Food and Agriculture Organization of the United Nations

84th JECFA - Chemical and Technical Assessment (CTA), 2017

BETA-CAROTENE-RICH EXTRACT FROM *DUNALIELLA SALINA*

Chemical and Technical Assessment (CTA)

Prepared by Kristie B. Laurvick

1. Summary

This Chemical and Technical Assessment summarizes data and information relevant to Beta-Carotene-Rich Extract from *Dunaliella salina* submitted to the 84th meeting of the Joint FAO/WHO Expert Committee on Food Additives (Committee) as well as published information. The product is extracted from the algae using an essential oil, which is subsequently removed and replaced by a vegetable oil.

Beta-Carotene-Rich Extract from *Dunaliella salina* is produced by *Dunaliella salina*, an extreme halotolerant alga, which inhabits natural and man-made salt lakes and ponds. The carotene-rich algae is harvested and concentrated, and the carotenoids are extracted using an essential oil rich in d-limonene. The resulting extract is saponified, purified, centrifuged, evaporated and finally mixed with a vegetable oil to obtain a commercial product with a carotene content of about 30% by weight. β-Carotene accounts for more than 95% of the carotene content of the extracted material as a mixture of trans and cis isomers in a ratio of approximately 2:1 by weight. The remainder of the carotene content includes α-carotene, lutein, zeaxanthin and cryptoxanthin. In addition to the colour pigments and vegetable oil used for standardization, d-limonene extracts of D. salina contain lipids and other fat-soluble components naturally occurring in the source material, such as fatty acids, long-chain alcohols, alkenes and waxes. The composition of these fat-soluble components is primarily a mixture of fatty acids common to vegetable oils used in foods..

Carotenes from natural sources were reviewed at the 18th, 31st and 35th meetings of the Committee. At the 35th meeting, the Committee assessed data for several carotene preparations from supposedly different algae of the genus *Dunaliella* and concluded that it was not possible to evaluate them individually or read across the dataset. At its 41st meeting, the Committee was informed that there was only one species being used for commercial purposes: *Dunaliella salina*. The previously used names *D. bardawil* and *D. kone* are synonyms for *D. salina*. The Committee assessed two different algal carotene preparations produced from this species: (1) Spray dried concentrated, lyophilized or dehydrated preparations of *Dunaliella salina*; and (2) Vegetable oil extract of *Dunaliella salina*.

At the 41st meeting, the Committee adopted specifications for Carotenes (Algae) which are obtained by solvent extraction of the dried *Dunaliella salina* (syn. *D. bardawil* and *D. kone*) [JECFA, 1993]. These specifications were not adopted by Codex Alimentarius because the 41st Committee meeting did not allocate an ADI to Carotenes (Algae).

Beta-Carotene-Rich Extract from *Dunaliella salina* is intended for use in a variety of food categories and beverages as a colour. The intended use levels range from 20 mg/kg to 1200 mg/kg, depending on the food category.

2. Description

Carotenoids are natural pigments, which are synthesized by plants and are responsible for the bright colours of various fruits and vegetables. Many different carotenoids are present in the foods we eat, and most of these have antioxidant activity. The most common carotenoid, beta-carotene, consists of a highly branched, unsaturated chain with identical substituted ring structures at each end (NOSB 2012).

Beta-Carotene-Rich Extract from *Dunaliella salina* occurs as an opaque, deep red, viscous suspension that is insoluble in water and soluble in oils. A solution of β-carotene in cyclohexane shows absorption maxima at approximately 448 – 457 and 474 – 486 nm. A spot of a solution of β-carotene in toluene (approximately 0.4 mg/mL) on filter paper turns blue 2-3 minutes after application of a spray or drop of a 20% solution of antimony trichloride in toluene.

Beta-carotene is widely distributed in nature; its various roles include provitamin A activity, absorption of light energy, triplet chlorophyll and singlet oxygen quenching, antioxidant activity, oxygen transport, and general colouration of many different organisms including plants and algae. Some plants and red-orange foods owe their typical colour principally to beta-carotenes (Shariati & Hadi 2011), including citrus fruits, carrots and tomatoes.

