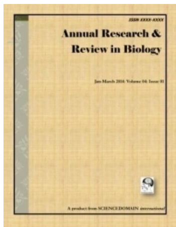

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Studies on Metabolism of Capsanthin and Its Regulation under Different Conditions in Pepper Fruits (*Capsicum spp.*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SNMS wrote the manuscript and performed the literature search. Authors SLT and ZHG designed the review. Author ZAG conceived the idea and author MHA contributed towards managing the analysis of the study. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Capsanthin is a natural pigment gains great attention because it used world widely in the food, cosmetic and dye industries and readily metabolized in the body. Its functions are anti oxidative, anti tumor, anti cancer and have inhibitory effect on colon carcinogenesis in the human body. The demand for capsanthin is increasing day by day in the growing industries, while its supply is low. So it required great attention to increase yield of capsanthin content to meet public demand. In this paper we have focus on chemistry of capsanthin, classical genetics, regulator genes in synthetic pathway and environmental stress. This paper will provide research review ideas for the researchers to improve capsanthin content and fruit quality.

Keywords: Capsanthin; regulation mechanism; capsanthin/capsorubin synthase (Ccs) gene.

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1. INTRODUCTION

Capsanthin (3,3´-dihydroxy-β,k-Caroten-6´-one) is one of major carotenoid of red pepper which accumulates in the thylakoid membranes of chromoplasts in the pericarp of ripe red pepper (Capsicum spp.) fruit and can contribute up to 60% of the total carotenoids [1,2] but its proportion varies among the cultivars in genus capsicum (Table1). It increases proportionally with advanced stages of ripeness of fruit and more stable [3] towards abiotic stress. Capsanthin has also been found in the red tepals (sepals and petals) of Asiatic hybrid lily (*Lilium* spp.) [4], in the fruit of *Berberis spp*. [5] and *Asparagus* spp. [6,7]. Dali, et al. [6] observed that the carotenoids biosynthesis process is same in *Asparagus officinalis* and *Capsicum spp.* It is a natural flavoring and coloring agent throughout the world and normally extracted with solvents from dry red capsicum [8]. Capsanthin production in china is about 2000 tons per year [9]. The interest for application of natural colorants in food products are increasing worldwide because they are more acceptable to consumers and readily metabolized [9]. In recent years, chemically synthesized pigments containing carcinogens has been prohibited gradually in food industry in many countries. Capsanthin as a natural pigment is being applied in food, cake, cosmetics industry. It is important to figure out the metabolic and regulation mechanisms of capsanthin in capsicum fruit and environmental factors how to affect synthesis of capsanthin.

Table 1. Capsanthin concentrations in pericarp of mature fully ripe Capsicum fruits. All concentrations are reported as mean of triplicate extractions (±SE) in mg/g DW [14].

Capsicum spp.	Cultivars	Fruits	Capsanthin	Total
annuum	Nambe	Red	3.03 ± 0.64	10.76 ± 2.54
	NuMex Nematador	Red	4.01 ± 2.86	9.07 ± 2
	Giant Thai	Red	1.15 ± 0.05	6.55 ± 0.34
	Pimiento	Red	2.98 ± 0.77	5.68 ± 0.46
	Andy	Red	0.75 ± 0.04	5.56 ± 0.52
	NuMex Garnet	Red	0.66 ± 0.45	5.34 ± 1.38
	Indian PC-1	Red	0.44 ± 0.12	3.76 ± 0.83
	Sandia	Red	0.56 ± 0.03	3.35 ± 0.56
	Blackbird	Red	0.76 ± 0.27	2.11 ± 0.42
	Big Red Cayenne	Red	0.23 ± 0	1.60 ± 0.15
	Hungarian Apple	Red	0.31 ± 0.05	1.52 ± 0.18
	Sweet Banana	Red	0.09 ± 0.04	0.69 ± 0.39
	NuMex Centennial	Red	0.04 ± 0.02	0.21 ± 0.08
	Gourmet Rainbow	Orange	0.95 ± 0	3.67 ± 0
	Costeno Amarillo	Orange	0.30 ± 0.04	3.52 ± 0.50
	Orange Thai	Orange	0.32 ± 0.07	2.45 ± 0.43
	Oriole	Orange	0.60 ± 0.12	1.44 ± 0.42
	Early Sunsation	Orange	0.13 ± 0.07	0.46 ± 0.23
	Blushing Beauty	Orange	0.14 ± 0.05	0.31 ± 0.01
	Alba	Orange	0.05 ± 0	0.15 ± 0.01
	Mandarin	Orange	0.08 ± 0.02	0.13 ± 0.01
	NuMex Thanksgiving	Orange	0.03 ± 0.01	0.05 ± 0.017
	NuMex Sunglo	Yellow	0.12 ± 0	0.30 ± 0
	Yellow Cheese Pimiento	Yellow	0.10 ± 0	0.31 ± 0
baccatum	Bolivian Yellow	Orange	0.92 ± 0.86	2.50 ± 1.20
chinense	Aji Dulce	Red	0.42 ± 0.22	1.10 ± 0.24
	Jamaican Hot Chocolate	Brown	0.10 ± 0.04	0.89 ± 0.43
	Jamaican Yellow	Yellow	0.21 ± 0.09	1.48 ± 0.70

The molecular formula of capsanthin is $C_{40}H_{56}O_3$ (Fig. 1) molecular weight is 584.871g mol^{-1}, with density 1.012 g ml^{-1} and melting point is 177-178ºC. Capsanthin has a long chain of conjugated double bond ending in one or two polar ketones efficiently absorb green light to give a red-orange hue. The hydroxyl group of ring structure is esterified with fatty acids into monoesters and diesters as well as appearing in the free form [10]. Capsanthin contains eleven conjugated double bonds, a conjugated keto group and a cyclopentane ring [11,12]. In the capsanthin molecule the double bond of the β-ionone ring is outside the polyene chain plane because of repulsion between the hydrogen atoms of the ring methyl groups and the hydrogen atoms of the polyene chain. While the carbonyl double bond in the other head group is very close to planarity with the polyene chain and no repulsion exist here [13].

