De Piscium Colore Carotenoidibus

The Role of Carotenoids in Coloring Fish

An Overview of Data from the Literature

Composit et scripsit:

Antonius H.M. Terpstra

Philosophiae Doctor Universitate Vadensi

Orando, Laborando et Cogitando Patefiet Verum

Θαυμασια η αρχη τησ φιλοσοφιησ (Plato)

The Netherlands, Anno Domini MMXV (2015)

The Role of Carotenoids in Coloring Fish

Summary

Xantophylles play an important role as an pigment in coloring fish. Xantophylles, such as astaxanthin, lutein etc. are oxy-carotenoids and are derived from the food. They can color both the skin and the flesh, but it depends on the fish species whether the skin or the flesh is colored. The pigments are located in the chromatophores which are highly branched dendrite like cells.

Types of Carotenoids

Carotenoids can be subdivided into:

1. **Carotenes** (oxygen-free carotenoids) such as lycopenes (in tomatoes) and α, β and γ carotenes (in carrots). Carotenes have a yellowish color but *do not color the fish*.
.
2. **Xanthophylls** or oxy-carotenoids (oxygen-containing carotenoids, xanthophylls means yellow leaves). These compounds have in contrast to carotenes one or more oxygen atoms. These compounds can color the fish, either the fillet or the skin and can also color egg yolks.

Examples of xanthophylls are:

- Astaxanthin (in shrimps and trout, used to color trout and salmon fillet, trade names Carophyll pink by DSM or Lucanthin pink by BASF)
- Canthaxanthin (in cantharelle mushrooms, used to color eggs, trade name Carophyll red by DSM)
- Capsanthin (in red pepper, the capsicum)
- Zeaxanthin (in Zea Mais, red mais or corn, spirulina, alfalfa, grass meal)
- Lutein (luteus means yellow, in mais or corn, spirulina, alfalfa, grass meal)
- Apo ester (used to color eggs, Carophyll yellow, Food Orange 7 E 160f, the ethyl ester of β – apo – 8'- carotenic acid). It is only found in small quantities in some plants, but is predominantly commercially produced from Beta – apo – 8 –carotenal, E 160e Food Orange 6, that is found in spinach and citrus fruits.

The Role of Carotenoids in Coloring Fish

Coloration of animals can be produced by structural colors or by true pigments. Structural coloration is the results of the physical structure of tissues or surfaces which reflect certain light wave lengths and eliminates others or which scatter the light. Certain colors of bird feathers are generated this way.

True colors are the result of pigments. The pigmentation of warm-blooded animals and humans is caused by melanocytes, cells that contain pigment granules (black melanin, red/yellow pheomelanin, and brown/black eumelanin), called melanosomes. The color of cold-blooded, poikilotherm animals like fish and crustaceans, on the other hand, is produced by so called chromatophores (“color bearing”) (*Bagnara et al., 1968, Fingerman 1966, Wallin, Wikipedia, see Fig 1*). Chromatophores are highly branched, dendrite like cells that contain differently colored pigment granules or chromatosomes. There are various types of chromatophores, melanophores (contain brown/black pigment melanine), erythrophores (contain red pigment (pteridines and carotenoids), granulas or erythrosomes), xantophores (contain red-yellow pigment (pteridines and carotenoids) granulas or xanthosomes), cyanophores (contain blue pigment granulas or cyanosomes), and leucophores (contain dull, white purine granulas or leucosomes).

