

# COLOUR IN FRUIT JUICES – CAROTENOIDS by Ray Mears

The colour and appearance of a fruit juice is an important feature of its anticipated organoleptic quality. Generally, the preference will always be for a bright, fresh looking colour devoid of darkening or browning. Colour in fruit juices arises from the presence of water or oil soluble pigments. In rare cases both classes of pigments can be present, where perhaps the best known example is of Blood Oranges. The water soluble colours occur as Anthocyanins or Betalains while the oil soluble ones are Carotenoids.

Colours in plants exist primarily for photosynthesis and for attracting pollinating insects. The green pigment chlorophyll that occurs in all plants is the principal agent of photosynthesis

This article deals with a third class natural colours, the oil soluble Carotenoids which occur in both fruits and vegetables and are amongst the most widely distributed pigments in plants.

1. Location.

Carotenoids are synthesized by many plants, (and some aphids, spider mites, algae, photosynthetic bacteria, fungi and yeasts), but not by animals. Carotenoids provide colours ranging from yellow to orange to dark red. While Carotenoids are dominant in 15 - 30% of tree species, they are not found in the leaves until the end of summer when they develop. For deciduous trees, carotenoids contribute to the brilliant autumn display of yellow and orange colours. (See also Colours in Fruit Juices (1) – Anthocyanins).

Carotenoid pigments are found in photosynthetic tissues, with the highest concentration of Carotenoids being in the leaves of most green plants. The light energy absorption drives photosynthesis while also protecting chlorophyll from oxidative damage.

As neither humans nor animals can synthesise Carotenoids, they obtain them through dietary ingestion. When in circulation, Carotenoids bind to lipoproteins and enter tissues such as liver, macula, lung, adipose, brain, prostate and skin.

2. <u>Types.</u>

Carotenoids comprise of two categories, Carotenes and Xanthophylls. The Carotenes are pure hydrocarbons and generally of red colour, while the Xanthophylls contain one or more Oxygen component and are yellow in character. There are now over 1200 identified Carotenoids, some of the more commonly occurring being:-

 $\frac{\text{Carotenes}}{\alpha\text{-Carotene}} \\ \beta\text{-Carotene} \\ Lycopene$ 

Xanthophylls

Lutein Zeaxanthin β-Cryptoxanthin

Carotenoids absorb light generally in the 400- 540nm (violet to green) range, resulting in the observed colours of yellow to orange to red. The long chain conjugated double bonds of all Carotenoids are responsible for colour, which is in turn affected by modifications to the basic molecular structure.

See Appendix 1 for further examples and general structures.



#### 3. Occurrence.

Carotenoids are found in many yellow and orange coloured fruits and vegetables as well as dark green vegetables, where the carotenoid colour is masked by the chlorophyll. Despite being lipophilic, carotenoids frequently occur in highly aqueous systems such as fruit and vegetable juices as dispersions or associated with proteins. In fruits, the concentration of Carotenoids is often higher in the peel than in the flesh, for example in oranges Carotenoids protect photosynthetic reactions against damage from a combination of light and oxygen. Carotenes derive their name from the Latin or Greek names for carrot, carota or karotan respectively. Carrots contain one of the highest levels of  $\beta$ -Carotene itself. Carotene was discovered around 1831 but its structure was not fully elucidated until 1930-31. The Xanthophylls are so called from the Greek xanthos for yellow and phyllon for leaves and were discovered as a yellow band in the chromatography of leaf pigments.

Pumpkin	Yams	Avocados	Swiss Chard	Peaches
Sweet Potato	Tomatoes	Tangerines	Watercress	Pistachio
Carrot	Bell Peppers	Corn	Romaine Lettuce	Prunes
Winter Squash	Oranges	Peas	Courgettes	Apples
Apricot	Papayas	Guavas	Brussel Sprouts	Lemons
Kale	Watermelons	Grapefruits	Broccoli	Limes
Spinach	Cantaloups	Asparagus	Kiwi Fruit	Pineapples
Collared Greens	Mangoes	Red Cabbage	Sea Buckthorn	Chanterelles
Turnip Greens	Spinach	Red Bell Peppers	Wolfberry (Goji)	Palm Oil
Vietnamese Gak	Paprika	Parsley	Rosehip	Nectarines
	Summer Squash	Yellow Carrots	Basil	Cauliflower
	Plums	Persimmon	Plantain	Apple

Carotenoids in some fruit and vegetables.

Note: Winter Squash is a generic description which may cover both use as an annual fruit or a vegetable, e.g. Pumpkin and Butternut Squash. Collared Greens includes Cabbage and Broccoli. Vietnamese Gak is a type of perennial melon fruit with high levels of β-Carotene and Lycopene.

#### Some carotenoids distributed in fruits

Fruit	β-Carotene	Lutein	Zeaxanthin	β-Cryptoxanthin	Lycopene	α- Carotene
Acerola	$\checkmark$	$\checkmark$		$\checkmark$		
Apple	$\checkmark$	$\checkmark$		$\checkmark$		$\checkmark$
Cherry	$\checkmark$	✓		$\checkmark$		$\checkmark$
Kiwi	$\checkmark$	✓				
Orange	$\checkmark$	✓		$\checkmark$		$\checkmark$
Рарауа					$\checkmark$	
Peach	$\checkmark$	✓		$\checkmark$		$\checkmark$
Pear	$\checkmark$	✓		$\checkmark$		
Persimmon	$\checkmark$	✓		$\checkmark$	✓	$\checkmark$
Pumpkin	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	✓	$\checkmark$
Red	$\checkmark$		$\checkmark$	$\checkmark$		
Capsicum						
Tomato	$\checkmark$				$\checkmark$	
Watermelon	$\checkmark$				$\checkmark$	

See Appendix2 for pictorial examples.



Note: Lutein and Zeaxanthin are stereoisomers and not always separated in analysis, so Zeaxanthin can be included with Lutein.

β-Carotene	Lycopene	Lutein	Zeaxanthin	β-Cryptoxanthin
(Most studied)	(Bright Red)	(Yellow)		
Pumpkin	Vietnamese Gak	Kale	Kale	Рарауа
Sweet Potato	Tomato	Spinach	Spinach	Mango
Carrot	Carrot	Turnip Greens	Turnip Greens	Orange
Winter Squash	Pumpkin	Summer Squash	Summer Squash	Corn
Apricot	Watermelon	Pumpkin	Pumpkin	Bell Peppers
Kale	Pink Guava	Paprika	Paprika	
Spinach	Pink Grapefruit	Yellow fleshed fruit	Yellow fleshed fruit	
Collared Greens	Рарауа	Swiss Chard	Avocado	
Turnip Greens	Asparagus	Collared Greens		
Рарауа	Red Cabbage	Watercress		
Mango	Red Bell Pepper	Peas		
	Parsley	Romaine Lettuce		
	Autumn Olive	Courgette		
	Sea Buckthorn	Brussel Sprouts		
	Wolfberry (Goji)	Pistachio Nuts		
	Rosehip	Broccoli		
		Persimmon		
		Carrot		
		Corn		
		Avocado		

#### Good dietary sources of Carotenoids. Plant Based.

Good dietary sources of Carotenoids. Animal based.