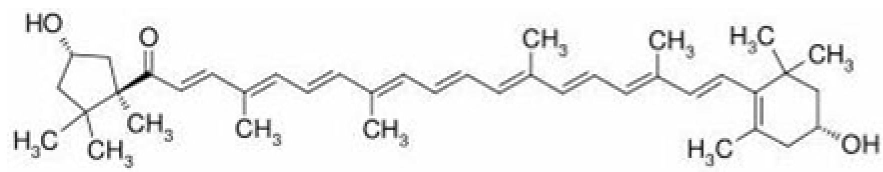

Fig. 1. Molecular formula of capsanthin

Capsanthin is bioavailable in animal after feeding because capsanthin increases apolipoprotein A5 (apoA5) levels and lecithin cholesterol acyltransferase（LCAT） activity in plasma [15,16]. Capsanthin colors the egg yolk when fed to laying hens and the skin of broilers. The capsanthin 16 mg for laying hens and for skin pigmentation of hens 50mg capsanthin kg^{-1} feed is enough [17]. Pigmentation is also one of the important quality attributes of the aquatic animal for consumer acceptability. Carotenoids are responsible for pigmentation of muscle in food fish, skin color in ornamental fish [18] and abdomen and exoskeleton of Pacific white shrimp [19]. Carotenoids can boost antioxidant protection and immune strength and enrich the color of sexual ornaments like feathers in birds. Capsanthin is a fat soluble and enhances liposolubility by esterifying with short-chain saturated fatty acids. Capsanthin is distributed at the polar surface of lipoproteins and its clearance is faster than lycopen in the human body. Pérez-Gálvez, et al. [1] found that the bioavailability of capsanthin from paprika oleoresin is very low in human sample while oleoresin is a suitable sources for the pro-vitamin A carotenoids β-carotene and β-cryptoxanthin and the macular pigment zeaxanthin. The studies showed that dietary capsanthin was absorbed in to the body and distributed to plasma lipoprotein [20] and disappeared from plasma at faster rate than lycopene, while capsanthin is transported in to plasma lipoproteins in larger amounts and may be metabolized in the human body more rapidly than lycopene [21]. In a study of Maeda, et al. [22] obesity is related to various diseases, such as diabetes, hyperlipidemia, and hypertension. Adipocytokine, which is released from adipocyte cells, affects insulin resistance and blood lipid level disorders. Further, adipocytokine is related to chronic inflammation in obesity condition adipocyte cells. They have reported that paprika pigments, which contain high proportion of capsanthin, affect the liver and improve lipid disorders of the blood. They have further suggested that paprika (Capsanthin) ameliorates chronic inflammation in adipocytes caused by obesity, adjusts adipocytokine secretion and affect antimetabolic syndrome diseases. Capsanthin has greater anti-oxidative activity than other xanthophylls [23] which is because of its structure particularly the keto groups by lowering the autoxidation. Capsanthin is regarded as an antipromoter of cancer [24] and anti-tumor-promotion activity, even though it exhibits no pro-vitamin A activity. The epidemiological studies showed that capsanthin have inhibitory effect on colon carcinogenesis. So capsanthin rich foods are helpful to keep healthy [25]. Capsanthin also inhibit the growth and toxic productions of some poisonous fungus. Aflatoxin is among the most potent mutagenic and carcinogenic compounds produced by *Aspergillus flavus* in nature, which is globally health hazard to humans and animals. Capsanthin completely inhibited both the growth and toxin production of *Aspergillus flavus* [26].

Capsanthin is an end product of carotenoids in pepper carotenoids biosynthesis pathway (Fig. 2). The carotenoid (capsanthin) biosynthetic pathway starts from geranylgeranyl pyrophosphate (GGPP). Phytoene synthase (PSY) convert two molecules of GGPP to phytoene. Phytoene is desaturated into lycopene through ζ- carotene, which is catalyzed by two enzymes phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS). The lycopene undergoes a cyclization reaction on both ends by lycopene β-cyclase (LCYB), thus producing β-carotene. β-carotene is then converted to β-cryptoxanthin, zeaxanthin and Antheraxanthin. The reactions are triggered by β-carotene hydroxylase (β-CH) and zeaxanthin epoxidase (ZE) respectively. Antheraxanthin is catalyzed by capsanthin-capsorubin synthase (CCS) to form Capsanthin (Fig. 2).

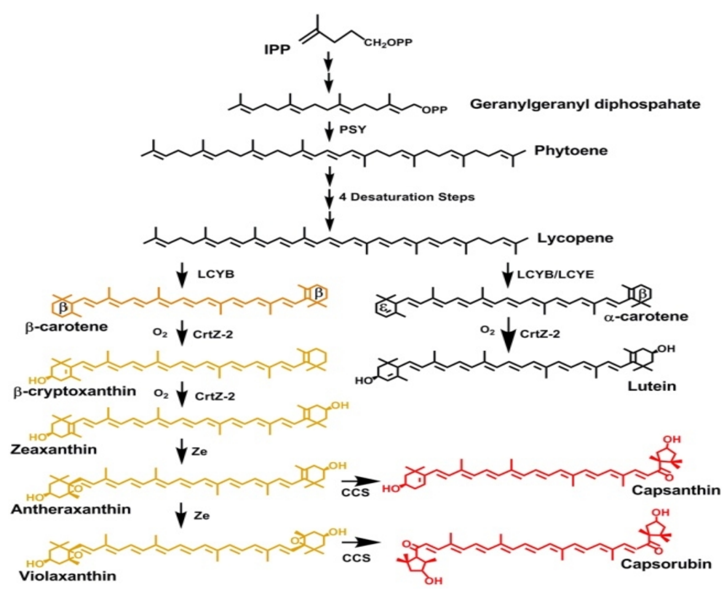

Fig. 2. Biosynthetic Pathway of Carotenoids (Capsanthin) in Capsicum [27]

2. THE RESEARCH OF CAPSANTHIN IN CLASSICAL GENETICS

The studies about the color of the pepper showed that the red, yellow or orange fruit color is control by three loci which are known as c1, c2 and y gene [28]. Kormos and Kormos [29] stated that the red pigment is determined by r^+ and yellow by r. They have mentioned that the precursor gene c^+ is required for the full expression of r^+ and r and in its absence the pigments are formed from the polyenes which accompany chlorophyll, while in the absence of c^+ and chlorophyll, only traces of pigment are formed. Later Lefebvre, et al. [30] find out that the red color is determined by the y+ dominant allele and yellow by y recessive allele. The red color is dominant over white and yellow in the F_1 cross of a red capsicum with a white and yellow capsicum producing only red F_1 progeny and it is controlled by a single gene corresponding to the y locus was subsequently determined to be the capsanthin-capsorubin synthase (*Ccs*) gene. The capsicum orange color is as result from the absence of the *Ccs* gene [31,32]. Thorup et al. [33] used the *Ccs* gene to determine the genotype of capsicums with different fruit colors at the y locus. They have find out that in BC1 segregants from a red × white cross, the red and peach-fruited progenies had the wild type allele at the *Ccs* locus, while the orange, yellow and white-fruited progenies had the mutant allele. Their experimental results showed that the capsanthin-capsorubin synthase (*Ccs*) locus, shown to cosegregate with Y, capsicum fruit color locus, mapped to capsicum chromosome 6.