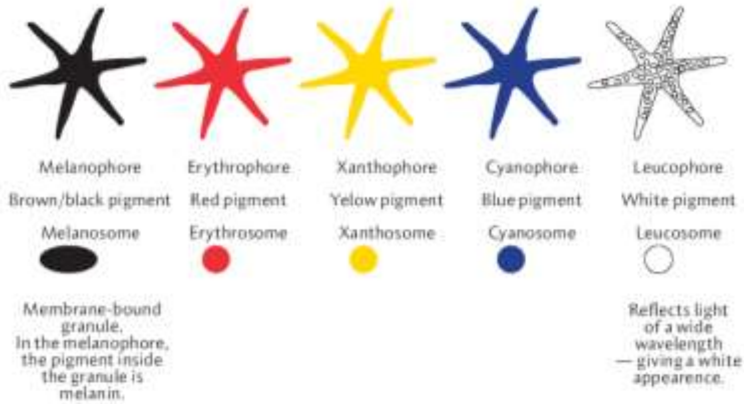


Figure 1

Further, there are also the iridophores, cells that contain purine and guanine crystals and reflect the light to give a silvery shiny color or to produce structural colors (e.g. blue) by scattering light. The pigment patterns in fish predominantly result from positioning of differently colored chromatophores. The pigment in the chromatophores can also be dispersed or agglomerated in the center of the chromatophores, which may be dependent on factors such as temperature, light, or hormones. Dispersion and agglomeration of the pigment granules result in a change of the color as seen in some (fish) species that are able to modulate their skin color (Fig. 2).

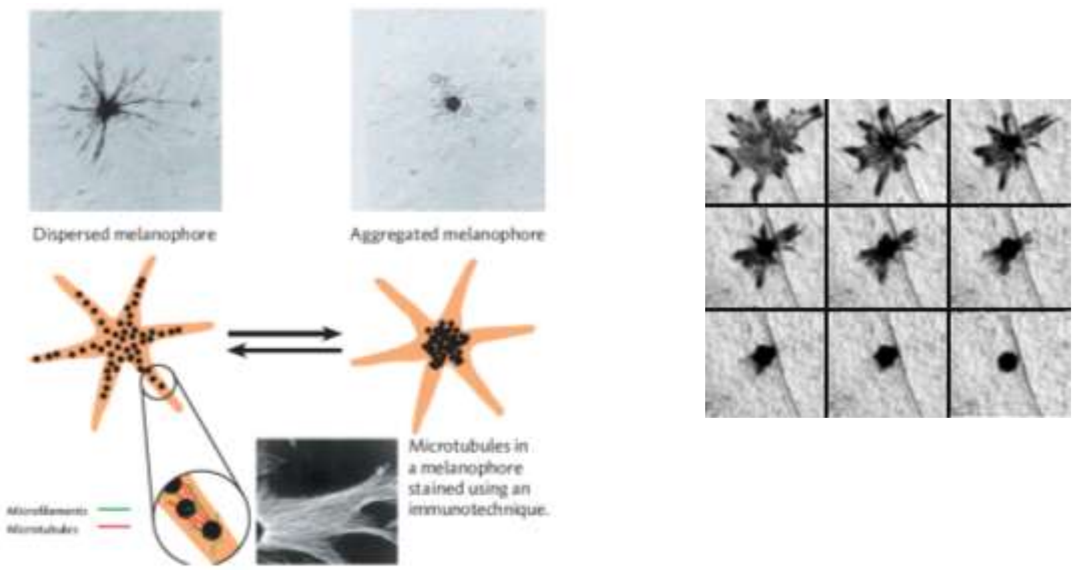


Figure 2

A single zebrafish melanophore imaged by time-lapse photography during pigment aggregation (right figure).

Sources of pigments: There are two ways of the formation of the pigments for the body coloration. (1) One is produced by synthesis of pigments such as pteridines, melanines, and purines in the integument of the body. (2) The other way is the uptake of pigments directly from foods, predominantly carotenoids. Carotenoids can be subdivided into carotenes (e.g. α- β-carotenes and lycopenes) and xanthopylles, which are oxy-carotenoids (e.g. astaxanthin, lutein and zeaxanthin) (See Appendix 1). Astaxanthin, the major pigment of salmonids (pigmentation of the muscles, flesh), is derived mainly from zooplankton and can also be synthesized by several microorganisms such as Phyaffia yeast and Haematococcus algae, whereas lutein (yellow) and zeaxanthin are predominantly derived from plant sources such as alphalpha, corn, yellow corn, and algae.