β-Carotene	Lycopene	Lutein	Zeaxanthin	β-Cryptoxanthin
(Most studied)	(Bright Red)	(Yellow)		
Enriched eggs		Egg Yolk. Animal fat.		Egg Yolk, Dairy product such as Butter.

While there are typically over 40 different Carotenoids identified from dietary intake, over 95% circulating in the blood are accounted for by just 6 of them, Lycopene,  $\alpha$ -Carotene,  $\beta$ -Carotene, Lutein, Zeaxanthin and  $\beta$ -Cryptoxanthin.

#### 4. Properties.

- 4.1 As hydrocarbons, Carotenes are lipophilic while the Xanthophylls are slightly less so due to the presence of Oxygen atoms, mostly in the form of Hydroxy groups. Carotenoids are then essentially insoluble in water, but can become solubilised in the hydrophobic areas of cells by association with proteins.
- 4.2 Carotenoids being antioxidants, or free radical scavengers, have many reported health benefits in humans. (To be an effective antioxidant requires that removal of free radicals results in creation of harmless products or the disruption of free radical chain reactions). In addition,  $\beta$ -Carotene and to a lesser extent  $\alpha$ -Carotene,  $\gamma$ -Carotene and  $\beta$ -Cryptoxanthin are



pre-cursors to Vitamin A, which is essential to health.  $\beta$ -Carotene is also known as Pro-Vitamin A.

(See Appendix 3 for information on Vitamin A, including the  $\beta$ -Carotene content of a range of fruit and vegetables with corresponding dietary Vitamin A contribution). Some claimed health benefits of Carotenoids are:-

Promotes good eye health (Lutein and Zeaxanthin). Probably effective against age related macular degeneration (AMD). Reduces incidence of cataracts and sensitivity to light. Increases the pigmentation of macular tissues and protects eyes from ionising (blue-ultra violet) radiation by absorption of short wavelength light.

Deactivating free radicals by antioxidant effect.

Cancer fighting properties, although mixed results against prostate cancer. (Except lung cancer for smokers and asbestos workers taking high doses from supplements). Anti-inflammatory properties.

Cardiovascular disease prevention e.g. risk of strokes (in men), anti-atherosclerotic. May improve skin health, possible protection against sunburn.

Reduce the risks of metabolic syndromes, such as high blood pressure, cholesterol and sugar, excess fat around the waist. Probably an antioxidant effect.

Some longevity benefits, such as from reduced risk of diabetes and respiratory disease. Part of a complex antioxidant network within cells.

Improves bone density to reduce the risk of osteoporosis.

Reduces liver diseases such as hepatitis and cirrhosis, likely through antioxidant effect.

Carotenoids are absorbed via fats and bile acids. For example, Lycopene from Tomato is far better absorbed after cooking. While Tomatoes have a lower Lycopene content than does Gak, the widespread availability of Tomato products accounts for over 55% of Lycopene intake for many people.

- 4.3 Note: while here are no Dietary Reference Intakes for Carotenoids, epidemiological studies indicate that a total Carotenoid level of <1000mol/L in blood carries an increased risk of chronic disease. However, there are levels suggested for Vitamin A. (See 4.1 and *Appendix 3*).
- 4.4 Since Carotenoids are water insoluble, unlike Anthocyanins and Betanins they are generally less affected by changes in pH over the range normally found in foods. At low pH, Carotenoids can undergo isomerization, giving rise to furanoid pigments and in a change in colour. In highly acidic conditions Carotenoids can undergo degradation, thought to be from ion pairs which then dissociate to form a carotenoid carbocation. Such reactions have been reported in model systems. (Sulphuric acid, pH 3 -3.5).
- 4.5 Carotenoids are subject to thermal degradation, resulting in loss of perceived colour. For example, in one study using 1 % of pumpkin extract as the source of carotenoids in a beverage base, there was a loss of β-Carotene of 30-32% after 42 days storage at 10° or 20°C in the dark. At storage temperatures of 35° or 45°C for 36 days, the loss of β-Carotene was 36 38%. The equivalent figures for Antheraxanthin were 14 19% and 33 -38%. Losses for Lutein, Violaxanthin and Neoxanthin were lower under the same conditions. In a similar study using orange juice, there was little change in total Carotenoids (6.49mg/L) for storage at 20° or 28°C over 32 weeks, but a 15% loss at 45°C over 8 weeks (of storage). However, there was a marked change in colour, with reduction of bright yellowness and appearance of more dull brown colouration. Visible changes occurred after 8 weeks storage at 20°C and after only 1 week at 45°C. Some of the colour changes were thought due to



Carotenoid isomerisation rather than oxidation reactions, with some 19 out of 30 peaks identified and levels of  $\zeta$ -Carotene increasing at all temperatures. Other factors contributing to the changes in colour were considered be those from Maillard reactions.

- 4.6 Carotenoids are also sensitive to light exposure. In the same conditions as in 4.5, but with exposure to light during storage the losses of carotenoids were accelerated. Other studies have, not surprisingly, demonstrated faster degradation of β-Carotene with higher energy light sources, such as those in the 450-485nm region The degradation mechanism involves free radical autoxidation and photo oxidation.
- 4.7 The presence of trace amounts of metals can accelerate the auto-oxidation of Carotenoids, through the formation of Carotenoid free radicals. For example, in the case of iron, there is a change of ferric to ferrous. The reaction is accelerated in the presence of near u/v light.
- 4.8 Dissolved oxygen (e.g. in the beverage of 4.5) can result in loss of Carotenoids through oxidative reactions. The Carotenoids can be protected from oxidation by the addition of another antioxidant, such as Ascorbic Acid which preferentially reacts with oxidative species, undergoing aerobic degradation in the process. Addition of 300ppm of Ascorbic Acid to the model pumpkin beverage base (4.4) before processing showed a 48% decrease after processing, with dissolved oxygen reducing from its' initial saturation level of 7.1 (6.5-7.3) mg/L to 1.6mg/L. Subsequent storage showed a far lower loss of β-Carotene than without the addition of ascorbic acid in both dark and light conditions. The level of ascorbic acid declined slowly during storage through oxidative degradation while protecting the Carotenoids and correlated with levels of dissolved oxygen. After processing and storage for 5 days, all the dissolved oxygen was consumed, meaning less oxygen was available for other reactions involving Carotenoid oxidation. [0.5 mole of oxygen reacts with 1 mole of ascorbic acid]. The results help to explain why products such as Orange Juice better retain their Carotenoid colouration because of the presence of naturally occurring ascorbic acid.
- 4.9 Degradation of carotenoids leads to the formation of many species, including ionones, damascones and damascenones some of which are odiferous and contribute for example to the flavour of black tea, aged tobacco and many fruits. Other degradation products can cause off flavours, such as those found in Carrots in the presence of oxygen. (See *Appendix 4* for a list of some of the large number of intermediate and final breakdown molecules).