3. THE RESEARCH ON RELEVANT GENETIC REGULATION IN THE CAPSANTHIN SYNTHESIS PATHWAY

A full understanding of the regulation of the carotenoid (capsanthin) pathway, both structural and regulatory genes is necessary in order to manipulate carotenoid levels in crops. The studies showed that capsanthin is major pigment in the red fruits and synthesized by enzyme capsanthin-capsorubin synthase (CCS), which was purified from a membrane fraction of capsicum fruits by Bouvier, et al. [34]. Expression studies on selected carotenoid structural genes show that pigment-related transcripts are detected as the fruit begins to ripen. Phytoene synthase (*Psy*), phytoene desaturase (*Pds*), and capsanthin/capsorubin synthase (*Ccs*) genes expression are high with high levels of total carotenoid in pepper [3]. In immature capsicum fruits Capsanthin/capsorubin synthase (*Ccs*) gene does not express and â-carotene and lutein are the main carotenoids. Capsanthin/capsorubin synthase (*Ccs*) gene begins to express and increased gradually with color-changed period of capsicum fruits and at the same time, the lutein disappear and capsanthin accumulation begin. A deletion of *Ccs* detected in yellow capsicums was considered to be causal in the fruit phenotype [30]. The capsanthin capsorubin synthase (*Ccs*) gene is specifically expressed during chromoplast development in fruits accumulating ketocarotenoids, but not in mutants impaired [34] in biosynthetic pathway of capsanthin. The chromoplasts indeed differentiate from preexisting chloroplasts while chromoplast development process is entirely independent of the chloroplasts [35]. The transcripts of nuclear genes coding for chloroplast proteins, such as the major chlorophyll a/b-binding protein (encoded by *cab)* and the small subunit of ribulose-l, 5-bisphosphate carboxylase (encoded by *rbcS)* were detected by Kuntz et al. [36] in chromoplast-containing bell pepper leaves, whilst they disappeared in bell pepper fruit chromoplasts.

According to Ha et al. [3] that capsicum yellow color at ripening is not because of deletion of *Ccs* gene but because of the nonsense-mediated transcriptional gene silencing of *Ccs*. Sequence analyses of the *Ccs* gene further revealed two structural mutations in yellow capsicums that may result in either a premature stop-codon or a frame-shift. So as a result the *Ccs* transcript is not detectable in yellow capsicums. A further analysis of the relationship between yellow ripe-fruit color and the capsanthin-capsorubin synthase (*Ccs*) gene in pepper (*Capsicum spp.*) was studied by Li et al. [37]. They have identified a new ccs variant in the yellow fruit cultivar CK7 which has low genetic similarity to other yellow *C. annuum* varieties. In the coding sequence of this ccs allele, they have detected a premature stop codon derived from a C to G change, as well as a downstream frame-shift caused by a 1-bp nucleotide deletion. Guzman et al. [14] cloned and sequenced four carotenoid biosynthetic genes of *Psy, Lcyb, CrtZ-2* and *Ccs* of capsicum cultivars (Valencia, Orange Grande, Oriole, Fogo, Dove, Canary and NuMex Sunset). They observed a new variant of *Ccs* by observing that *Lcyb* and *Ccs* contained no introns but did exhibit polymorphisms resulting in amino acid changes. The Lang et al. [31] find out that in orange color of capsicum phenotype the deletion of amino terminus of CCS is consider being causal. They have crossed red fruit (cv.

227 long) and orange fruit (cv. Ms GTY-1) pepper and observed a polymorphism of *Ccs* gene in the segregation population in the PCR pattern. In the orange fruit they have found a deletion of upstream region of the *Ccs* gene. They have noted in orange fruits that 211-bp of the downstream region of the gene was conserved but no transcript of the *Ccs* gene was detected by RT-PCR. This showed that *Ccs* gene determines the fruit color by changing the carotenoid composition. A team of research confirmed in the capsicum cv. “Fogo” transcripts of *Ccs-3* gene but carrying the mutant *Ccs*-3 allele and no CCS protein accumulated because the premature stop termination in ccs-3 prevented expression of the biosynthetic activity to synthesize the capsanthin and capsorubin. They have also observed that in orange-colored capsicum cultivars (Orange Grande, Oriole and Canary) transcripts for all four (*Psy, LcyB, CrtZ-2* and *Ccs*) of the wild-type carotenogenic enzymes were readily detected yet no CCS protein appeared to be accumulated and no red carotenoids were synthesized. This confirmed that mutations in *Psy* and *Ccs* have been identified as the loci controlling color in the fruit [38]. Xu et al. [39] cloned capsanthin/capsorubin synthase homologous gene from orange (*Citrus sinensis*). The complete sequence is 3788 bp long with a coding sequence of 1512 bp, which encodes a polypeptide of 503 amino acids. The 5' upstream sequence is 1721 bp long and the 3' downstream sequence is 555 bp long. They have mentioned that the amino acid sequence of this gene is 78% and 69% identical to the genes from carrot and pepper, respectively. Kumagai et al. [40] verified that *Ccs* genes regulate capsanthin synthesis by using RNA viral vector containing capsanthin-capsorubin synthase (CCS) cDNA. The cDNA encoding CCS was placed under the transcriptional control of a tobamovirus subgenomic promoter. They have observed that leaves from transfected plants expressing CCS developed an orange phenotype and accumulated high levels of capsanthin (up to 36% of total carotenoids).