3. Manufacturing

Natural carotenoids are obtained from the alga *Dunaliella salina* which is grown in large salt water lakes under the combination of high salinity, high light intensity and high temperature and with the addition of fertilizers to promote *D. salina* growth.

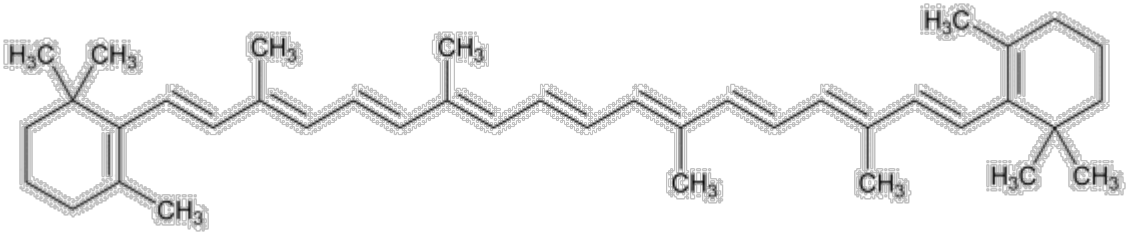
The primary recovery process begins when the maximum number of *Dunaliella salina* cells per ml (containing maximum levels of beta-carotene per cell) is reached. Harvested algae are concentrated using mechanical methods. The concentrated algae are extracted with a natural essential oil (e.g. from orange or citrus) containing d-limonene that is suitable for use in foods. The extract is decanted to remove most of the solids from the essential oil extract. The resulting primary algal concentrate is saponified and purified by washing followed by centrifugation to remove residual water. After the extract is centrifuged, it is evaporated to increase the beta-carotene concentration.

After further evaporation, food grade vegetable oil (e.g. soybean oil, olive oil, sunflower oil) or medium chain triglyceride oil is added to replace the essential oil previously used for extraction. The product may be further homogenized with particle size reduction via ball milling and standardized to a specified carotenoid content with vegetable oil.

4. Chemical characterization

Beta-carotene has the formula of $C_{40}H_{56}$, a molecular weight of 536.9, eleven conjugated double bonds and a typical dark red colour. It has been assigned Chemical Abstract Service (CAS) number 7235-40-7. Numerous isomers of beta-carotene are naturally occurring and present in Beta-Carotene-Rich Extract from *Dunaliella salina*, but the major carotenoid component is all-*trans*-beta-carotene, as depicted in Figure 1.

Figure 1. Structural formula of all-trans-ß-Carotene



The Beta-Carotene-Rich Extract from *Dunaliella salina,* prepared as above, is > 95% beta-carotene (isomers including all-*trans*-, 9-*cis*-, 13-*cis*- and one of the three di-*cis*-beta-carotenes), of which about 65% is all-*trans*-beta-carotene. Lower amounts of *cis*-beta-carotene, alpha-carotene, lutein, zeaxanthin and cryptoxanthin may also be present, as well as a small amount of unknown carotenoids. Unknown carotenoids are present at levels less than 1.5% individually and 3% as a sum. Beta-Carotene-Rich Extract from *Dunaliella salina* consists mainly of beta-carotene and the vegetable oil.

The manufacturing process enriches lipids and other fat-soluble components such as fatty acids, long-chain alcohols, alkenes or waxes. The non-pigment part of the extract consists of lipids of which the carrier vegetable oil contributes between 35 to 50%, and the remaining 20 to 35% are carried over from the algae primary extract. Included in the typical composition of Beta-Carotene-Rich Extract from *Dunaliella salina* are the following components: D-limonene (< 0.3%), chlorophyll (< 200 mg/kg), phytol (approximately 2%), 8-heptadecene (approximately 7 – 9%), and ash (< 1%). The 8-heptadecene, $C_{17}H_{35}$ (C17:1) is a decarboxylated oleic acid (C18:1) and the presence in algae is described in literature (Satoh et al. 2010, Zhou et al. 2012). Phytol is an acyclic diterpene alcohol. Often, it occurs esterified to chlorophyll. In the saponification step during production, much of the chlorophyll is separated but a small amount of both chlorophyll and phytol remain in the final product.