4.10 It can be seen that food processing will inevitably alter Carotenoid profiles.

## 5. <u>Uses.</u>

5.1 Carotenoids are widely used as colourants in food, in the UK are regulated by The Food Additives Regulations 2009 S.I. 3238 (as amended).

Specifications can be found in Commission Regulation (EU) No 231/2012 of 9 March 2012.

EC No.	Colour	Colour Index No.	Colour
E 160a	Carotenes	75130	Yellow-Orange to
	(i) Mixed carotenes		Brown
E 160a	(ii) Beta-carotene	40800	Variously described as yellow-orange to orange red.
E 160b	Annatto, bixin, norbixin	75120	Orange
E 160c	Paprika extract, capsanthin, capsorubin		Red
E 160d	Lycopene	75125	Bright to deep Red
E 160e	Beta-apo-8'-carotenal (C30)	40820	Orange-Red to Yellow



E 161b	Lutein
E 161g	Canthaxanthin

40850

Orange-Red to Yellow Violet

In the US, the use of Carotenoids in foods in governed by The US Code of Federal Regulations Title 21 / Chapter I / Subchapter A / Part 73. (Lutein is not permitted as a Food Colour in the US, but may be added to animal feed).

5.2 Historically, highly coloured fruit and vegetable juices or their extracts have been used to add colour to foods. Examples are from pumpkin, carrots, tomatoes and red palm oil, although concomitant addition of flavour can be an issue. Current extraction and purification techniques of Carotenoids from fruits, vegetables or other substrates (eg *Blakeslea trispora*) provide sources of refined natural Carotenoids without the flavour carryover. A range of Carotenoids, notably β-Carotene and β-apo-8'-carotenal, are available as Nature Identical materials from chemical synthesis.

For fat based foodstuffs such as butter or margarine, oil soluble Carotenoid colourants can readily be incorporated. Typically,  $\beta$ -Carotene and Lycopene are used in cheese spreads, yoghurts, soups, sauces, and bakery products, with  $\beta$ -Carotene often added to beverages and ice cream.

For aqueous products, such as fruit juices and soft drinks, the addition of purified Carotenoids presents more difficulty. There are two principal methods of addition, the first being to dissolve the Carotenoid material in a suitable oil, which often involves some application of heat, followed by dispersion into all or a portion of the aqueous phase. The mix is then subjected to high pressure homogenisation, to form an emulsion of evenly distributed fine particle sized oil droplets, typically of 1µm. The second procedure is to use the Carotenoid material pre-distributed on to a solid medium such as gelatin or modified starch as carrier, which allows direct dispersion into the juice or drink without the need for heating. Each method results in a slightly differing colour rendition for the same Carotenoid material. Other more complex methods involve the use of encapsulation, multiple emulsions and nanotube technology.

When used in an essential oil containing soft drink, such as those prepared with citrus fruit comminutes, the stability of the drink itself has a profound influence on the distribution of added Carotenoids. If the citrus essential oil emulsion is not properly stabilised the droplets will migrate upwards during storage, extracting the Carotenoids during their ascent and resulting in the dreaded, intensely coloured oil ring.

5.3 Because of the many health claims made for Carotenoids, there is also widespread use of them in the food supplements industry (Nutraceuticals). In particular the Pro-Vitamin A,  $\beta$ -Carotene is widely used. When ingested,  $\beta$ -Carotene is stored in the liver and body fat and converted as required in the small intestine to Retinol, then by oxidation to Retinal and Retinoic Acid. As noted in 4.2,  $\alpha$ -Carotene,  $\gamma$ -Carotene and  $\beta$ -Cryptoxanthin (the only known Xanthophyll with a  $\beta$ -lonone ring) are also pre-cursors to Vitamin A, but having only one  $\beta$ -lonone ring at the end of their molecules, produce only half the amount of Vitamin A as does  $\beta$ -Carotene. None of the other Carotenoids have any Vitamin A activity. Vitamin A is required in humans for many processes, particularly those involved in vision. Anecdotally, to safeguard knowledge of miniature radar in British aircraft during WW2, the Ministry of Aviation briefed the press that the success of night-time RAF defences was due to the pilots having a diet high in carrots, so maintaining the myth that eating carrots helps to see in the dark.



5.4 Carotenoids also find many applications in the cosmetics industry, such as in sunscreen products where they protect skin and skin lesions against oxidation and UV radiation. Conversely, Cantaxanthin has been promoted as a skin tanning agent in oral ingestion formulations.

5.5 Carotenoids such as  $\alpha$ -Carotene,  $\beta$ -Carotene, Lycopene, Lutein, Zeaxanthin and  $\beta$ -Cryptoxanthin intended for use in food, dietary supplements and pharmaceuticals are often synthetically derived, but there is increasing demand for naturally produced materials. The largest market segment being for natural  $\beta$ -Carotene, particularly for use in pharmaceuticals.

5.6 Carotenoids are also used extensively in animal feedstuffs, where they impart colour for example to egg yolk. Lutein and Zeaxanthin added to corn feed also serve to maintain and protect poultry health in the process. Astaxanthin is used in salmon, trout and sea bream farming and also finds application in ornamental fish.

5.7 The global Carotenoid market was estimated at \$760 million in 2007. Between 2016 and 2021 the market grew by approximately 4% pa and subsequently is expected to grow by around 5% pa, to reach \$2 billion by 2031.

The approximate market split (2022 estimate) was:-

β-Carotene	23.2%
Lutein	21.5%
Astaxanthin	17.3%
Annatto (Bixin & Norbixin)	10.6%
Capsanthin	10.4%
Lycopene	5.85%

The remainder (11.15%) is divided between Canthaxanthin,  $\beta$ -apo-8-Carotenal, Zeaxanthin, and  $\beta$ -apo-8'-Carotenal.

Some 46% is used by the animal feed sector, with 54% by the food, supplements, cosmetics and pharmaceutical industries.

# 6. Extraction/Production.

#### 6.1 Plant Origin.

Historically, extractions have been from the green parts of plants, flowers, fruits, seeds, roots and tubers with the most common sources being carrots. Carrot usage yields predominantly  $\beta$ -Carotene. Extraction of tomatoes provides mainly Lycopene. The process is generally of cleaning, shredding, juice pressing, protein coagulation, sedimentation, centrifugation, organic solvent or CO<sub>2</sub> extraction, deodourisation, evaporation and crystallisation. Extraction efficiency can be improved by firstly fermenting the starting material, drying or greater reduction of particle size to disrupt cell walls. The main disadvantage of production from plant based materials is one of high cost, followed by seasonality. Yields are typically low, with around 40gm of crystalline Carotenoid mix obtained per tonne of carrots. Efforts are being made to increase plant Carotenoid content through genetic modification. Secondary disadvantages are of variable colour and low basic stability.

