalents (TE) per gram on dried basis (mol TE/g, db) [15]. Antioxidant activities (mol α -TE/mol) of β -carotene isomers and metabolites are given in Table 2 (last column). Table 3 illustrates the FRAP activity of β -carotene.

Table 2. Simple illustration of FRAP assay

Sample	Antioxidant ability (mol α -TE/mol)	Source of data
β -Carotene isomers standardized with respect to α -tocopherol measurement	0.0	[16]
β -Carotene metabolites standardized with respect to α -tocopherol measurement	1.3	[16]

Table 3. Effect of β carotene concentration variables on FRAP assay production

Concentration of β carotene (μ g/mL)	Antioxidant ability (μ M Fe (II)/ μ g)	Source of data
6.71	10.70 \pm 0.24	[17]
3.36	4.27 \pm 0.03	[17]
1.68	1.07 \pm 0.13	[17]
0.84	-1.28 \pm 0.64	[17]

7. Extraction Method of Carotene

According to the results of previous studies, the most commonly mentioned methods for extracting β -Carotene are enzyme-assisted extraction (EAE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and ultrasonic-assisted extraction (UAE). Table 4 shows the comparison of yields of β -Carotene extracted by different methods.

7.1. Enzyme-assisted Extraction (EAE)

Enzyme-assisted extraction selects appropriate hydrolytic enzymes (cellulases, hemicellulases, pectinases, etc.) to increase the yield of the outcome. Each enzyme has its specific function, and these enzymes are used to hydrolyze the cell walls in order to increase their permeability. The enzyme-assisted extraction method is sometimes used to extract β -Carotene from fungi and microalgae, and food flavoring from ginger, pepper, and chili [18].

Merits of enzyme-assisted extraction method are mainly about: (1) extracts with less time and steps; (2) environmentally friendly and renewable; (3) less energy consumption and reduce the use of organic solvent; (4) higher yield; (5) can completely eliminate the solvent when using oil as solvents [19, 20]. In contrast, the drawback is that the costs of enzymes are high, and it needs optimal operating conditions.

7.2. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction uses supercritical CO₂, which fluids at a temperature and pressure higher than its limit as the extracting solvent to extract high purity thermolabile compounds from the solid or liquid matrix. The extraction yield is high since the supercritical CO₂ has a high diffusion coefficient and low viscosity [21].

Goto et al. illustrated that the use of water-miscible solvents can eliminate the requirement of the process of drying in the supercritical fluid extraction method [22]. While sometimes they also need to adjust the polarity of CO₂ in order to match it of the carotenoid, as there is a strong connection between the both of them.

Merits of supercritical fluid extraction include: (1) the use of solvent is non-toxic and recyclable; (2) provides carotenoids with high purity; (3) rapid and efficient [21]. But it is not useful for the polar

carotenoids such as xanthophylls, and for the samples which contain a large amount of water [23]. Besides, the equipment cost will be higher [21].

7.3. Microwave-assisted Extraction (MAE)

The microwave frequencies from 300 MHz to 300 GHz acts on the cell structure of the materials, and the separation of the substance is achieved by adjusting the parameters and heat generation caused by electromagnetic radiation [24]. Although it can extract β -Carotene with high speed through ordinary equipment [25]. It may still cause thermal degradation and cis-trans isomerization of carotenoids [21]. This method can be operated in two situations. One is to extract the target product in open vessels with low temperature and atmospheric pressure, the other is to extract in close vessels with high temperature [24].

7.4. Ultrasonic-assisted Extraction (UAE)

Acoustic cavitation of ultrasound leads to cell disruption, which increases the mass transfer of extractants [21]. Ultrasound from 400 kHz to 2 MHz is redefined to use in UAE to enhance separation. In recent years, ultrasonic-assisted extraction has been frequently used in the extraction of β -Carotene from microalgae and seaweeds. It's not thermal but efficient, but with the aging of ultrasonic probe, it may affect the extraction efficiency [21].

7.5. Other Extraction Methods

Atmospheric liquid extraction (ALE) is a method that uses solvents at a specific temperature and pressure to extract the target compound. The fracture of the cell wall allows the solvent to enter the cell to dissolve carotenoids. This method can provide high extraction yields with simple types of equipment. But it is limited because of its high cost and toxic which may contaminate the final product.