Interconversion of xantophylles: Carotenoids can also be modified and converted into each other in the fish. For example, (1) crustaceans have a high conversion capability and are able to convert e.g. the algal carotenoids alpha- and betacarotene into lutein and zeaxanthin, respectively, and subsequently into astaxanthin (*Latscha*). The astaxanthin in the exoskeleton of crustaceans is stored in the form of mono and di-esters and in the form of complexes with proteins; heating results in degradation of these complexes and the astaxanthin gets a bright reddish color. (2) Goldfish, red carp, and golden-red carp are also able to convert zeaxanthine and lutein, and possibly to a lesser extent also β-carotene into astaxanthin (*Latcha, Ohkubo 1999*). (3) Salmonids have more limited modificatory abilities, but may be able to convert astaxanthin into zeaxanthin and possibly lutein. The skin of trout contain a relative high level of yellow xantophylles, although lutein and zeaxanthine are absorbed 10 – 20 times less efficient than astaxanthin in the trout; these yellow xantophylls in the skin may be generated from astaxanthin (*Schiedt et al 1985*). Further, astaxanthin, canthaxanthin and zeaxanthin can be converted into vitamin A in aquatic animals (*Schiedt et al, 1985*).

Absorption of xantopyhlles: The absorption of the various xantophylles is dependent on the source and the fish species studied. In salmonids, free astaxanthin is absorbed more efficiently than astaxanthin in the esterified form. Salmonids appear to have a limited capability to hydrolyze esterified astaxanthin in the digestive tract. Carotenoids are transported in the plasma in the unesterified form. Further, in salmonids, astaxanthin and cantaxanthin are absorbed 10 – 20 times more efficiently than lutein and zeaxanthin, whereas in chicken and gold fish, zeaxanthin is 3 times more efficiently absorbed than astaxanthin (absorption preference is zeaxanthin, astaxanthin and lutein for goldfish and chicken (*Schiedt et al. 1985*).

Efficacy to color skin or flesh: There is a large variety of carotenoids and the efficacy of a carotenoid to color the skin or the flesh (muscles) of a fish is dependent on the type of carotenoid and on the fish species. The location of carotenoid deposition is also related to the fish species. For instance, feeding the carotenoid astaxanthin to trout or salmon will mainly result in coloration of flesh and deposition in (white) muscle tissues, whereas feeding astaxanthine to gilthead seabream does not color the flesh or muscles, but predominantly the skin (*Gouveia et al. 2002, Gomes et al. 2002, Ibrahim et al. 1984*). Similary, feeding xanthophylles to tilapia (e.g. corn gluten) does not color the fillets. Lobsters for instance, have a red exoskeleton due to astaxanthin, but their meat is white and more than 80% of the astaxantine in shrimp is localized in the head and the shell (*Hertramp 2000*). Further, astaxanthin is stored in the flesh as free astaxanthin, but stored in the skin as esters.

<u>Gold fish:</u> Various studies have examined the effects of dietary carotenoids on skin coloration of fish. In goldfish, feeding astaxanthin increases the skin color of the goldfish (*Xu, 2006, Paripatananont, 1999, Gouveia 2003*). There was an increase in the total skin carotenoid concentrations upon feeding astaxanthin to goldfish. It is not clear whether astaxanthin or metabolites of astaxanthin are deposited in the skin, but it is known that goldfish have a high capability to metabolize carotenoids and goldfish skin contains a large

variety of carotenoids (*Ohkubo et al 1999*). Goldfish may be able to convert various carotenes and carotenoids into astaxanthin (see Discussion *Chatzifotis et al. 2005*). Further, Xu (*2006*) reported that feeding astaxanthin to gold fish resulted predominantly in accumulation of carotenoids in the skin whereas little was retained in the flesh.