Other factors affecting extraction efficiency are:

- the choice of solvent, eg hexane extracts non-polar species while acetone extracts the polar species. A mix of acetone, ethanol and hexane is commonly used to extract both types.
- supercritical carbon dioxide (CO<sub>2</sub>) gives a low yield of polar Carotenoids (Xanthophylls) which can be enhanced by the addition of ethanol as a co-solvent. In one study using



optimum conditions of 59°C, 350bar, 15gm/min CO<sub>2</sub> with 15.5% ethanol for/and 30 minutes extraction time resulted in >90% recovery of Carotenoids from 15 different fruits and vegetables.

- the use of microwave and ultrasonic assisted extractions, both which disrupt plant cell walls and break down the associations of Carotenoids with such species as fatty acids and proteins, enhance accessibility of the solvent. Tomato peels subjected to ultrasonic treatment of 100W at 40kHz for 30 minutes at 50°C extracted with n-hexane and acetone in a 3:1 v/v mix yielded a material with total Carotenoids of 261.74mg/g dry weight and a Lycopene content of 166.71mg/gm dry weight).
- the application of cellulolytic and pectolytic enzymes. As an example, a high yield of Lycopene was obtained from industrial tomato waste using ethyl acetate solvent following enzyme treatment. (The yield was of 11.5g Lycopene/gm oleoresin from a 1:1 enzyme mix with a dosage of 0.2ml/gm of tomato waste at 40°C for 5 hours, followed by extraction using ethyl acetate at 5ml/gm substrate for 1 hour).

Processing of fruits and vegetables generates considerable waste material and disposal is both of cost and environmental concern. In particular the wastes from citrus and tomato processing are rich in Carotenoids and could be used for the recovery of the Carotenoids.

6.2 Synthesis.

Some Carotenoids can be produced synthetically at lower cost, better stability and standardised colour levels than their natural counterparts obtained from plants. Synthesis was made possible following identification of molecular structures.  $\beta$ -Carotene was first isolated in 1831 by Wackenroder, but its empirical formula not determined until 1907 by Willstatter and Mieg. The structure of  $\beta$ -Carotene was finally established by Karrer in 1930-31, for which he received a Nobel Prize. The first synthesis of  $\beta$ -Carotene occurred in 1950 (by Karrer and Eugster, Inhoffen *et al* and Milas *et al*) with commercial production started by Roche in 1954. (See *Appendix 5* for further information). There are now many methods known, and beta-Carotene is produced industrially on a large scale. The initial use for the synthetic  $\beta$ -Carotene was mostly for colouring butter and margarine.

The relatively few Carotenoids produced synthetically include Lycopene, Cantaxanthin, Astaxanthin,  $\beta$ -Carotene,  $\beta$ -apo-Carotenal,  $\beta$ -apo-8'-Carotenal and Cytranaxanthin For application in drinks and juices, the most commonly used synthetic (NI) Carotenoids are  $\beta$ -Carotene (or mixed Carotenes) and  $\beta$ -apo-8'-Carotenal. The products are typically supplied as water dispersible powders, with the Carotenoid held on a carrier such as a modified starch or gelatin. Antioxidants such as  $\alpha$ -tocopherol and ascorbic acid are commonly added to prolong the product shelf life.

6.3 Microbiological Origin.

With the increasing interest and pressure to find economically available natural colours and the desire to have 'clean label' declarations for products. (See 'Colour in Fruit Juices [1] – Anthocyanins), there has been much effort to obtain Carotenes from microbiological biosynthesis. Additionally, Carotenoids derived from biotechnology have better bioavailability and greater consumer trust.

Investigations have been carried out based on the use of yeasts, moulds, bacteria and algae. Yeasts in particular have received much attention because of their high growth rate. Industrial production of  $\beta$ -Carotene has centred on the mould *Blakeslea trispora* and the alga *Dunaliella salina*. The process is typically of inoculum multiplication followed by biomass biosynthesis and separation by centrifugation. The material is then dried and pressed to give a yeast/alga mass containing Carotenoids, or is extracted, purified and crystallised to give a solid material. For yeast and mould usage, the required carbon source



can be provided by corn or wheat flour, corn syrup, glucose, fruit, liquid hydrocarbons or by-products of other processes such as glycerol. Use of the algae requires intense sunlight and saline water of around 24% sodium chloride (compared to a seawater average of 3.5%) which must be of low heavy metal concentrations. A typical yield is 30 – 40g dry mass/day/m<sup>2</sup> of tank.

*D. salina* can accumulate up to 15% of  $\beta$ -Carotene (dry cell weight) and *Haematococcus* pluvialis (*H. pluvialis*) can accumulate up to 7% Astaxanthin (dcw) and theses microalgae are used in commercial production of the Carotenoids. From a circular bioeconomy perspective, the reuse of seawater reverse osmosis brine to produce  $\beta$ -carotene from *D. salina* has been successfully investigated. The main commercial supplies of natural  $\beta$ -Carotene are currently from the alga *Dunaliella salina*. *Blakeslea trispora* Thaxt is also used in the production of Lycopene.

Others in commercial production are:-

Microalgae	Product
Chlorella zofingiensis Dönz	Canthaxanthin
Scenedesmus Sp. Meyen	Lutein
Botryococcus braunii Kützing	Echinenone
Tisochrysis lutea Bendif & Probert and	Fucoxanthin
Phaeodactylum tricornutum Bohlin	

# 7 Measurement / Analysis

7.1 As in the case of the Anthocyanin colours, both the intensity and indicative quality is measurable by colourimetric or spectrophotometric techniques. The Carotenoids generally show light absorption in the range 350 – 550nm, depending on the solvent. The wavelength of maximum absorption ( $\lambda_{max}$ ) and the shape of the curve (spectral fine structure) are characteristic of the chromophore. Most Carotenoids show three absorption peaks in their spectra. The more double bonds there are in the Carotenoid, the higher the  $\lambda_{max}$  value. For example, the most unsaturated acyclic Carotenoid, Lycopene, with 11 conjugated double bonds absorbs at the higher wavelengths of 440, 470 and 502nm and is red in colour. Carotenoids need a minimum of 7 conjugated double bonds to show perceptible colour, the 7 conjugated double bond acyclic  $\zeta$ -Carotene has  $\lambda_{max}$  values of 278, 400 and 425nm and is of a light yellow colour. The two Carotenoids that precede  $\zeta$ -Carotene in biosynthesis, Phytoene and Phytofluene with 3 and 5 conjugated double bonds respectively, are colourless, having  $\lambda_{max}$  values at 276, 286 and 297nm and 331, 348 and 367nm respectively. The presence of cyclic end groups in Carotenoids causes steric hindrance in the structure, resulting in a shift of  $\lambda_{max}$  values to lower wavelengths (hypsochromic shift), a reduction in absorbance (hypochromic effect) and a loss of fine structure.  $\beta$ -Carotene has the same number of conjugated double bonds as Lycopene but is yellow-orange in colour with only an inflexion at 425nm and  $\lambda_{max}$  values at 450 and 477nm. The monocyclic  $\gamma$ -Carotene also having 11 conjugated double bonds has  $\lambda_{max}$  values of 437, 462 and 494nm intermediate between Lycopene and β-Carotene and is of red-orange colour. The presence of hydroxy or methoxy groups does not affect the chromophore, so