The *Psy* gene catalyzes geranylgeranyl pyrophosphate (GGPP) into phytoene. *Psy* and C_2 gene in the genetic model of capsicum fruit located on the fourth chromosome, which was confirmed by RFLP molecular markers and high-performance liquid chromatography (HPLC) test, while this gene is rate-limiting factor of carotenoid synthesis in fruit ripening of capsicum [33]. The orange fruit formation of capsicum is due to decrease activity of *Psy* gene, which leads to reduced accumulation of phytoene. The mechanism leading to the production of yellow color fruit may be not as complex as that leading to orange fruit production. Li et al. [37]. The decrease of *Crtz* gene activity leads to the reduction of â-carotene and its downstream products. Consequently, there is no sufficient substrate available for the next step in biosynthetic pathway of carotenoid and therefore impeding the formation of capsanthin [3].

4. DIFFERENT ENVIRONMENTAL FACTORS AFFECT ACCUMULATION OF CAPSANTHIN

4.1 Light and Capsanthin

Previous studies have showed that light affect the capsanthin synthesis. Light can produce high or lower carotenoids [41]. Lopez et al. [42] investigated highest carotenoids content in the capsicum fruits under the full sun, while lowest in the shade fruits. They have find out that capsanthin synthesis was inhibited in all treatments (white, yellow, red, blue cellophane filter were used for shade). The carotenoids harvest light energy in photosynthesis and protect plants from photosensitized oxidative damage [43]. Carotenoids are to serve as antenna pigments which enable plants to capture photons at wavelengths not efficiently absorbed by chlorophylls [44]. Minguezmosquera et al. [45] experiment showed that during the drying of pepper (cv. Bola) there is a period of carotenogenesis after harvesting. They have mentioned that this biosynthetic period is strongly favor by favored by light, while in darken, this process is not as fast (Table 2). But Carnevale et al. [46] find out that sunlight probably promotes oxidative degradation during drying. Minguezmosquera and Jarengalan [47] find out that the light accelerates the degradation reaction (discoloring) of carotenoids (β-carotene, di-esterified capsanthin and capsanthin) without changing the aspects of the reactions. So discoloring is the first step of the carotenoid degradation. The studies showed that Capsanthin lose their color when exposed to oxidizing agents such as light [48] but capsanthin are more stable than capsorubin and diesters of these xanthophylls are more resistant than monoesters to oxidative degradation [49]. Many authors said that esterified capsanthins are more stable to light than capsanthin itself in paprika powder and paprika extract. During the study of lauroylmyristoyl capsanthin and capsanthin showed no significant differences from each other in photo stability. Esterification makes the xanthophylls more liposoluble and therefore, it is generally believed that the esterified capsanthins accumulate more easily into the lipophilic globules of fruit chromoplastes [50]. The researchers mentioned that when samples contained a significant amount of geometrical isomer of lauroylmyristoyl capsanthin (esterified capsanthin), an additional amount of lauroylmyristoyl capsanthin was generated by photo-irradiation. So the degradation of lauroylmyristoyl capsanthin became delayed with compared to capsanthin [51]. This previous studies showed that biosynthetic period of carotenoid (capsanthin) don't stop after the harvesting of fruits and remain continues during drying of fruits and light accelerate this reaction. Some studies also showed that light accelerates the degradation reaction of carotenoids. So here is a question raised that when the light start the degradation reaction in the carotenoid, which need to be solved for the researchers.

Table 2. The capsanthin concentration (mg/kg of dry matter) during the drying in light and darkness of Var. Bola Peppers [45]

Drying	Capsanthin	Total	Dry-	Capsanthin	Total	Dry-
0	1750.2	3197.0	12.0	1750.2	3197.0	12.0
25	2052.5	3816.9	18.4	1646.3	2889.4	18.5
65	2217.9	4002.9	28.4	2002.4	3455.6	44.7
90	2770.2	4700.6	34.2	2221.3	3669.9	76.2
161	1750.6	3273.8	91.7	2185.5	3720.3	94.5
233	1867.6	3801.3	94.2	2099.6	3539.0	95.0
329	1939.6	3616.0	95.7	1999.0	3538.1	96.1

4.2 Other Abiotic Stress and Capsanthin Accumulation

During field cultivation, plants are exposed to stress from cultural, biological and environmental sources, which often affect plant development, yield and carotenoid metabolism. Plants exposed to less than optimum water can produce less or higher carotenoids levels. Studies have showed that drought could induce fib (fibrillin) gene expression and caused chromoplast to change and to induce carotenoid accumulation. Carotenoids levels also rise when pepper leaves and fruits are wounded. The wound induced transcriptional activation of *fib,* which is light and oxygen dependent [52]. The fibrillins, providing a protein coat to the chloroplast plastoglobules (PGs) and preventing coalescence of the PGs. PGs are oval or tubular lipid-rich structures present in all types of plastid [53]. The salinity level significantly modified the lycopene content in the pepper fruits [54]. A team of researchers find out that the activity of Geranylgeranyl Pyrophosphate (GPP) synthase depends on presence or absence of Mn^{2+} in soil. When soil in plant root zone contains Mn^{2+}, GGPP metabolized towards carotenoid. So Mn^{2+} is very important to plant carotenoid synthesis [55].

4.3 Different Methods of Drying and Dehydration of Capsicum Fruit and Capsanthin Accumulation during Post-harvest

Many factors affect the capsanthin proportion in the pepper apart from maturity, variety, and genetic diversity [45]. Previous studies have showed that drying methods and process are also one of the factors in the capsanthin loss in paprika [56]. Capsanthin quantity is affect by dry methods and milling process (Table 3). The capsanthin concentrations of sun-dried paprika are higher than those of dehydrated paprika. During sun-dried paprika 79.58% capsanthin loss occur, while in dehydrated paprika 85.73%. The decrease of capsanthin concentration may be that drying and milling processes increase the rate of oxidation reaction and also addition of high portion of seeds, salt and oil results dilution to make a more homogeneous product with regard to color and texture [57]. But the red pigments are highly stable, with minimal degradation due to process during the processing of paprika [58]. The studies showed that drying process induces the synthesis of red pigment from their yellow precursors and that new carotenoid biosynthesis is associated with the incomplete maturation of fruit depending on its drying time (Table 2) and temperature [59,47]. Topuz, et al. [60] used different drying methods such as freeze drying, oven drying, and natural convection drying for capsicum drying process. The freeze drying, oven drying techniques have showed decrease carotenoid content, while natural convection drying technique showed increased carotenoid content (Table 4). The protocols of drying methods are:

Gernal: Cut the fresh pepper into small pieces and then ground with seeds into fine puree using a blender and stored at -18ºC in screw caped plastic bottles until use. After thawing they have used Freeze drying (FD), Oven drying (OD), Refractance Window Drying (RWD) and Natural convective drying (NCD) [57].