Literature describes the presence of fatty acids in *Dunaliella salina*. Most of them are simple fatty acids with C16 and 18 carbons and the total amount of unsaturated fatty acids is higher than saturated fatty acids (Ben-Amotz et al. 2009, Fakhry & Maghraby 2013, Can et al. 2016). Full characterization of a lipid extract continues to present challenges to analytical chemistry: substances are extracted based on their solubility in nonpolar solvents. Solubility depends on the properties of components e.g. presence or absence of functional groups, chain length, and molecular structure. Most chromatographic techniques are based on such features and are not suitable if differences between the components are small. In addition, any analytical method employing higher temperatures such as GC/MS may result in thermal degradation and generation of artefacts.

Dunaliella salina is not known to synthesise any toxic secondary metabolites of concern that could be carried over into the finished product. Minerals and heavy metals will remain in the water phase and the saponification step will hydrolyse any polar lipids such as phospholipids and chlorophylls, reducing possible chelating agents for metal cations.

5. Proposed specifications

The proposed specifications are based on the manufacturing process of Beta-Carotene -Extract from *Dunaliella salina* and differ from specifications for synthetic β-carotene or other extracts. The assay is intended to define the content of total carotenes (calculated as β-carotene) by spectrophotometry at not less than declared in the final product. Purity specifications include limits for arsenic (not more than 1 mg/kg), lead (not more than 2 mg/kg) and d-limonene (not more than 0.3%). The analytical

methods for the proposed specifications for total carotenes calculated as β-carotene, arsenic, and lead are based on general tests for identity and purity published in the Combined Compendium of Food Additive Specifications, Vol. 4.

The proposed method for the determination of d-limonene is a gas chromatographic (GC) method which utilizes a flame ionization detector. The content of limonene is determined using a series of standard limonene solutions with terpinene added as an internal standard.

Specifications proposed for content of total carotenes calculated as β-carotene and data supporting the composition of individual carotenoids present in Beta-Carotene-Rich Extract from *Dunaliella salina* are also based on analyses performed using a high-pressure liquid chromatographic (HPLC) technique run on samples of the finished product under typical conditions for analysis of carotenoids using a C30 bonded silica based reversed-phase column optimized for carotenoid analysis. Standard reference materials for β-carotene, α-carotene, lycopene, lutein and β-cryptoxanthin are used for the determination. Peak areas are recorded and calculated at 450 nm for all carotenoids except lycopenes for which 472 nm are used. The method is used to quantify the proportion (by area %) of single carotenoids including some isomers but not for absolute quantification.

6. Functional use

Beta-Carotene-Rich Extract from *Dunaliella salina* is intended for use as a food colour in foods and beverages including cider, malt beverages, water-based flavoured drinks, margarines, cheeses, cake fillings, custards, yogurts, processed nuts, pre-cooked pastas and noodles and other products. Intended use levels range from 20 mg/kg to 1200 mg/kg, depending on the food item or category. The intended uses and use levels of Beta-Carotene-Rich Extract from *Dunaliella salina* in food categories of the Codex General Standard for Food Additives are provided in Appendix 1.

7. Reactions and fate in foods

Carotenoids are highly unsaturated compounds, thus degradation is mainly due to oxidation, which is accelerated by temperature, metal presence, light and enzymes, and it is reduced when antioxidants are added. Carotenoids can fade in the presence of oxygen resulting in an inactive, colourless oxidation product (Melendez-Martinez et al. 2004). Carotenoids are stable between 2 to 8 pH ranges, but degrade rapidly under high heat and / or direct sunlight. They are often supplied in oil dispersions rather than in crystalline form in order to reduce the amount of degradation caused by exposure to oxygen, heat, or light. Carotenoids supplied in oil dispersion are also less susceptible to damage from handling during transportation. Stability of commercial formulations of 30% Beta-Carotene-Rich Extract from *Dunaliella salina* in olive oil (held at 25°C) was evaluated for a period of five years. The results consistently indicated that beta-carotene was stable in these products for the entire study period.