their influence on spectral characteristics is minimal. The spectra of Lutein, Zeinoxanthin and  $\alpha$ -Cryptoxanthin resemble that of  $\alpha$ -Carotene, while those of  $\beta$ -Cryptoxanthin and Zeaxanthin are identical to that of  $\beta$ -Carotene. Other structural changes in the range of Carotenoids introduce further complexity to their spectra. Consequently, spectral analysis alone is generally insufficient for accurate quantitative analysis of Carotenoids and is also dependent on the solvent used.

- 7.2 Chromatography of Carotenoids is widely used for their separation and identification. The classical open-column (OCC) procedure enables collection of the separated bands for subsequent identification by chemical or physical means. Such chromatography can be used to obtain purified material for use as standards in other forms of analysis. As with light absorption properties, structural differences in the Carotenoids lead to differences in adsorption behaviour. Adsorption affinity is also dependent on the chemistry of the column material, although for the same column packing, the order of elution remains the same for the same solvent. The method is low cost and relatively simple.
- 7.3 While thin layer chromatography (TLC) can be useful for monitoring the progress of analyses for identification purposes, it is not suitable for quantitative analysis.
- 7.3 The generally preferred method for the separation and quantification of Carotenoids is by High Performance (or Pressure) Liquid Chromatography (HPLC) with reversed phase, where the elution is the generally in the reverse order to that when using normal phase OCC. Again, results are dependent on both the column material and solvent system used. Isocratic elution is relatively rapid and uses simple equipment, whereas gradient elution provides both better resolution and sensitivity but requires more sophisticated equipment. Detection of eluted material is usually by u/v-vis absorption, providing absorption spectra with  $\lambda_{max}$  values and curve shape for initial identification and quantification of the Carotenoid. Better absorption characteristics are obtained using a photodiode array detector which captures the spectra online for further comparisons with known standards. The application of mass spectrometry (MS) provides yet further confirmation of separated material from its molecular mass. Increasingly nuclear magnetic resonance (NMR) is being used, particularly in food analysis, to yield definitive structural data for the Carotenoid fractions.
- 7.4 A major concern in any analysis of Carotenoids is their inherent instability. As noted, Carotenoids are susceptible to isomerisation and oxidation and are adversely affected by exposure to heat, light, acids, enzymes, metals and co-oxidation with lipid hydroperoxides, particularly when extracted or isolated from their natural environment. Further, adsorption on to an active chromatographic column material, such as alumina, can promote isomerisation of the normally found *trans*-Carotenoids to *cis*-Carotenoids. For meaningful results, analysis has to be conducted in the shortest time possible and with due regard to all the factors affecting stability.
- 7.5 As with the Anthocyanins, the Carotenoid content of a juice can be used to assess its authenticity. The AIJN has developed standards of identity for a number of fruit juices, given in *Appendix 6*

In conclusion, Carotenoids contribute to the colour of many fruits and have beneficial health properties in their own right. To maintain good colour quality, the processing and storage of fruit juices needs to minimise exposure to all the factors that influence Carotenoid stability, which are broadly the same considerations as for achieving a good quality juice. In dietary term, fruits and their juices are a valuable source of Carotenoids.



#### APPENDICES.

- 1. Structures of some Carotenoids.
- 2. Examples of Carotenoids in some fruit and vegetables. (Ramesh Kumar Saini et al).
- 3. Vitamin A information, with the β-Carotene content of a range of fruit and vegetables and corresponding dietary Vitamin A contribution.
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#### Appendix 1. Carotenoid examples and structures.

Carotenes are a large family of polyunsaturated hydrocarbons with a backbone of 40 Carbon atoms and a variable number of Hydrogen atoms only. Structurally, Carotenes are comprised of tetraterpenes synthesised from four 10 carbon terpene units, which in turn are created from eight 5 carbon isoprene units. The isoprene units are joined end to end to form a conjugated chain and the two central isoprene units are joined head-to-head instead of tail-to-tail, so that the chain has a central symmetry. The two central methyl groups are in a 1,6-positional relationship (methyl groups separated by 6 C atoms) and the remaining non-terminal methyl groups are in a 1,5-positional relationship (methyl groups separated by 5 C atoms).

Some Carotenes have hydrocarbon rings at their terminals (cyclized), either at one or both ends and have various hydrogenation levels, or possess oxygen-containing functional groups. Lycopene and  $\alpha$ -



Carotene are examples of acyclized and cyclized Carotenoids, respectively. The structure of Carotenes gives them their shape, chemical reactivity, and light-absorbing properties. All Carotenes are coloured because of their long chain conjugated double bonds. Those of only Isoprene units are CAROTENES, such as  $\alpha$ -,  $\beta$ -  $\gamma$ -Carotene and Lycopene. Others containing oxygen, usually as hydroxy, epoxy or keto groups are XANTHOPHYLLS, such as Lutein and Zeaxanthin. Oxygen when present at the ionone ring can be esterified with long chain fatty acids to form esterified, or bound Carotenoids. Both groups are collectively (generally referred to) known as CAROTENOIDS. As noted, the Carotenoids  $\beta$ -Carotene,  $\alpha$ -Carotene,  $\beta$ -Cryptoxanthin and  $\gamma$ -Carotene with unsubstituted  $\beta$ -ionone rings have Vitamin A activity, as they can be converted to Retinol.

In biosynthesis, the C<sub>5</sub> primer (of Isoprene units) undergoes successive additions of C<sub>5</sub> units giving the sequence C<sub>10</sub> to C<sub>15</sub> to C<sub>20</sub> compounds. Dimerization of the C<sub>20</sub> compound then yields Phytoene, the first C<sub>40</sub> Carotenoid. Nearly all Carotenoids are biosynthesised from Phytoene, following a series of modifications firstly to Phytofluene then to  $\zeta$ - Carotene then to Neurosprene. For example, Neurosporene is the precursor of Lycopene which itself the precursor of the  $\alpha$  to  $\varepsilon$ -Carotenes.

The two main types of Carotenes found in plants are  $\alpha$ -Carotene and  $\beta$ -Carotene. Lesser amounts of  $\gamma$ -,  $\delta$ -,  $\epsilon$ - and  $\zeta$ - Carotenes also occur. The predominant naturally occurring isomers are in the all *trans* (all-E) configuration. (Minute amounts of *cis-trans* (Z-E) isomers can be found in bread and durum wheats).  $\beta$ -Carotene is a secondary metabolite synthesized by plants, it is a polyene.

