

Apocarotenoids: Sources, Classification and Their Potential Application as Food Additives

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ABSTRACT

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Apocarotenoids are the degradation product of the enzymatic and or non enzymatic cleavage of carotenoids. Apocarotenoids are present in plants, fungi, bacteria and a certain animal species. There have been large numbers of apocarotenoids identified as the consequence of the great variety of naturally occurring carotenoids. Apocarotenoids can be found as volatile, semi-volatile or non-volatile compounds based on their chemical structures, while based on the number of the carbon atoms contained in their structure, apocarotenoids is classify into triporoids, cyclofarnesoids and methylhexanoids. Bibliometric analysis performed on publication having "apocarotenoids" keywords informs that crocin, picrocrocin, saffron and crocus sativus nodules appear in the overlay visualization. The appearance is attributed to the great potential application and commercialization of crocin which is originated from saffron. The bibliometric analysis also shows that there is food additive nodule appear which imply the strong correlation and potential application of apocarotenoids as food additive. The potential utilization and the commercial production of apocarotenoids are challenged by the the genetic diversity as well as the high cost, time consuming and seasonal production of natural apocarotenoids isolation process. Chemical synthesis, engineering biosynthesis and bio-manufacturing using microbes provide a promising and economical alternative.

Keywords: ocarotenoids, classification, source, bibliometric, food additive

1. INTRODUCTION

Apocarotenoids, a class of terpenoid compounds with less than 40 carbon molecules, are the result of the degradation of carotenoids which occurs through the carbon atoms loss placed at the side chain of the carotenoids structure. The obtained apocarotenoids having shorter fragment than the one of the precursor carotenoids as one or both ends of the carotenoids fragments are cleavage [1]–[3]. The oxidative cleavage of caretonoids can be enzymatic (site-specific) or non-enzymatic (non-site specific) [1].

The enzymatic cleavage generally performed by carotenoid-specific cleavage oxygenases (CCOs), a family of non-heme iron-type enzymes that cleaves double bonds in the carotenoids conjugated carbon chain allowing the formation of specific apocarotenoids [4]. The CCOs including carotenoid cleavage dioxygenases (CCDs)

and 9- cis epoxydioxygenases [3]. CCDs which contain retinal segment epithelial membrane protein (RPE65) domain responsible for binding Fe²⁺ typically act by incorporating oxygen atoms between adjacent carbon atoms along the backbone of the conjugated carotenoid [5]. Moreover, the non-enzymatics oxidation involves the exposure of carotenoids to reactive oxygen species (ROS).

2. APOCAROTENOID SOURCES

Apocarotenoids are present in plants, fungi, and bacteria [4]. Ever since the biosynthesis of carotenoids in animal kingdom is rare, therefore the synthesis of apocarotenoids in animals is only described to perform only in a few species. It is reported that a few arthropods such as pea aphids, spider mites and gall midges are species that have ability to synthesize carotenoids [1], [6]. The phylogenetic analyses imply that numerous modern

aphid species inherited the genes from fungus through the ancestral gene transfer [1], [4], [6], [7]. Pea aphid, *Acyrtosiphon pisum* (*A. pisum*), displays a red-green color. Assay on carotenoids of *A. pisum* revealed that γ -carotene, β -carotene, and α -carotene are the three major carotenoids of green clone of *A. pisum*, whereas the red clone contains red torulene and dehydro- γ,ψ -carotene in addition to the previous three carotenoids [7]. It was then reported that the conversion of carotenoids into apocarotenoids is tailored by the enzymes β -carotene-15,15'-oxygenase (CMOI) and β -carotene-9',10'-oxygenase (CMOII) [1]. Apocarotenoids of animals have different functions such as for energy source and for camouflage as part of defense mechanism [1].

Plant apocarotenoids are present in different types of plastids, such as amyloplasts, leucoplasts, chloroplasts, etioplast and chromoplasts. They are also present in different parts of plant including, seed, fruits, barks, flowers and roots [3], [8]. Apocarotenoids function is diverse and varied including act as repellents, chemoattractants, growth simulators and inhibitors; provide colours to flowers, fruits, and seeds that support plant reproduction and protection from pathogen infections; attract animals for flower pollination and seed dispersal; increases the photooxidative stress tolerance; upregulation of lipid peroxidation; playing important role in plant's response to drought and other abiotic stress conditions; establishing plant's shoot and root architecture; increases the osmolyte accumulation; promotes cell division in root meristems and stimulates lateral root branching; as well as took part in intra-species and inter-species communication [3], [8]–[10].

Fungi such as Arbuscular mycorrhiza [11], *Phycomyces blakesleeanus* [2], [12], *Blakeslea trispora* and *Mucor mucedo* [2] are reported as sources of apocarotenoids. Mycorradicin and blumenol C cellobioside are two types of apocarotenoids found in arbuscular mycorrhizal (AM) maize roots [11]; while trisporic acid, trisporin, trisporol, cyclofarnesine T, (2S,8S,E)-cyclofarnesoid, (2S,8R,E)-cyclofarnesoid are some of apocarotenoids isolated from different cultures of *Phycomyces blakesleeanus*, *Blakeslea trispora* and *Mucor mucedo* [2].