Although carotenes have a long history of use in food applications, information about the applications of Beta-Carotene-Rich Extract from *Dunaliella salina* in food, is not available, however general information about the use of carotenes from other sources have been found which is representative and applicable to Beta-Carotene-Rich Extract from *Dunaliella salina*.

In food, the main causes of carotenoid degradation are various oxidative reactions mainly involving oxygen, hydroperoxides and peroxy- radicals, and anaerobic thermal degradation (principally) beta-carotene leads to formation of volatile aromatic hydrocarbons. It has also been reported that coloured

degradation products may arise from the degradation of trans-beta-carotene during extrusion cooking, and that reversible stereoisomerization was deemed important in the formation of both non-oxidized volatiles and oxidation products. Since many of the non-volatile degradation products of beta-carotene possess chemically reactive groups such as carbonyls, they are likely to react with amino acids and/or their degradation products during the thermal processing of foods (Scotter & Castle 2004).

Studies on specific foods, such as the stability and nutrient contribution of beta-carotene on selected bakery products (yellow cake, sugar cookies, and bagels), showed that pre-baking processing steps had no adverse effect on the stability or isomeric distribution of carotene. Carotene losses during baking ranged between 20% - 30%. During baking, the all-*trans* isomer was reduced from 91% to 85.77% and 74%. No significant additional losses or isomeric transformations occurred during the typical shelf life of the products, and serving of each product provided about 1mg of beta-carotene after processing (Rogers et al. 1993).

8. References

Ben-Amotz et al. 2009: Ben-Amotz A, Polle J E W, Subba Rao D V, 2009. The alga Dunaliella. Biodiversity, physiology, genomics and biotechnology / editors, Ami Ben-Amotz, Jürgen E.W. Polle, D.V. Subba Rao. Enfield, N.H.: Science; Plymouth: NBN [distributor].

Fakhry & Maghraby 2013: Fakhry E M, Maghraby D M E, 2013. Fatty Acids Composition and Biodiesel Characterization of *Dunaliella salina*. Journal of Water Resource and Protection, 2013, 5, 894-899.

JECFA 1993: Evaluation of certain food additives and contaminants (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.

Melendez-Martinez et al. 2004: Melendez-Martinez A J, Vicario I M, Heredia F J, 2004. Estabilidad de los pigmentos carotenoides eri los alimentos. Archivos Latinoamericanos de Nutricion, Vol 54 No 2, 2004.

NOSB 2012: Beta-carotene technical evaluation report. Technical Services Branch for the USDA National Organic Programelem.

Rogers et al. 1993: Rogers D E, Malouf R B, Langemeier J, Gelroth J A, Ranhotra G S, 1993. Stability and Nutrient Contribution of β-Carotene Added to Selected Bakery Products. Cereal Chem. 70(5):558-561.

Satoh et al. 2010: Satoh A, Kato M, Yamato K, Ishibashi M, Sekiguchi H, Kurano N, Miyachi S, 2010. Characterization of the Lipid Accumulation in a New Microalgal Species, Pseudochoricystis ellipsoidea (Trebouxiophyceae). Journal of the Japan Institute of Energy, 89, 909-913 (2010).

Shariati & Hadi 2011: Shariati M, Hadi M R, 2011. Microalgal Biotechnology and Bioenergy in Dunaliella. In Angelo Carpi (Ed.): Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications.
Available online at:
http://www.intechopen.com/books/progress-in-molecular-and-environmental-bioengineering-from-analysis-and-modeling-to-technology-applications/microalgal-biotechnology-and-bioenergy-in-dunaliella.

Scotter & Castle 2004: Scotter M J, Castle L, 2004. Chemical interactions between additives in foodstuffs: a review. Food Additives and Contaminants, Vol. 21, No 2 (February 2004), pp. 93–124.