The six most prevalent Carotenes in the human diet are:- $\alpha$ - Carotene,  $\beta$ - Carotene,  $\beta$ - Cryptoxanthin, Lutein, Lycopene and Zeaxanthin.

Example Structures.

Isoprene C<sub>5</sub>H<sub>8</sub>.

# Phytoene C<sub>40</sub>H<sub>64</sub>.



Uncyclized  $\psi$ -group at each end. (7,7,8,8',11,11',12,12'-octahydro- $\psi$ ,  $\psi$ -Carotene).

# Zeta-Carotene C<sub>40</sub>H<sub>60</sub>.



Uncyclized  $\psi$ -group at each end. (7,8,7',8'-tetrahydro- $\psi$ ,  $\psi$ -Carotene).

#### Neurosporene C40H58.



Uncyclized  $\psi$ -group at each end. (7,8-dihydro- $\psi$ ,  $\psi$ -Carotene).

# Lycopene C40H56



Uncyclized  $\psi$ -group at each end. ( $\psi$ - $\psi$ -Carotene)

# Beta-Carotene C<sub>40</sub>H<sub>56</sub>.



with numbering sequence.



The two ends of the  $\beta$ -carotene molecule are structurally identical and are known as  $\beta$ -rings.

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**M**<sup>c</sup>DONALD



 $(\beta-\beta-Carotene)$ . Specifically, the group of nine carbon atoms at each end form a  $\beta$ -ring.

# Alpha-Carotene C40H56.



β-ring at one end with an ε- ring at the other. (α-rings do not exist). (β, ε-Carotene).

Differs from  $\beta$ -Carotene in the position of the double bond of the cyclic group at one end of the structure.

# Gamma-Carotene C40H56.



 $\beta$ -ring at one end with a  $\psi$ -group (psi, uncyclized) at the other. ( $\beta$ ,  $\psi$ -Carotene).

Alternative rendition of molecule below to demonstrate ring unfolding.



# Delta-Carotene C40H56.



ε- ring at one end with a ψ-group (uncyclized) at the other. (ε, ψ-Carotene).

Alternative rendition of molecule below to demonstrate ring unfolding.





## Epsilon-Carotene C<sub>40</sub>H<sub>56</sub>.



 $\epsilon$  - rings at both ends. ( $\epsilon$ ,  $\epsilon$ -Carotene).

The naming of the end groups of Carotenoid molecules provides the basis for systematic nomenclature. For example:

CAROTENOID	STRUCTURE
Alpha-Carotene	β, ε-Carotene
Beta-Carotene	β, β-Carotene
Gamma-Carotene	β, ψ-Carotene
Delta-Carotene	ε, ψ-Carotene
<b>Epsilon-Carotene</b>	ε, ε-Carotene
Lycopene	ψ, ψ-Carotene

All of molecular formula  $C_{40}H_{56}$ .

# $\beta$ - Cryptoxanthin C<sub>40</sub>H<sub>56</sub>O.



(3R-β-β-Carotene-3-ol)





# Zeaxanthin C<sub>40</sub>H<sub>56</sub>O<sub>2</sub>.



 $(\beta$ - $\beta$ -Carotene-3,3'-diol).

Lutein and Zeaxanthin are sterioisomers.



# Appendix 2. Carotenoids in some fruit and vegetables.

## Appendix 3. Vitamin A.

Vitamin A is needed by the retina of the eye in the form of retinal, which combines with protein opsin to form rhodopsin, the light-absorbing molecule necessary for both low-light (scotopic vision) and colour vision.



Vitamin A in the human diet is occurs in two varieties. Preformed Vitamin A, Retinol and Retinyl Ester such as Retinyl Palmitate, is derived from animal based foods such as dairy, meat and fish products. Pro-Vitamin A is contributed by four types of Carotenoids largely originating from fruits and vegetables, the most important of which is  $\beta$ -Carotene; all have a  $\beta$ -ionone ring with a retinyl group of an isoprenoid chain attached.  $\beta$ -Carotene itself has two such connected retinyl groups and so provides twice the amount of Vitamin A than do the single  $\beta$ -ionone ring Carotenoids of  $\alpha$ -Carotene,  $\gamma$ -Carotene and  $\beta$ -Cryptoxanthin (which is the only known Xanthophyll with a  $\beta$ -ionone ring). When ingested,  $\beta$ -Carotene is stored in the liver and body fat and converted as required in the small intestine to Retinol, then by oxidation to Retinal and Retinoic Acid.

None of the other Carotenoids have any Vitamin A activity.

To compare the relative contributions of Carotenoids to dietary intake of Retinol, the original (pre 2001) system of International Units (IU) listed the equivalences as:-

1 International Unit = 0.3µg of Retinol  $\equiv$  0.60µg of  $\beta$ -Carotene  $\equiv$  1.2µg of other Provitamin-A Carotenoids.

Or,

1 Retinol Equivalent (RE) = 1µg Retinol  $\equiv$  2µg of  $\beta$ -Carotene dissolved in oil or 6µg in normal food  $\equiv$  12µg other Provitamin-A Carotenoids.

Later, it was found that absorption of Provitamin-A was only half that previously accepted. A new measurement, Retinol Activity Equivalent (RAE) was introduced in 2001 as:-1 Retinol Activity Equivalent (RAE) =  $1\mu g$  Retinol =  $2\mu g$  of  $\beta$ -Carotene dissolved in oil or  $12\mu g$  in

normal food  $\equiv 24\mu g$  of the other three Provitamin-A Carotenoids.

Unit	Retinol	β-Carotene, µg	β-Carotene, μg	β-Carotene, μg	Other pro Vit A
			dissolved in oil.	in normal food.	Carotenoids. µg
1 IU	0.3	0.60µg			
1 RE	1		2	6	12
1 RAE	1		2	12	24

Dietary absorption of Provitamin A is highly dependent on the amount of fat co-consumed.

The US Recommended Daily Allowance (RDA) of Vitamin A varies according to age and differs between males and females. For adult females the RDA is 700 RAE while for adult males it is 900 RAE. European standards set by the European Food safety Authority (EFSA) refer to Population Reference Intakes (PRI) rather than RDA, with the value for female adults set 650 µg RE/day and for adult males 750 µg RE/day.

Supplements of Vitamin A are also available commercially, usually consisting of Retinyl Acetate or Palmitate, others may also contain or be solely of  $\beta$ -Carotene.

To put into perspective the importance of plant based Carotenoids, one US study found that the proportion of the intake of Vitamin A from Carotenoids was typically 26% for men and 34% for women.

 $\beta$ -CAROTENE in Selected Fruits and Vegetables. (USDA). For an adult male at 900 $\mu$ g RAE = 10800 $\mu$ g  $\beta$ -CAROTENE.