Freeze drying (FD): Spread paprika purees on 30 × 40cm glass tray, to a thickness of about 1cm and freeze it for -70ºC. Then place the trays in freeze dryer at 40 mmHg for 8 days to dry the purees [57].

Oven drying (OD): (1) Spread paprika purees on 40× 50 cm tray and placed in dryer using parallel air flow at 60ºC and 0.76 m s^{-1} for 7 ± 0.5 h [57].

(2) In this method the pepper fruits are chopped into 9mm square shape. The chopped peppers are dehydrated in a tunnel dryer with a hot air steam for 90 minutes. The temperature of the air at the entrance of the tunnel should be 70ºC. After drying, crush them in the mill to get powder form of the pepper [57].

Refractance Window™Drying (RWD): (Figs. 3a &3b). Spread paprika purees on the conveyor belt of the dryer and move the plastic belt on hot water about 94ºC to dry the purees. The thickness of paprika puree on belt should be about 1mm with velocity of the belt in range 0.45–0.58 m/min [57].

Natural convective drying (NCD): In this method the paprika pods are longitudinally cut into two pieces and dried by natural convection on strings. The drying process is carried out under gloomy condition at room temperature for 8–10 days [57].

Sun-Drying: Most of the farmers traditionally used this method because of low cost. Spread the pepper fruits in the open sun light for 5-7 days. After drying, clean the impurities of the dried fruits before milling. Use sieves (<1 or 1-3 mm) of require particles size to remove the coarse particles and re-mill it [60].

Table 3. Quantitative changes in the capsanthin concentration (mg/kg of dry matter) during the processing of pepper fruit (*Capsicum annuum*) [56]

Variety	Fresh fruit		Dry fruit		Percentage of loss	
	mg/kg	%	mg/kg	%	Drying	Milling
Bola	1885.73	39.92	2796.61±158.10	55.79	-48.30	42.70
Agriaulce	3461.67 ±	48.09	3242.16±198.26	48.48	5.88	50.95

Mean ± standard deviation

Table 4. Capsanthin concentration (Milligrams per Kilograms of Dry Matter) in the fresh red pepper (*Capsicum annuum*) and changes during the drying process

Variety	Drying Methods	Capsanthin	Loss/Increase (%)
Unnamed	Fresh fruit	4005.8±175.9	-------
Turkish	sun-dried	818.12±24.04	79.58 (Loss)
paprika [57]	dehydrated	571.57±14.13	85.73(Loss)
Jalapeno [60]	Puree (not dried)	1297.6b±22.7	------
	Freeze drying	902.1c±59.2	30.48(Loss)
	Oven drying	837.1c±43.5	35.49(Loss)
	Refractance Window™Drying	876.2c±16.3	32.48(Loss)
	Natural convective drying	1494.4a±50.4	15.17 (Increase)

Mean value ± standard error

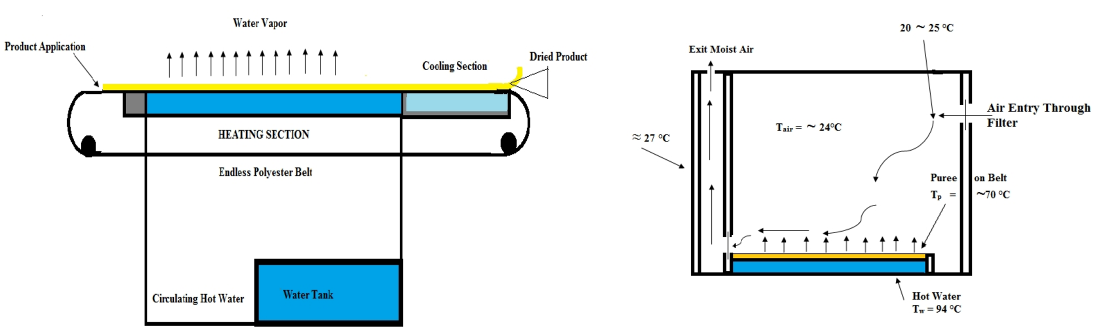

Fig. 3. (a) & (b) Method of Refractance Window™Drying

4.4 Effect of Temperature, Steam and Irradiation during Storage Period on Capsanthin

Storage temperature has a great influence on the quality attributes of pepper [61,62]. The applied temperature and time regime significantly affect the carotenoid content in red pepper during the dehydration process [63]. High temperature darkens the paprika color and decrease the intensity of reddish color which leads to decrease its shelf life [64]. According to Rico et al. [65] experiment the degree of redness of steamed pepper was low and become darker, which showed that capsanthin are degraded with steaming pepper fruits. They have also find out that value of red color slightly increase of steamed pepper if stored in refrigerated conditions but significantly lower than if store at room temperature. Capsanthin accumulated in the pepper fruits if store at 8ºC (not heat treated) and decrease at 4ºC [66]. Irradiation with a with a maximum overall average of 10 kGy is widely use in dried spices for destroying of bacteria and moulds without affecting the quality attributes of different spices, while in Australia and USA, up to 30-kGy dose is allowed [67,68]. The value of redness of irradiated pepper is lower after storage at room temperature then at refrigerated conditions. The red color of dried pepper without irradiation treatment if stored at 4 ± 2ºC is significantly higher than irradiated pepper. So capsanthin content become lower in irradiated dried pepper [65]. The Byeong-Keun et al. [69] findings showed that irradiation degrade the capsanthin pigment of pepper. The concentration of capsanthin 11.1% reduced in the γ-irradiated (10 kGy) paprika [57]. The storage period is more influential in the color changes of red pepper powder [65] and stability of capsanthin in the red pepper fruit [70]. The 10 months storage of paprika reduced 42.1% capsanthin content at ambient temperature [57].