3. APOCAROTENOIDS CLASSIFICATION

To date, there have been more than 850 of naturally occurring carotenoids had been reported [13]. Consequently, the large number of carotenoids results in the great variety of apocarotenoids. Based on their chemical structures, apocarotenoids can be found as volatile, semi-volatile or non-volatile compounds [14].

Volatile apocarotenoids are comprise of β -ionone, pseudoionone, β -Cyclocitral, cis-6 geranyl acetone, 2-hydroxy- β -ionone, neryl acetate, β -Damascenone, geranyl acetate, geranylacetone and 6-methyl-5-hepten-2-one [20], [25]. Farnesylacetone is one example of semi-volatile linear C18 apocarotenoid found in tomato [14]. Non-volatile apocarotenoids identified in tomato comprise of aglycons and glycosylated apocarotenoids. There are 6 aglycons apocarotenoids of tomato are reported, including hydroxy- β -cyclocitral, hydroxy- β -damascenone1, hydroxy- β -damascenone2, hydroxy- β -ionone, 3-hydroxy-5,6-epoxy- β -ionone, and 6-hydroxy-3-oxo- α -ionone [14]. Additionally, six glycosylated hydroxy-apocarotenoids compounds were detected and quantified in tomato which are hydroxy- β -cyclocitral-glucoside, hydroxy- β -damascenone-glucoside, hydroxy- β -ionone-glucoside1, 3-hydroxy-5,6-epoxy- β -ionone-glucoside1, 3-hydroxy-5,6-epoxy- β -ionone-glucoside2, and hydroxy- β -ionone-glucoside2 [14]. Meanwhile, non volatile apocarotenoids are including β -apo-8'-carotenal, β -apo-10'-carotenal, β -apo-12'-carotenal, β -apo-14-carotenal', β -apo-15-carotenal and β -apo-13-carotenone [8]. Additionally, beta-carote-5,6-epoxide, beta-carotene-5,8-epoxide, mutachrome, and aurochrome are other examples of non volatile apocarotenoids [27]. Some example of apocarotenoids and their CCD enzymes involved, precursor carotenoids, sources and biological activities is tabulated on Table 1, while the scheme of the apocarotenoids classification is illustrated on Figure 1.

Table 1. The CCD enzymes involved, precursor carotenoids, sources and biological activities of apocarotenoids

Apocarotenoids	Carotenoids precursor/ CCD enzymes involves	Sources	Biological Activities	References
β -Ionone	β -carotene/ dioxxygenase CCD1	<ul style="list-style-type: none"> • Osmanthus fragrans • Petunia hybrid, Rosa bourboniana • Lawsonia inermis L. • Myrtus communis, Cistus ladanifer • Montpellier cistus • Thevetia peruviana • Fruits: carrot, tomato, apple, plum, melon, raspberry, apricot, 	<ul style="list-style-type: none"> • Anti inflammatory • Cancer preventing • Antibacterial, • Antifungal • Antileishmanial 	[15]
Pseudoionone	β -carotene/ CCD1 lycophene/ CCD1	<ul style="list-style-type: none"> • Tomato • Watermelon 		[16]
β -Cyclocitral	B-carotene/CCD4	<ul style="list-style-type: none"> • Camellia sinensis • Solanum lycopersicum • Oryza sativa • Cyanidium caldarium • Chlorella pyrenoidos • Piper nigrum • Arabidopsis thaliana 		[17]
β -Damascenone	Neoxanthin	Rose Grape	Antispasmodic	[18]
Geranylacetone	β -carotene/CCD1	<ul style="list-style-type: none"> • Fruits i.e tomat, grape, melon • Petunia 	Antitrypanosomal activity	[19]–[21]
Crocin	<ul style="list-style-type: none"> • Crocus/ CCD2, • Gardenia/ CCD4a • Buddleja/CCD4 	<ul style="list-style-type: none"> • Saffron (Crocus sativus) • Gardenia (Gardenia jasminoides) • Buddleja davidii • Nyctanthes arbortristis, 	<ul style="list-style-type: none"> • Anti-tumor • Antioxidant • Anti-inflammatory • Detoxification activities 	[19], [22]
Zaxinone	CCD	<ul style="list-style-type: none"> • Rice 		[23], [24]
β -citraurin	β -cryptoxanthin, zeaxanthin/CCD4	<ul style="list-style-type: none"> • Citrus fruit 		[25]
Anchorene	CCD	<ul style="list-style-type: none"> • Rice • POMocapes caprae 	Antispasmodic	[23], [26]