<u>*Koi Carps:*</u> To our knowledge, there is only one study that has examined the effect of astaxanthin on skin coloring of kois (*Gouveia et al. 2003*). Various types of kois (Kawari, Showa, and Bekko) were fed with astaxanthin from various sources (synthetic, spirulina, Chlorella, Haematococcus pluvialis). The studies indicated that there was an accumulation of carotenoids in the skin, but the magnitude of the accumulation was also dependent on the type of koi. The authors conclude that feeding astaxanthin may enhance the coloration of kois.

<u>*Seabream:*</u> In seabream, skin coloration is also important. Gomes et al. (*2002*) fed gilthead seabream (*Sparus auratus*) diets with various sources of carotenoids, i.e. synthetic astaxanthin (carophyll pink), canthaxanthin (Carophyll red), a synthetic carotene (Carophyll yellow) and (synthetic) lutein (Flora glo). Total carotenoid content of skin increased on all the diets, but the muscle carotenoid level was not affected and was very low. Further, they found that the composition of the carotenoids in the plasma were similar to that in the diet, but the composition in the skin was different; the skin of the seabream fed the astaxanthin and canthaxanthin contained predominantly lutein and epilutein esters, and the skin of the seabream fed the carotene and lutein contained exclusively lutein. In trout, lutein is a putative reductive metabolite of astaxanthin, and a similar reductive pathway may also take place in seabream. Further, this study suggests that the seabream is also able to convert carotene into lutein. Gouveia et al (*2002*) fed seabream with astaxanthin or with Chlorella Vulgaris. The pigments in the Chlorella were lutein (0.3%), β-carotene (1.2%), canthaxanthin (36.2%), astaxanthin (55.0%), and other pigments (7.2%). The carotenoid pigments were substantially deposited in four defined skin zones during the feeding trial: the forefront between the eyes, the opercule, along the dorsal fin, and the abdominal area. However, the amount of deposition of pigments in the muscle (flesh) was very low. Ibrahim et al (*1984*) studied the effect of raw krill, krill meal and a aceton extract from krill meal to red seabream. The reported that feeding raw kril resulted in a higher deposition of carotenoids and a distinct coloration of the red seabream, whereas feeding krill meal or an aceton extract from krill meal resulted in a varied concentration of skin carotenoids and a faint skin coloration.

<u>*Red Porgy:*</u> Chatzifotis et al (2005) studied the effect of astaxanthin, β-carotene and lycopene on coloration of the red porgy (*Pagrus pagrus*). Only astaxanthine significantly increased the total carotenoid levels of the skin and had an effect on skin coloration. Thus, this study suggests that β-carotene and lycopene are not deposited in the skin of *Pagrus pagrus* and are also not converted into other types of carotenoids.

<u>***Sources of xantophylles:***</u> Feed ingredients that are rich in carotenoids are algae (spirulina), red paprika (capsanthin) extracts and alphalpha (xantophylles). Further, astaxanthin, a major carotenoid coloring the flesh of salmon and trout but also coloring the skin of seabream and goldfish, can be derived from shrimps and krill which contain astaxanthin in their exoskeleton. In addition, astaxanthin derived from several microorganisms such as Phyaffia yeast and Haematococcus algae and synthetic astaxanthin can be used as a coloring agent for the flesh or the skin. Further, feed ingredients such as yellow corn, corn gluten and alphalpha are rich in carotenoids (xantophylles). Marigold is also rich in carotenoids. Thus, all these ingredients may enhance the coloring of (ornamental) fish, since they are rich in carotenoids.

Literature.

General

Aakermann, T, Skulberg, O.M. & Liaanen-Jensen, S. (1992) A comparison of the carotenoids of strains of Oscillatoria and Spirulina (Cyanobacteria). Biochemical systems and Ecology 20: 761-769.

Bagnara, J.T. (1966) Cytology and cytophysiology of non-melaphore pigment cells. International Review of Cytology 20: 173-205.