5. CONCLUSION

Capsanthin is one of major red color pigment in carotenoids. It is natural flavoring and coloring agent and used globally in many important industries. It can play important role in the nutrition, health and reproduction of animals. In human capsanthin is regarded as an anti-promoter of cancer, anti-tumor-promotion activity, control of obesity and improve the immune system. Capsanthin is synthesized by enzyme capsanthin-capsorubin synthase (CCS) and accumulate in the pericarp ripe red pepper (Capsicum spp.) fruit, which control by gene namely capsanthin-capsorubin synthase (*Ccs*). Capsanthin biosynthesis and accumulation is influenced by genetic, environmental and some abiotic factors. The capsanthin quantity is also affected during storage, drying and milling process.

6. PROSPECT

The regulation of capsanthin content in the capsicum fruits, the focus should not be only on the crossbreeding and *Ccs* gene but also to be considering other factors. Shan, et al. [71] isolated broccoli orange gene from orange cauliflower (*Brassica oleracea* var. *botrytis*) by using map-based cloning method, and found that *orange* (*Or*) gene can be used to induce accumulation of carotenoids. Their results showed that *Or* gene encodes a plastid-associated protein containing a DnaJ Cys-rich domain. The *Or* gene mutation is due to the insertion of a long terminal repeat retrotransposon in the *Or* allele. *Or* appears to be plant specific and is highly conserved among divergent plant species. They have showed that *Or* is associated with a cellular process that triggers the differentiation of pro-plastids or other non-colored plastids into chromoplasts for carotenoid accumulation. They have showed that *Or* gene can be used as a novel genetic tool to induce carotenoid accumulation in a major staple food crop. They have also mentioned that controlling the formation of chromoplasts is an important mechanism by which carotenoid accumulation is regulated in plants. Kolotilin et al. [72] found that accumulation of carotenoids is mainly associated with the number and size of plastid in tomato fruit during the research of the tomato highpigment 1 (hp1) and highpigment 2 (hp2) mutant genes. Galpaz, et al. [73] found that the carotenoid increase is due to larger plastid size to enhance carotenoid biosynthetic and storage capacity during the research of *zeaxanthin cyclase* (ZEP) gene mutation. Now the question raise that capsanthin synthesis is also related with number and size of the plastid or not? Geranylgeranyl Pyrophosphate (GGPP) is not only the precursor substance of â-carotene or capsanthin synthesis, but also vitamin E, gibberellin and chlorophyll. If a large proportion of GGPP is involved in synthesis of carotenoids, it will certainly affect synthesis of other substances. A question will be raising how to solve the mutual interference phenomenon of the several metabolic regulations. Perhaps we can increase number or size of plastid in capsicum fruit because plant carotenoids (capsanthin) are synthesize in the plastid. It will also be a great significance for improving the capsanthin content and yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Perez-Galvez A, Martin HD, Sies H, Stahl W. Incorporation of carotenoids from paprika oleoresin into human chylomicrons. Br J Nutr. 2003;89:787-93.
2. Suzuki K, Mori M. Carotenoid composition of new cultivar of *Capsicum annuum* during maturation and its high capsanthin content. J Jpn Socr Food Sci Tech, Japanese. 2003;50:324-26.
3. Ha SH, Kim JB, Park JS, Lee SW, Cho KJ. A comparison of the carotenoid accumulation in capsicum varieties that show different ripening colours: Deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. J Exp Bot. 2007;58:3135-44.
4. Yamagishi M, Kishimoto S, Nakayama M. Carotenoid composition and changes in expression of carotenoid biosynthetic genes in tepals of asiatic hybrid lily. Plant Breeding. 2010;129:100-07.
5. Bubicz M. Occurrence of carotenoids in fruits of the genus berberis. Bull Acad Pol Sci Biol. 1965;13:251-5.
6. Deli J, Matus Z, Toth G. Carotenoid composition in the fruits of *Asparagus officinalis*. J Agric Food Chem. 2000;48:2793-96.
7. Deli J, Molnar P, Osz E, Toth G. Analysis of carotenoids in the fruits of *Asparagus falcatus*: Isolation of 5,6-diepikarpoxanthin. Chromatogr. 2000;51:S183-S87.
8. Uquiche E, del Valle JM, Ortiz J. Supercritical carbon dioxide extraction of red pepper (*Capsicum annuum* l.) oleoresin. J Food Eng. 2004;65:55-66.
9. Zhao G, Zhang W, Zhang G. Production of single cell protein using waste capsicum powder produced during capsanthin extraction. Lett Appl Microbiol. 2010;50:187-91.
10. Locey CL, Guzinski JA, Paprika. In Natural food colorants: Science and technology, ed. FJF Gabriel J. Lauro:97–114. New York: Marcel Dekker Incorporated; 2000.
11. Hirayama O, Nakamura K, Hamada S, Kobayasi Y. Singlet oxygen quenching ability of naturally-occurring carotenoids. Lipids. 1994;29:149-50.
12. Narisawa T, Fukaura Y, Hasebe M, Nomura S, Oshima S, Inakuma T. Prevention of n-methylnitrosourea-induced colon carcinogenesis in rats by oxygenated carotenoid capsanthin and capsanthin-rich paprika juice. Proc Soc Exp Biol Med. 2000;224:116-22.
13. Requena A, Ceron-Carrasco JP, Bastida A, Zuniga J, Miguel B. Density functional theory study of the structure and vibrational spectra of beta-carotene, capsanthin, and capsorubin. J Physl Chem A. 2008;112:4815-25.
14. Guzman I, Hamby S, Romero J, Bosland PW, O'Connell MA. Variability of carotenoid biosynthesis in orange colored *Capsicum spp*. Plant Sci. 2010;179:49-59.
15. Aizawa K, Inakuma T. Dietary capsanthin, the main carotenoid in paprika (*Capsicum annuum*), alters plasma high-density lipoprotein-cholesterol levels and hepatic gene expression in rats. Br J Nutr. 2009;102:1760-66.