Soxhlet is also a kind of atmospheric liquid extraction, but it needs a large amount of time and solvents in the process of extraction.

Conversely, pressurized liquid extraction (PLE) is a method that extracts compounds at constant high pressure to improve cell permeability and the penetration of the solvents. Strati et al. found that high pressure causes the denaturation of carotenoid-binding protein which improved the yield of carotenoids [26]. After comparing the yield of carotenoid extracted by pressurized liquid extraction and solvent extraction, it was demonstrated that the pressurized liquid extraction method is better than the other one, as it can use less solvent and less time to achieve a similar yield. While solvent extraction is always concerned as its one-off and toxic resources, and it will also cause the greenhouse effect.

Table 4. Extraction methods of β -Carotene.

Extraction method		Extraction yield	Source of data
EAE	1:4 flower: hexane ratio, 0.3% (v/w flower), Viscozyme/HT-Proteolytic, 45°C, 5h, 700 rpm	97%	[27]
SFE	390 bar, 35 mL/min flow rate, 70°C, 190min	90.12%	[28]
SFE	300 bars, 30°C, 90min	47%	[23]
SFE	35 MPa, 50-70°C, combination of 10% water and 10% olive oil or 10% ethanol and 10% olive oil	-	[29]
UAE	76 min, 50°C, 250W	269 mg/100g	[30]
UAE	30°C, 167 W/cm ² ultrasonic intensity, 61.5% duty cycle, 8min	47.10%	[31]
UAE	2:10 carrot: oil ratio, 22.5 W/cm ² ultrasonic intensity, 40°C, 20min	334.75 mg/L	[32]
UAE	ethyl acetate, 200W, 80min	263 mg/100 g DW	[33]
MAE	ethyl acetate, 120W, 25min	262 mg/100 g DW	[33]
MAE	1:1:1 methanol: ethyl acetate: light petroleum, 400W, 50°C, 1 bar pressure, 15min	629 μ g/g	[24]

7.6. β -Carotene Industrial Use

The high bioactivity of β -Carotene and its widespread application affect the development of its industrial use. In the food industry, β -Carotene can be used as a pigment and a nutritional fortification in many products. It is the most widespread and stable natural pigment and is now widely used as an orange-red pigment instead of oil-soluble tar-based pigments. Beta-carotene has been authorized as food additive in the European Union (EU) and has been evaluated previously by the Joint FAO/WHO/Expert Committee on Food Additives (JECFA) in 1966 [34]. The FDA gives β -Carotene GRAS definition and The JECFA established an Acceptable Daily Intake (ADI) of 0-5 mg/kg bw/day. As well as being used in products such as creams, cheeses and mayonnaise, which already contain carotene, β -Carotene has a wide range of uses. It covers almost all shades from red to yellow, as differences in concentration can alter its color. This, together with another advantage: it is an edible oil soluble pigment, making β -carotene quite popular with the food industry and ideal for developing oil-based and protein-based products.

In the pharmaceutical industry, in addition to being used as a coloring agent for tablets, β -Carotene is also used as a vitamin AD medicine. Beta carotene, as a vitamin A supplement, prevents damage to the eye caused by a lack of vitamin A due to slow adaptation to dark vision and damage to the eye caused by bright light after dark vision [1]. It also prevents night blindness, dry eyes, corneal ulcers and corneal softening. Beta carotene is the most active source of theoretical vitamin A. β -Carotene can be stored in the liver and gradually converted to vitamin A according to the body's needs, without causing harm to the body by excess vitamin A. In addition, β -Carotene has a mild but continuous sweating effect and improves blood circulation, thus treating skin disorders such as xerosis cutis and acne [35]. For the adjuvant treatment of cancer, β -Carotene maintains the integrity of microsomal membranes. Chemotherapeutic drugs can kill cancer cells while mutating normal cells, and β -Carotene has an anti-mutagenic effect, thus reducing its toxic side effects.