Bagnara, J.T., Taylor, J.D., & Hadley, M.E. (1968) The dermal chromatophore unit. Journal of Cell Biology 38: 67-79.

Bickoff, E.M., Livingstone, A.L., Baily, G.F. & Thompson, C.R. (1954) Alfalfa carotenoids. Xanthophylls in fresh and dehydrated alfalfa. Journal of Agriculture and food Chemistry 2(11): 563 – 567. *Gives a composition of the carotenoids in alfalfa, 35% lutein.*

Breithaupt, D.E. & Schwack, W. (2000) Determination of free and bound carotenoids in paprika (Capsicum Annuum L.) by LC/MS European Food Research Technology 211: 52-55. *This article described that Paprika contains various carotenoids like capsanthin, β-cryptoxanthib, capsorubin, zeaxantin, β-carotene.*

Fingerman, M. (1965) Chromatophores. Physiological Reviews 45: 269 – 339.

Fuji, R. (1993) Cytophysiology of fish chromatophores. International Review of Cytology 143: 191-255..

Halver, J.E. & Hardy, R.W. (2002) Fish Nutrition, Academic Press. ISBN 0-12-319652-3 (Hard Copy). Page 528

Hertrampf, J.W. & Piedad-Pascual, F. (2000) Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers. ISBN 0-412-62760-4. (Page 142 - 156). *The levels of various carotenoids in ingredients are given, and many literature references are also presented*.

Hsu, W.J., Rodriguez, D.B. & Chichester, C.O. (1972) The biosynthesis of astaxanthin VI The conversion of 14 lutein and 14 beta carotene in goldfish. Int J Biochem 2-333-1972 (PDF)

Katayama, T., Yokoyama, H. & Chichester, C.O. (1970) the biosynthesis of astaxanthin I. The structure of alpha-doradexanthin and beta-doradexanthin. (PDF)

Katsuyama, T., Komori, T. & Matsumo, T (1987) Metabolism of three stereoisomers of astaxanthin in the fish, rainbow trout and tilapia. Comparative Biochemistry and Physiology 86B: 1-5 (PDF)

Latscha T. Carotenoids in Animal Nutrition: Their nature and significance in animal feeds. Hoffmann La Roche LTD ISBN 3-906507-03-3 (Hard Copy)

Moros, E.E., Darnoko, D., Cheryan, M., Perkins, E.G. & Jerrell, J. (2002) Journal of Agriculture and food Chemistry 50: 5787-5790 (PDF)

Nutrient Requirements of Fish (1993). National Academy Press, Washington, D.C. ISBN 0-309-04891-5, page 35. This book can also be read at the internet (www.nap.edu/books/0309048915/html/1.html)

Rodrigues – Amaya – D.B. (2001) A guide to carotenoid analysis in food. On the internet: http://www.docstoc.com/docs/7250465/A-GUIDE-TO-CAROTENOID-ANALYSIS-IN-FOODS

Schiedt, K., Leuenberger, F.J., Vecchi, M., & Glinz, E. (1985) Absorption, retention, and metabolic transformations of carotenoids in rainbow trout, salmon and chicken. Pure and Applied Chemistry 57:685-692.

Wallin, M. Natures palette, how animals, including humans, produce color. http://www.bioscience-explained.org/

Wikepedia: http://en.wikipedia.org/wiki/Chromatophore

Coloring Red Porgy and Red Snapper

Booth, M.A., Warner-Smith, R.J., Allan, G.L. & Glenross, B.D. (2004) Effect of dietary astaxanthine sources and light manipulation on the skin color of Autralian snapper (Pagrus auratus). Aquaculture Research 35: 458-464.

Cejas, J.R., Almansa, E., Tejera,N., Jerez, S., Bolannos, A. & Lorenzo, A. (2003) Effect of dietary supplementation with shrimp on skin pigmentation and lipid composition of red porgy (Pagrus pagrus). Aqaculture 318: 457-469.