Appendix 1

Codex Alimentarius Global Standard Food Additives, version 12.08.2016
Levels for Beta-Carotene-Rich Extract from *Dunaliella salina* in the different food categories derived from the group "Carotenoids" which includes e.g. beta-carotene, synthetic (160a(i)) and beta-carotene from *Blakeslea trispora* (160a(iii)), complemented by food categories allowed for carotenes (vegetable) (160a(ii)) with usually lower levels

Food category number	**Food category name**	**mg/kg**
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, whey based drinks)	150
01.3.2	Beverage whiteners	100
01.4.4	Cream analogues	20
01.5.2	Milk and cream powder analogues	100
01.6.1	Unriped cheese	100
01.6.2.1	Ripened cheese, includes rind	100
01.6.2.2	Rind of ripened cheese	500
01.6.2.3	Cheese powder (for reconstitution; e.g., for cheese sauces)	100
01.6.4	Processed cheese	100
01.6.5	Cheese analogues	200
01.7	Dairy-based desserts (e.g., pudding, fruit or flavoured yoghurt)	100
02.1.2	Vegetable oils and fats	25
02.1.3	Lard, tallow, fish oil, and other animal fats	25
02.2.1	Butter	25
02.2.2	Fat spreads, dairy fat spreads and blended spreads	35
02.3	Fat emulsions mainly of type oil-in-water, including mixed and/or flavoured products based on fat emulsions	200
02.4	Fat-based desserts excluding dairy-based dessert products of food category 01.7	150
03.0	Edible ices, including sherbet and sorbet	200
04.1.2.3	Fruit in vinegar, oil, or brine	1000
04.1.2.4	Canned or bottled (pasteurized) fruit	200
04.1.2.5	Jams, jellies, marmelades	200
04.1.2.6	Fruit-based spreads (e.g., chutney) excluding products of food category 04.1.2.5	500
04.1.2.7	Candied fruit	200
04.1.2.8	Fruit preparations, including pulp, purees, fruit toppings and coconut milk	100
04.1.2.9	Fruit-based desserts, including fruit-flavoured water-based desserts	150
04.1.2.10	Fermented fruit products	500
04.1.2.11	Fruit fillings for pastries	100
04.2.2.1	Surface-treated fresh vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	500
04.2.2.2	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	1000

04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds in vinegar, oil, brine, or soybean sauce	50
04.2.2.4	Canned or bottled (pasteurized) or retort pouch vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), and seaweeds	50
04.2.2.5	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g., peanut butter)	50
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g., vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5	50
04.2.2.7	Fermented vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweed products, excluding fermented soybean products of food categories 06.8.6, 06.8.7, 12.9.1, 12.9.2.1 and 12.9.2.3	50
05.1.3	Cocoa-based spreads, including fillings	100
05.1.4	Cocoa and chocolate products	100
05.1.5	Imitation chocolate, chocolate substitute products	100
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	100
05.3	Chewing gum	100
05.4	Decorations (e.g., for fine bakery wares), toppings (nonfruit) and sweet sauces	100
06.3	Breakfast cereals, including rolled oats	100
06.4.2	Dried pastas and noodles and like products	200
06.4.3	Pre-cooked pastas and noodles and like products	1200
06.5	Cereal and starch based desserts (e.g., rice pudding, tapioca pudding)	150
06.6	Batters (e.g., for breading or batters for fish or poultry)	500
07.1.2	Crackers, excluding sweet crackers	1000
07.1.3	Other ordinary bakery products (e.g., bagels, pita, English muffins)	100
07.1.4	Bread-type products, including bread stuffing and bread crumbs	200
07.1.5	Steamed bread and buns	100
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	100
08.1.2	Fresh meat, poultry, and game, comminuted	100
08.2	Processed meat, poultry, and game products in whole pieces or cuts	100
08.3.1	Non-heat treated processed comminuted meat, poultry, and game products	20
08.3.1.1	Cured (including salted) non-heat treated processed comminuted meat, poultry, and game products	100
08.3.1.2	Cured (including salted) and dried non-heat treated processed comminuted meat, poultry, and game products	20
08.3.1.3	Fermented non-heat treated processed comminuted meat, poultry, and game products	20
08.3.2	Heat-treated processed comminuted meat, poultry, and game products	20
08.3.3	Frozen processed comminuted meat, poultry, and game products	100
08.4	Edible casings (e.g., sausage casings)	100
09.1.1	Fresh fish	300
09.1.2	Fresh mollusks, crustaceans, and echinoderms	100