		Calculated for			Calculated for
FRUITS	<b>B-CAROTENE</b>	adult Male	FRUITS	<b>B-CAROTENE</b>	adult Male
TROTTS		%RDA/100g	TRONS		%RDA/100g
	MB/ 1008	/010/1/ 2008	Melons.	MB/ 1008	/01/2/ 1008
Apples.	27	0.3	honeydew.	30	0.3
Apricots	1094	10.1	, Mulberries.	9	0.1
Avocados.	62	0.6	Nectarines.	150	1.4
Bananas.	26	0.2	Olives.	198	1.8
Blackberries.	128	1.2	Oranges.	71	0.7
Blueberries.	32	0.3	Orange juice.	33	0.3
Boysenberries.	52	0.0	orange juice.	55	0.0
frozen.	40	0.4	Papayas.	274	2.5
Cherimoya.	2	0.0	Passionfruits.	743	6.9
/ -			Passionfruit,		
Cherries, sour red.	770	7.1	purple, juice.	419	3.9
Cherries, sweet.	38	0.4	Peaches, yellow.	162	1.5
Cranberries.	38	0.4	Pears.	14	0.1
Currants, red and					
white.	25	0.2	Persimmon, Fuyu.	253	2.3
Dates, Medjool.	89	0.8	Pineapples.	35	0.3
Durian.	23	0.2	Plantains.	457	4.2
Feijoa.	2	0.0	Plums.	190	1.8
Figs.	85	0.8	Prickly Pears.	25	0.2
Grapefruit.	552	5.1	Raspberries	12	0.1
Grapefruit juice.	188	1.7	Rhubarb.	61	0.6
Grapes	59	0.5	Rowal	230	2.1
Guavas	374	3 5	Sanote Mamey	82	0.8
lackfruit	61	0.6	Sourson	1	0.0
Jackhan.	01	0.0	Starfruit	1	0.0
Kiwifruits.	52	0.5	(Carambola).	25	0.2
Lemons.	3	0.0	Strawberries.	7	0.1
Limes	30	03	Tamarinds	18	0.2
Lime luice	30	0.3	Tangerines	155	1 4
Loganberries.	50	0.0	rungernies.	100	
frozen.	21	0.2	Tangerine juice.	38	0.4
Mangoes.	640	5.9	Watermelon.	303	2.8
Melons,					-
cantaloupe.	2020	18.7			
		Calculated for			Calculated for
VEGETABLES	β-CAROTENE	adult Male.	VEGETABLES	β-CAROTENE	adult Male.
	µg/100g	%RDA/100g		µg/100g	%RDA/100g
			Peppers, Bell,		
Asparagus.	449	4.2	Sweet Red.	1624	15.0
Beans, Green					
snap, raw.	379	3.5	Potatoes, Sweet.	8509	78.8
Beet Greens, raw.	3794	35.1	Pumpkin, cooked.	2096	19.4
Broccoli.	361	3.3	Spinach.	5626	52.1
Brussel Sprouts,					
raw.	450	4.2	Squash, Butternut.	4226	39.1
Cabbage, Savoy.	600	5.6	Swiss Chard.	3580	33.1
Carrots.	8285	76.7	Tomatoes.	449	4.2
Collards.	2991	27.7	Turnip Greens.	6952	64.4
Kale.	2873	26.6	Watercress.	1914	17.7
Lettuce, Green					
leaf.	4443	41.1			

Peas.

449

4.2



Structurally, Vitamin A can be seen to resemble half of the  $\beta$ -Carotene from which it can be derived, illustrating why  $\beta$ -Carotene has twice the Vitamin A potential of other Carotenoids.

## <u>β-Carotene</u>



<u>Retinol</u>



Breakdown of  $\beta$ -Carotene can be by cleavage from the centre, or as is thought to occur biologically, by breakdown from one end.

## Appendix 4. Some products of Carotenoid degradation.

There are 11 main routes involved in the degradation of Carotenoids.

- I. <u>Oxidation.</u> Reaction products resulting from the influence of oxidising materials can react together resulting in a plethora of new materials. In foods the effects can manifest in a loss of quality such as rancidity, colour change and bioactivity. The incorporation of antioxidants can reduce the effects.
- II. <u>Autooxidation.</u> Autooxidation can occur through contact with air and more than twenty breakdown products have been identified. The mechanism involves the formation of epoxides, carbonyls and various (unidentified) oligomers, then further oxidation resulting in short chain carbonyls, carbon dioxide and carboxylic acids.
- III. <u>Thermal Degradation</u>. In the presence of oxygen, heating can result in the formation of both volatile and larger non-volatile compounds. All double bonds of  $\beta$ -Carotene are capable of being oxidised, usually beginning at the ends and working towards the centre of the molecule. Various radicals are thought to be produced which can react with oxygen to give peroxyls which then further react (propagation reactions) with other Carotenoids. The presence of metals may also catalyse oxidation of  $\beta$ -Carotene.
- IV. <u>Photodegradation.</u> Exposure to light causes degradation, for which a number of mechanisms have been proposed, for example through the formation of Carotenoid radical cations. In some solvents such as chloroform, rapid bleaching occurs from excitation of β-Carotene which immediately reacts with the solvent, forming either a radical by hydrogen removal or a Carotenoid-solvent adduct.



- V. <u>Singlet Oxygen.</u> A similar mechanism to Photodegradation, with not fully understood chemical pathways. For β-Carotene, reaction products have been identified as beta-apo-14' Carotenal, beta-apo-10' Carotenal, beta-apo-8' Carotenal, beta-lonone and beta-Carotene 5,8-endoperoxide.
- VI. <u>Acid.</u> The action of acids can result in the formation of ion pairs, which dissociate to give a Carotenoid carbocation. Studies have been carried out using model systems, such as Carotenoids dissolved in organic solvents with addition of strong acid such as trifluoracetic acid, rather than for more complex food systems.
- VII. <u>Iron.</u> Investigations using ferric chloride have shown to cause degradation of Carotenoids, with the formation a Carotenoid radical cation and ferric reduced to ferrous chloride. The reaction is accelerated in the presence of near-UV to visible light. (The milder oxidising agent iodine can also cause carotenoid degradation resulting in cation radicals).
- VIII. <u>Free Radicals.</u> In foods, oxidation of fats and other reactions can produce free radicals and curiously, Carotenoids can act as either anti- or pro-oxidants in such circumstances dependant on the Carotenoid, solvent polarity and oxygen concentration.
- IX. <u>Electron Transfer.</u> Neutral Carotenoids such as β-Carotene, Canthaxanthin, Zeaxanthin, Astaxanthin and Lycopene have been found to form radical cations, as described in VII and VIII, by electron transfer reactions with tryptophan radical cations.
- X. <u>Hydrogen Abstraction.</u> Similarly, neutral Carotenoids reacting with a radical can have a hydrogen atom removed to create a resonance stabilised Carotenoid radical, which in turn may react with the original radical to form non-radical compounds.
- XI. <u>Adduct Formation</u>. Reaction of Carotenoids with radicals can also form radical adducts.