Chatzifotis, S., Pavblidis, M., Jimeno, C.D., Vardanis, G., Sterioti, A. & Divanach, P. (2005) The effect of different carotenoid sources on skin coloration of cultured red porgy (Pagrus pagrus). Aquaculture Research 36:1517-1525.

Kalinovski, C.T., Robaina, L.E., Fernandez-Palacios, H., Schuchardt, D. & Izquierdo, M.S. (2005) Effects of carotenoid sources and their dietary levels on red porgy (Pagrus pagrus) growth and skin color. Aquaculture 244: 223-231.

Szisch, V., van der Salm, A.L., Wendelaar-Bonga, S.E. & Pavlidis, M. (20020 Physiological colour changes in the red porgy Pagrus pagrus following adaptation to blue lighting spectrum. Fish Physiology and Biochemistry 27: 1-8.

Coloring Seabream

Gomes,E., Dias, J., Silva, P., Valente, L., Empis, J., Gouveia, L., Bowen, J. & young, A. (2002) Utilization of natural and synthetic sources of carotenoids in the skin of gilthead seabream (*Sparatus aurata*). European Food Research Technology 214: 287-293. *Sea bream was fed with astaxanthin, canthaxantin, apocarotenoic acid ethyl ester (synthetic, yellow) and lutein. Total carotenoids in skin increased but not in the muscle tissue. Plasma carotenoid composition was related to that in the diet, but lutein and epilutein esters were the major carotenoids In the skin, irrespective of the dietary carotenoid composition*.

Gouveia, L., Choubert, G., Pereira, N., Santinha, J., Empis, J. % Gomes, E. (2002) Pigmentation of gilthead seabream, Sparus aurata (L.1875), using Chlorella vulgaris (Chlorophyta Volvocales) microalga. Aqaculture Research 33: 987-993. *Seabream of about 145 grams were fed with Chlorella vulgaris, synthetic astaxanthin and a mixture of both. Carotenoid pigments were deposited in four defined skin zones, but the deposition of carotenoids in the muscle was very low (less than 1 mg/kg).*

Ibrahim, A., Shimizu, C., & Kono, M. (1984) Pigmentation of cultured red seabream *Chrysophrys Major*, using astaxanthin from antarctic krill, *Euphausia Superba*, and a Mysid, *Neomysis sp*. Aquaculture 38: 45-57. *Red seabream were fed with krill meal and a aceton extract from krill meal, and with raw krill and mysid. Feeding raw krill meal and mysid resulted in distinct coloration of the seabream whereas feeding the krill meal and the aceton extract of krill meal resulted in a faint coloration of the skin of the red seabream.*

Coloring Gold Fish

Gouveia, L., Rema, P., Pereira, O. & Empis, J. (2003) Coloring ornamental fish (Cyprinus Carpio and Carassius auratus) with microalgal biomass. Aquaculture Nutrition 9: 1230129

Ohkubo, M., Tsushima, M., Maoka, T. & Matsuno, T. (1999) Carotenoids and their metabolism in the goldfish Carassius Auratus (Hibuna). Comparative Biochemistry and Physiology Part B 124: 333-340. *This study provides evidence for the conversion of lutein and zeaxanthin to astaxanthin in the goldfish.*

Paripatananont, T, Tangtrongpairoj, B., Saqilasuta, A., & Chansue, N. (1999) Effect of astaxanthine on the pigmentation of goldfish Carassius auratus. Journal of the World Aquaculture Society 30 (4): 454-460.