09.2	Processed fish and fish products, including mollusks, crustaceans, and echinoderms	100
09.2.3	Frozen minced and creamed fish products, including mollusks, crustaceans, and echinoderms	100
09.2.4.1	Cooked fish and fish products	100
09.2.4.2	Cooked mollusks, crustaceans, and echinoderms	100
09.2.4.3	Fried fish and fish products, including mollusks, crustaceans, and echinoderms	100
09.2.5	Smoked, dried, fermented, and/or salted fish and fish products, including mollusks, crustaceans, and echinoderms	100
09.3	Semi-preserved fish and fish products, including mollusks, crustaceans, and echinoderms	100
09.3.1	Fish and fish products, including mollusks, crustaceans, and echinoderms, marinated and/or in jelly	100
09.3.2	Fish and fish products, including mollusks, crustaceans, and echinoderms, pickled and/or in brine	100
09.3.3	Salmon substitutes, caviar, and other fish roe products	100
09.3.4	Semi-preserved fish and fish products, including mollusks, crustaceans, and echinoderms (e.g., fish paste), excluding products of food categories 09.3.1 - 09.3.3	100
09.4	Fully preserved, including canned or fermented fish and fish products, including mollusks, crustaceans, and echinoderms	100
10.1	Fresh eggs	1000
10.2	Egg products	100
10.4	Egg-based desserts (e.g., custard)	150
11.4	Other sugars and syrups (e.g., xylose, maple syrup, sugar toppings)	50
12.2.2	Seasonings and condiments	500
12.4	Mustards	300
12.5	Soups and broths	300
12.6	Sauces and like products	500
12.6.1	Emulsified sauces and dips (e.g., mayonnaise, salad dressing, onion dip)	100
12.6.2	Non-emulsified sauces (e.g., ketchup, cheese sauce, cream sauce, brown gravy)	100
12.6.3	Mixes for sauces and gravies	100
12.7	Salads (e.g., macaroni salad, potato salad) and sandwich spreads excluding cocoa- and nut-based spreads of food categories 04.2.2.5 and 05.1.3	50
13.3	Dietetic foods intended for special medical purposes (excluding products of food category 13.1)	50
13.4	Dietetic formulae for slimming purposes and weight reduction	50
13.5	Dietetic foods (e.g., supplementary foods for dietary use) excluding products of food categories 13.1 - 13.4 and 13.6	300
13.6	Food supplements	300
14.1.4	Water-based flavoured drinks, including "sport," "energy," or "electrolyte" drinks and particulated drinks	100
14.2.1	Beer and malt beverages	100
14.2.2	Cider and perry	200
14.2.4	Wines (other than grape)	200
14.2.6	Distilled spirituous beverages containing more than 15% alcohol	200

14.2.7	Aromatized alcoholic beverages (e.g., beer, wine and spirituous cooler-type beverages, low alcoholic refreshers)	200
15.1	Snacks - potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	100
15.2	Processed nuts, including coated nuts and nut mixtures (with e.g., dried fruit)	100
15.3	Snacks - fish based	100

Appendix 2

Carotenoids present “Beta-carotene from Dunaliella salina” (determined by HPLC – values in % of area)

Carotenoid	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 5**	**Batch 6**
beta-Carotene (sum)	96.18	97.33	96.28	95.39	95.89
trans-beta-Carotene	67.36	70.96	67.11	66.08	65.89
9-cis-beta-Carotene	13.52	10.59	13.24	13.12	14.01
13-cis-beta-Carotene	6.77	5.89	5.97	6.04	6.32
15-cis-beta-Carotene	0.70	0.56	0.80	0.64	0.60
di-cis-beta-Carotenes (sum)	7.83	9.33	9.15	9.51	9.06
alpha-Carotene	-	0.35	0.47	0.60	-
trans-Lutein	0.54	0.56	0.68	0,67	0.59
6-cis-Lutein	0.64	0.59	0.64	0.58	0.61
beta-Cryptoxanthin	0.52	0.50	0.72	-	-
Sum of unknown other carotenoids	2.11 (n=3)	0.68 (n=1)	1.22 (n=2)	2.76 (n-3)	2.91 (n=3)