The degradation of Carotenoids leads to the formation of many species, such as ionones, damascones and damascenones which are odiferous, contributing for example to the flavour of black tea, aged tobacco and many fruits.

Oxidation is the major cause of Carotenoid degradation, resulting ultimately in the formation of short chain molecules such as acetaldehyde, 2-methylpropanol, butanol, diacetyl and pentanol. Epoxides such as the apo-Carotenoids are also formed and isomerization from the *trans* to the *cis* forms of molecules can occur. All Carotenoids react rapidly with oxidising agents and free radicals.

Most studies have been conducted on model systems, with far less carried out in food systems.

Oxidation	
Autooxidation	beta-Carotene-5,6-epoxide, 13-cis-beta-Carotene, 9-cis-beta-Carotene,
	<i>trans</i> and <i>cis</i> -beta-Carotene -15,15'-epoxide, long chain di-oxygenated
	Carotenes, dihydrofurans, beta-Carotene-5,6,5',6'-diepoxide, beta-
	Carotene-5,8-epoxide, beta-Carotene-5,6,5',8'-diepoxide, Carotene-5,8-
	endoperoxide, beta-apo-8'-Carotenal, beta-apo-12'-Carotenal, beta-apo-
	13-Carotenal, beta-apo-14'-Carotenal, Retinal, Beta-Ionylidene

Some products formed during the degradation of Carotenoids.



	Acetaldehyde, beta-lonone, beta-lonone-5,6-epoxide, beta-cyclocitral,				
	dihydroactinidiolide, 4-oxo-beta-lonone, 4-oxo-carbonyls, 4-oxo-13-				
	dihydroactinidiolide, 2,2,6-trimethylcyclohexanone, 1,1,6-				
	trimethyltetrahydronaphthalene				
Thermal degradation	beta-apo-13-Carotenal, beta-apo-15-Carotenal, beta-apo-14'-Carotenal, beta-apo-12'-Carotenal, beta-apo-10'-Carotenal, beta-apo-8'-Carotenal, beta-Carotene-5,6-epoxide, beta-Carotene-5,6,5',6'-diepoxide, beta- Carotene-5,6,5',8'-diepoxide, beta-Carotene-5,8-epoxide, polyene carbonyl, 13,13'-di-cis-beta-Carotene, 9,13'-di-cis-beta-Carotene, 15,-cis- beta-Carotene, 13,-cis-beta-Carotene, 9,9'-di-cis-beta-Carotene, 9,-di- cis-beta-Carotene, beta-Carotene-4-one, mutatochrome [5,8-epoxy-β- carotene], autochrome, 2,6,6-trimethylcyclohexanone, 2-methyl-2hepten- 6-one, 2-hydroxy-2,6,6-trimethylcyclohexanone, beta-cyclocitral.				
Photodegradation	neutral Carotenoid, free Radical adduct.				
Singlet Oxygen	neutral Carotenoid, beta-Carotene-5,8-endoperoxide, beta-Carotene-				
	5,6-epoxide, beta-apo-14'-Carotenal, beta-apo-10'-Carotenal, beta-apo-				
	8'-Carotenal, beta-Ionone.				
Acid	Carotenoid radical cations <sup>+</sup> , deprotonation, Carotenoid dimers and				
	didehydrodimers, Carotenoid radical dications <sup>2+</sup> .				
Iron					
Free Radicals					
Electron transfer	5,8-endoperoxide beta-Carotene, Carotenoid radical cations.				
Hydrogen abstraction	Resonance stabilised Carotenoids, non-radical compounds.				
Adduct formation	mation 12-formyl-11-nor-beta-Carotene, 15'-formyl-15-nor-beta-Carotene, 19-				
	oxomethyl-10-nor-beta-Carotene, 5,6-epoxy-5,6-dihydro-beta-Carotene,				
	13,15'-cyclo-12,15-epoxy-11,12,15,15'-tetrahydro-beta-beta-Carotene,				
	beta-Carotene-5,6-epoxide, 15',13-epoxyvinylene-13,15'-dihydro-14,15-				
	dinor-beta-beta-Carotene, 11,15'-dihydrooxepin-beta-beta-Carotene,				
	12-formyl-11-nor-beta-beta-Carotene, 15'-formyl-15-nor-beta-beta-				
	Carotene.				

#### Appendix 5. Synthesis of β-Carotene.

There are many methods for the synthesis of  $\beta$ -Carotene, by joining together 2 or more molecules of symmetric or non-symmetric carbon skeletons, for example  $C_{16} + C_8 + C_{16}$  and  $C_{25}+C_{15}$  respectively. The two principal routes are based on the Wittig and the Grignard reactions. In the Wittig reaction, alkenes are produced from aldehydes and ketones. One such route combines two phosphonium salts each of  $C_{15}$  with one dialdehyde of  $C_{10}$ , followed by isomerization to give symmetric molecules of  $C_{40}$  chains.  $\beta$ -Carotene, Lycopene and Astaxanthin are amongst those Carotenoids produced. The method can be represented as  $C_{15} + C_{10} + C_{15}C_{15} + C_{10} + C_{15}$ . Another Wittig route utilises a  $C_{16} + C_8 + C_{16}$  combination. The synthetic  $\beta$ -Carotene produced by BASF uses the Wittig procedure, combining  $C_{20+}C_{20}$  molecules. The Grignard reaction involves organometallic compounds to produce the  $C_{40}$  structure from two methanol molecules with one diketone molecule.  $\beta$ -Carotene made by synthesis offered commercially by Roche is produced by a Grignard reaction, which can be represented as  $C_{19} + C_{2} + C_{19}$ .



## Appendix 6. Some AIJN Standards for Carotenoids in fruit juices.

JUICE	Total Carotenoids mg/L	Hydrocarbons, as β-Carotene. % of Total Carotenoids	Carotenoid esters. % of Total Carotenoids	Xanthophyl ester. % of Total Carotenoids
ORANGE	Normal 2 – 5 Max 15	Normal 0.5 – 5 Max 5	Normal 6 – 10 Max 15	Normal 6 – 10 Max 15
MANDARIN	Normal 10 – 25	Normal 3 - 8 Max 10	Min 20	Max 13
PASSIONFRUIT	Min 7 Max 28	Min 30 Max 70	Max 12	

Note: Values for Orange do not apply to Blood Orange.

#### By way of comparison, Standards for Carrot are:-

	Total Carotenoids mg/L	Hydrocarbons, as β-Carotene. % of Total Carotenoids	β-Carotene mg/Kg	
CARROT	Min 30 Max 300 Special var 600	Min 50 Normal 70 - 80	Min 20 (Purees 50) Max 250	

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#### Appendix 8. Regulatory

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