Moreover, based on the number of the carbon contained in their structure, there are three groups of apocarotenoids including triporoids, cyclofarnesoids and methylhexanoids. Triporoids are apocarotenoids which consists of C18 compounds or 19 (C19) if they contain a methyl ester group [2], [12]. Triporic acid, triporin, triporol and methyl triporate B are triporoids isolated from different cultures of *Phycomyces blakesleeanus*, *Blakeslea trispora* and *Mucor mucedo* as listed and summarized by Borrero et al. [2]. The second group of apocarotenoids which have 15 carbon atoms (C15) is now called as cyclofarnesoids and formerly denominated as apotriporoids. Cyclofarnesine T, (2S,8S,E)- cyclofarnesoid, and (2S,8R,E)-cyclofarnesoid are cyclofarnesoids isolated from *Phycomyces blakesleeanus*, while (3S,5S,8R,E)-cyclofarnesoid U is isolated from *Blakeslea trispora*. The third apocarotenoids type is methylhexanoids which have 7 carbon atoms (C7) and 4-Dihydrocyclofarnesine T is one example of methylhexanoids isolated from *Phycomyces blakesleeanus* [2]. It is indicated that the indicated that the three groups of apocarotenoids derive from three separate fragments of β -carotene [12].

4. APPLICATION OF APOCAROTENOIDS AS FOOD ADDITIVES

As described previously, the diverse type as well as source of carotenoids as the precursor implies on the myriads type of apocarotenoids produced. As seen on Table 1, there are several types of biological activities of apocarotenoids including antispasmodic, anti cancer, anti tumor, anti oxidant, anti bacterial, anti fungal, antitrypanosomal activity as well as antileishmanial [15], [19], [21].

In order to estimate the research trend and the future potential application of apocarotenoids, we perform bibliometric analysis on publication of scopus indexed journal having "apocarotenoids" keywords. The overlay visualization shows that the red nodule represents keywords that appear on the recent date publications. Therefore, we can focus on the keywords of red nodule. There is several interesting information revealed from Figure 2 which shows that crocin, picrococin, saffron and crocus sativus nodules appear bigger than the other ones. The appearance of crocin is might be due to the great commercial value of crocin.

Together with bixin, crocin is stated as the two most important apocarotenoids [3]. Furthermore, interestingly there is red nodule represents food additives in the overlay visualization of keyword occurrence in publication with "apocarotenoids" keywords. Therefore, this review paper was then followed by presenting the correlation and the available information regarding the utilization and potential application of apocarotenoids as food additives. In term of food additives, apocarotenoids is already applied as food colorants. Crocin and bixin are the two most important colorants from apocarotenoids class and widely utilized as additives in food and cosmetic industry [19], [28]. Additionally, crocins and picrococin are water soluble apocarotenoids derived from the cleavage of zeaxanthin at 7,8:7_8_ positions and tailored by a specific carotenoid cleavage dioxygenase enzyme (CsCCD2L) [29]. They are high-value hydrophilic pigments found mainly in *Crocus sativus*, an herbaceous plant known as saffron that belongs to family Iridaceae, and found in a lesser quantity in *buddleja*, and *gardenia* [29], [30]. Bixin, a polyene derived from enzymatic cleavage of the central part of a C40 lycopene, imparts bright red hue to specific tissues of an annatto bush native to Central and South America, *Bixa orellana* L. Bixin is a FDA-approved food additive labeled with E160 b between 2013 and 2018 [28]. However, in general, the commercial production of apocarotenoids is challenged due to the genetic diversity as well as the high cost, time consuming and seasonal production of natural apocarotenoids isolation process. Therefore, there are alternative efforts such as chemical synthesis, engineering biosynthesis and bio-manufacturing using microbes which provides a promising and economical alternative.

5. CONCLUSION

Review conducted on term of apocarotenoids gave us information regarding the definition, synthesis mechanism as well as natural source of apocarotenoids. There a great variety of natural occurring carotenoids which serve as the precursor for the synthesis of apocarotenoids. Therefore, as the consequences there have been large numbers of apocarotenoids identified. Classification of apocarotenoids based on the chemical structures as well as the number of the carbon atoms also presented. Apocarotenoids is classify into volatile,

semi-volatile or non-volatile compounds based on their chemical structures, while based on the number of the carbon atoms contained in their structure, apocarotenoids is classify into triporoids, cyclofarnesoids and methylhexanoids. The review on term of apocarotenoids also completed with bibliometric analysis which performed on publication having "apocarotenoids" keywords. The bibliometric analysis inform that crocin, picrocrocin, saffron and crocus sativus nodules appear in the overlay visualization. The appearance is attributed to the great potential application and commercialization of crocin which is originated from saffron. The bibliometric analysis also shows that there is food additive nodule appear which imply the strong correlation and potential application of apocarotenoids as food additive. Crocin and bixin are the two most important colorants from apocarotenoids class and widely utilized as additives in food and cosmetic industry. However, in general, the commercial production of apocarotenoids is challenged due to the genetic diversity as well as the high cost, time consuming and seasonal production of natural apocarotenoids isolation process. Alternative efforts such as chemical synthesis, engineering biosynthesis and bio-manufacturing using microbes are provide a promising and economical alternative.

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CONFLICT OF INTEREST

No conflict of interest was reported by the author(s).

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Indah Hartati: Conceptualization, Methodology, Investigation, Formal analysis, Writing-original draft

Vita Paramita : Data curation, Formal analysis, Validation, Writing-original draft

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