In clinical applications in medicine, β -Carotene was approved by the US FDA in 1975 for the treatment of erythropoietic protoporphyria, a genetic disorder in which β -Carotene has shown significant efficacy [36]. Although plasma and erythrocyte protoporphyrin levels do not change significantly following treatment with β -carotene, patients' sunlight tolerance increases. The mechanism of action leading to this result is probably based on the quenching of the excited state of the reactive oxygen radical by β -carotene, with the photosensitization reaction being further blocked. Alternatively, the mechanism of action may be that the β -Carotene acts as a barrier by reaching the same maximum absorption spectrum as the porphyrin. In general, patients taking β -Carotene capsules orally, a side effect of the medication is a possible yellowing of the skin during the course of the treatment. However, there are no other adverse effects, and the yellow tinge may also subside spontaneously when the intake of β -carotene capsules is stopped or reduced. In addition, beta-carotene is partially effective in the treatment of other photosensitive skin conditions, like polymorphic sun rash and photosensitive dermatitis. β -Carotene is a promising therapeutic agent for clinical applications with good control effects, few adverse effects and abundant sources. In fact, several dosage forms of β -Carotene are now available for clinical treatment. Another widespread use of β -Carotene is in the treatment of mouth ulcers with its powder, which has also shown good clinical results. A daily intake of β -Carotene has a beneficial effect on the stability of cell membranes. At the same time, carotenoids have hematopoietic functions and can also replenish the body with the blood it needs to quickly mechanize the fibrous tissue inside the surface of ulcers to form capillaries, speeding up blood circulation to improve repair.

In the cosmetics industry, β -Carotene is often used as a bioactive ingredient in facial creams. Beta carotene increases the activity of red blood cells. As peroxy radicals accelerate the ageing process, β -Carotene neutralizes them in the body and thus has a slowing effect on ageing. In addition to protecting skin lesions from oxidation, it also reduces the effects of UV radiation. β -Carotene is a molecule with the right structure to absorb UV light and prevent direct damage to cellular targets. It has not only a preventive molecular effect but also an intercepting and repairing effect [14]. That is to say, it delays the inflammation caused by sun exposure. The addition of β -Carotene to sunscreens

increases the skin's basic defense against UV exposure, providing longer-term protection and helping to maintain skin health and appearance

7.7. Availability of β -Carotene in vivo

The bioactivity of the commercialized β -Carotene needs to undergo in vivo test to prove its availability in living organisms like mouse and humans. In vivo test has been done to prove the intake of β -Carotene in human [37]. Radiocarbon labelled β -Carotene was used to test for the absorption rate [37]. By measuring the urinal C^{14} contain, 84.3% of the β -Carotene has shown to be absorbed by human when taking in β -Carotene supplement [37]. In addition, evidence has shown that the effect sites of β -Carotenes vary across different β -Carotene species [38, 39]. The variation is caused mainly by the carrier differences in different organs [38]. The half-life of β -Carotene also varies according to sites of intake [39]. β -Carotene tends to stay longer in tissues like the testes and liver long than that in plasma [39]. Besides, the quantity of β -Carotene intake also affects its effect as an antioxidant [40]. The experiment has shown that 30 mg/day of β -Carotene supplement intake can effectively improve facial wrinkles and elasticity, increase type I procollagen mRNA transcription, and lower UV-induced thymine liver staining [40]. Overall, β -Carotene functions differently in different tissues, and the antioxidation property of the compound is highly dependent on the amount of daily intake.

8. Conclusion

β -Carotene has been widely used across various industries. It becomes a raising concern to maximize the extraction yield of the compound to optimize the economic benefits and minimize the environmental impact at the same time. Across all the extraction methods identified in different research articles, enzyme-assisted extraction showed maximal efficiency. Therefore, we suggest that future research can mainly focus on the EAE to get the maximum yield. Furthermore, the antioxidation property of β -Carotene makes it suitable for usage across food, pharmaceutical, clinical, and cosmetic industries. The mechanisms of β -Carotene delivering antioxidation function differ by products according to their recipient sites. In addition, the amount of usage and type of β -Carotene used can be optimized to enhance the desired beneficial effect by testing the bioactivity of β -Carotene antioxidation ability in different tissues of different organisms.

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