16. Breithaupt DE, Weller P, Grashorn MA. Quantification of carotenoids in chicken plasma after feeding free or esterified lutein and capsanthin using high-performance liquid chromatography and liquid chromatography-mass spectrometry analysis. Poultry Sci. 2003;82:395-401.
17. Panel EF. Opinion of the scientific panel on additives and products or substances used in animal feed on the request from the european commission on the safety of use of colouring agents in animal nutrition part ii. Capsanthin, citranaxanthin, and cryptoxanthin. EFSA J. 2006;386:1-40.
18. García-Chavarría M, Lara-Flores M. The use of carotenoid in aquaculture. Res J Fisheries and Hydrobiol. 2013;8:38-49.
19. Arredondo Figueroa JL PIR, Ponce Palafox JT, Vernon Carter EJ. Pigmentation of pacific white shrimp (*Litopenaeus vannamei*, boone 1931) with esterified and saponified carotenoids from red chili (*Capsicum annuum*) in comparison to astaxanthin. Revista Mexicana de Ingeniería Química. 2003;2:101-08.
20. Etoh H, Utsunomiya Y, Komori A, Murakami Y, Oshima S, Inakuma T. Carotenoids in human blood plasma after ingesting paprika juice. Biosci Biotech Biochem. 2000;64:1096-98.
21. Oshima S, Sakamoto H, Ishiguro Y, Terao J. Accumulation and clearance of capsanthin in blood plasma after the ingestion of paprika juice in men. J Nutr. 1997;127:1475-79.
22. Maeda H, Saito S, Nakamura N, Maoka T. Paprika pigments attenuate obesity-induced inflammation in 3t3-l1 adipocytes. ISRN inflammation. 2013;2013:763758.
23. Perez-Galvez A, Minguez-Mosquera MI. Degradation of non-esterified and esterified xanthophylls by free radicals. Biochim Biophys Acta. 2002;1569:31-34.
24. Mori T, Ohnishi M, Komiyama M, Tsutsui A, Yabushita H, Okada H. Growth inhibitory effect of paradicsompaprika in cancer cell lines. Oncol Rep. 2002;9:807-10.
25. Kim S, Ha TY, Hwang IK. Analysis, bioavailability and potential healthy effects of capsanthin, natural red pigment from *Capsicum spp*. Food Rev Int. 2009;25:198-213.
26. Thanaboripat D. Control of aflatoxins in agricultural products using plant extracts. KMITL Sci Technol J. 2011;11:35-42.
27. Rodriguez-Burruezo A, Gonzalez-Mas MD, Nuez F. Carotenoid composition and vitamin a value in aji (*Capsicum baccatum* L.) and Rocoto (C-pubescens R. & P.), 2 pepper species from the andean region. J Food Sci. 2010;75:S446-S53.
28. Smith HH-HaPG. Inheritance of mature fruit color in *Capsicum annuum* L. J Hered. 1985;76:211-13.
29. Kormos JKK. The genetic types of the carotenoid system of the red-pepper fruit. Acta Bot Hung. 1960;6:305-19.
30. Lefebvre V, Kuntz M, Camara B, Palloix A. The capsanthin-capsorubin synthase gene: A candidate gene for the y locus controlling the red fruit colour in pepper. Plant Mol Biol. 1998;36:785-89.
31. Lang YQ, Yanagawa S, Sasanuma T, Sasakuma T. Orange fruit color in capsicum due to deletion of capsanthin-capsorubin synthesis gene. Breeding Sci. 2004;54:33.
32. Popovsky S, Paran I. Molecular genetics of the y locus in pepper: Its relation to capsanthin-capsorubin synthase and to fruit color. Theor Appl Genet. 2000;101:86-89.
33. Thorup TA, Tanyolac B, Livingstone KD, Popovsky S, Paran I, Jahn M. Candidate gene analysis of organ pigmentation loci in the solanaceae. Proc Natl Acad Sci USA. 2000;97:11192-97.
34. Bouvier F, Hugueney P, Dharlingue A, Kuntz M, Camara B. Xanthophyll biosynthesis in chromoplasts - isolation and molecular-cloning of an enzyme catalyzing the conversion of 5,6-epoxycarotenoid into ketocarotenoid. Plant J. 1994;6:45-54.

35. Cheung AY, McNellis T, Piekos B. Maintenance of chloroplast components during chromoplast differentiation in the tomato mutant green flesh. Plant Physiol. 1993;101:1223-29.
36. Kuntz M, Evrard J-L, d'Harlingue A, Weil J-H, Camara B. Expression of plastid and nuclear genes during chromoplast differentiation in bell pepper (*Capsicum annuum*) and sunflower (*Helianthus annuus*). Mol Gen Genet. 1989;216:156-63.
37. Li Z WS, Gui X-L, Chang X-B, Gong Z-H. Analysis of the relationship between yellow ripe-fruit color and the capsanthin-capsorubin synthase gene in pepper (*Capsicum* spp.). PLoS ONE. 2013;8.
38. Rodriguez-Uribe L, Guzman I, Rajapakse W, Richins RD, O'Connell MA. Carotenoid accumulation in orange-pigmented *Capsicum annuum* fruit, regulated at multiple levels. J Exp Bot. 2012;63:517-26.
39. Xu CJ, Chen DM, Zhang SL. Molecular cloning of full length capsanthin/capsorubin synthase homologous gene from orange (*Citrus sinensis*). Shi yan sheng wu xue bao. Chineses. 2001;34:147-50.
40. Kumagai MH, Keller Y, Bouvier F, Clary D, Camara B. Functional integration of non-native carotenoids into chloroplasts by viral-derived expression of capsanthin-capsorubin synthase in nicotiana benthamiana. Plant J. 1998;14:305-15.
41. Russo VM, Howard LR. Carotenoids in pungent and non-pungent peppers at various developmental stages grown in the field and glasshouset. J Sci Food Agric. 2002;82:615-24.
42. Lopez M, Ma Emilia Candela, Sabater F. Carotenoids from *Capsicum annuum* fruits: Influence of spectral quality of radiation. Biol Plant. 1986;28:100-04.
43. Palozza PKNI. In In methods in enzymology, ed. P Lester, 213:403. New York: Academic Press. 1992; Number of 403 pp.
44. Bouvier F, dHarlingue A, Camara B. Molecular analysis of carotenoid cyclase inhibition. Arch Biochem Biophys. 1997;346:53-64.
45. Minguezmosquera MI, Jarengalan M, Garridofernandez J. Competition between the processes of biosynthesis and degradation of carotenoids during the drying of peppers. J Agric Food Chem. 1994;42:645-48.
46. Carnevale J, Cole ER, Crank G. Photocatalyzed oxidation of paprika pigments. J Agric Food Chem. 1980;28:953-56.
47. Minguezmosquera MI, Jarengalan M. Kinetics of the decolouring of carotenoid-pigments. J Sci Food Agric. 1995;67:153-61.
48. Biacs PA, Daood HG, Huszka TT, Biacs PK. Carotenoids and carotenoid esters from new cross-cultivars of paprika. J Agric Food Chem. 1993;41:1864-67.
49. Biacs PA, János Bodnár, Ágoston Hoschke, Anna Cs. Pavisa, Hussin Daood, Felix Hajdu, Natalia Kiss-Kutz. Separation and identification of carotenoid-esters in red pepper (*Capsicum annuum*) during ripening. The Metabolism, Structure, and Function of Plant Lipids. 1987;10.1007/978-1-4684-5263-1_23:135-38.
50. Matsufuji H, Nakamura H, Chino M, Takeda M. Antioxidant activity of capsanthin and the fatty acid esters in paprika (*Capsicum annuum*). J Agric Food Chem. 1998;46:3468-72.
51. Goda Y, Nakamura H, Sakamoto SS, Ishikawa K, Maitani T, Yamada T. Photo-stability of coloring constituents in paprika color. J Food Hyg Soc Japan. 1997;38:240-47.
52. Chen HC, Klein A, Xiang MH, Backhaus RA, Kuntz M. Drought- and wound-induced expression in leaves of a gene encoding a chromoplast carotenoid-associated protein. Plant J. 1998;14:317-26.
53. Ytterberg AJ, Peltier JB, van Wijk KJ. Protein profiling of plastoglobules in chloroplasts and chromoplasts. A surprising site for differential accumulation of metabolic enzymes. Plant Physiol. 2006;140:984-97.

54. Navarro JM, Flores P, Garrido C, Martinez V. Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chem. 2006;96:66-73.
55. Dogbo O, Laferriere A, D'Harlingue A, Camara B. Carotenoid biosynthesis: Isolation and characterization of a bifunctional enzyme catalyzing the synthesis of phytoene. Proc Natl Acad Sci USA. 1988;85:7054-8.
56. Minguezmosquera MI, Horneromendez D. Comparative-study of the effect of paprika processing on the carotenoids in peppers (*Capsicum annuum*) of the bola and agridulce varieties. J Agric Food Chem. 1994;42:1555-60.
57. Topuz A, Ozdemir F. Influences of gamma-irradiation and storage on the carotenoids of sun-dried and dehydrated paprika. J Agric Food Chem. 2003;51:4972-77.
58. Minguezmosquera MI, Jarengalan M, Garridofernandez J. Effect of processing of paprika on the main carotenes and eesterified xanthophylls present in the fresh fruit. J Agric Food Chem. 1993;41:2120-24.
59. Minguezmosquera MI, Horneromendez D. Formation and transformation of pigments during the fruit ripening of *Capsicum annuum* cv. Bola and agridulce. J Agric Food Chem. 1994;42:38-44.
60. Topuz A, Dincer C, Ozdemir KS, Feng H, Kushad M. Influence of different drying methods on carotenoids and capsaicinoids of paprika (cv., jalapeno). Food Chem. 2011;129:860-65.
61. Toivonen PMA JRD. Physiology of fresh-cut fruits and vegetables. In Fresh-cut fruits and vegetables: Science, technology, and market, ed. L O:91-125. Boca Raton, Florida CRC Press. 2002;91-125.
62. Watada AE, Ko NP, Minott DA. Factors affecting quality of fresh-cut horticultural products. Postharvest Biol Technol. 1996;9:115-25.
63. Gallardo-Guerrero L, Perez-Galvez A, Aranda E, Isabel Minguez-Mosquera M, Hornero-Mendez D. Physicochemical and microbiological characterization of the dehydration processing of red pepper fruits for paprika production. Lwt-Food Science and Technology. 2010;43:1359-67.
64. Almela L, Nieto-Sandoval JM, Lopez JAF. Microbial inactivation of paprika by a high-temperature short-x time treatment. Influence on color properties. J Agric Food Chem. 2002;50:1435-40.
65. Rico CW, Kim GR, Ahn JJ, Kim HK, Furuta M, Kwon JH. The comparative effect of steaming and irradiation on the physicochemical and microbiological properties of dried red pepper (*Capsicum annum* L.). Food Chem. 2010;119:1012-16.
66. Raffo A, Baiamonte I, Paoletti F. Changes in antioxidants and taste-related compounds content during cold storage of fresh-cut red sweet peppers. Eur Food Res Technol. 2008;226:1167-74.
67. Farag SEA, Aziz NH, Attia ESA. Effect of irradiation on the microbiological status and flavoring materials of selected spices. Z. Lebensm Unters Forsch. 1995;201:283-88.German.
68. Onyenekwe PC, Ogbadu GH. Radiation sterilization of red chili-pepper (*capsicum-frutescens*). J Food Biochem. 1995;19:121-37.
69. Joong-Ho B-KKKTKD-HK. Effects of gamma-irradiation and fumigation on microbial growth, color and absorption properties of dried red pepper during storage. Korean J Food Preserv. 2005;12:48-53. Korean.
70. Morais H, Rodrigues P, Ramos C, Almeida V, Forgacs E, Cserhati T, et al. Note. Effect of blanching and frozen storage on the stability of beta-carotene and capsanthin in red pepper (*Capsicum annuum*) fruit. Food Sci Technol Int. 2002;8:55-59.

71. Shan LJVE, Zhou X, Lopez AB, O'Halloran DM, Cosman KM, Conlin BJ, et al. The cauliflower or gene encodes a dnaj cysteine-rich domain-containing protein that mediates high levels of β-carotene accumulation. The Plant Cell. 2006;18:3594-605.
72. Kolotilin I, Koltai H, Tadmor Y, Bar-Or C, Reuveni M, Meir A, et al. Transcriptional profiling of high pigment-2(dg) tomato mutant links early fruit plastid biogenesis with its overproduction of phytonutrients. Plant Physiol. 2007;145:389-401.
73. Galpaz N, Ronen G, Khalfa Z, Zamir D, Hirschberg J. A chromoplast-specific carotenoid biosynthesis pathway is revealed by cloning of the tomato white-flower locus. Plant Cell. 2006;18:1947-60.

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