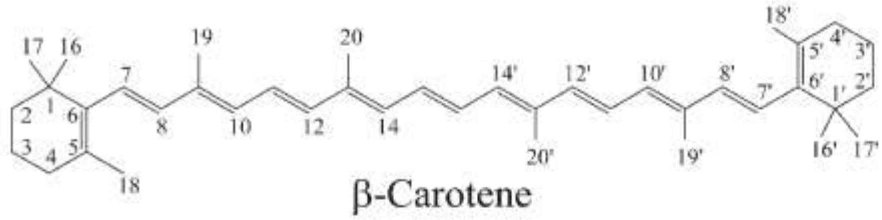
Xu, X. (2006) Effect of astaxanthine from Xanthophyllomyces dendrorhous on the pigmentation of goldfish, Carassius Auratus. Journal of the World Aquaculture Society 37 (3): 282-282

<u>Coloring Koi Carps</u>

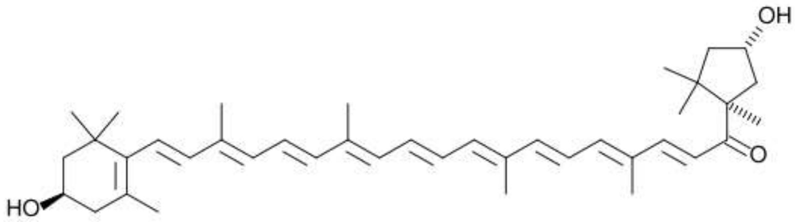
Gouveia, L., Rema, P., Pereira, O. & Empis, J. (2003) Coloring ornamental fish (Cyprinus Carpio and Carassius auratus) with microalgal biomass. Aquaculture Nutrition 9: 123-129.

Appendix

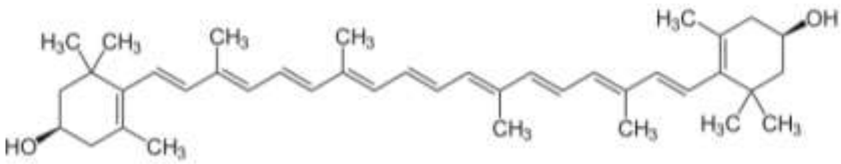
The chemical structure of various types of xanthophylles



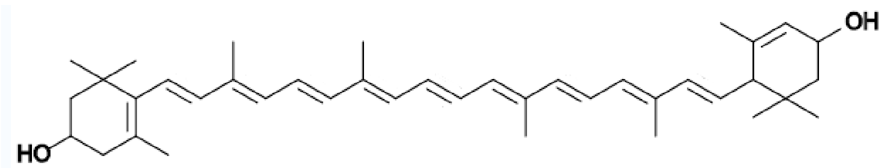
Beta carotene



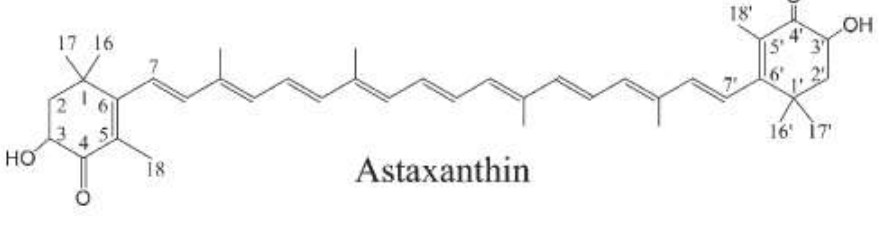
Capsanthin
(in red peppers or paprika)



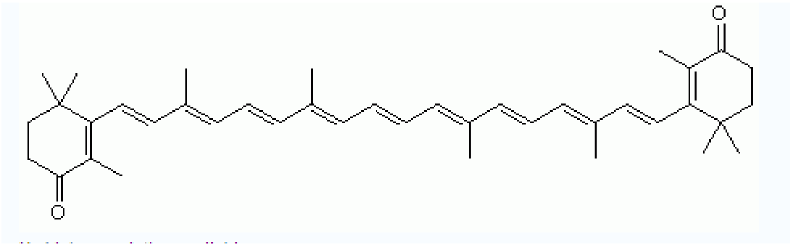
Zeaxanthin (orange color)
(in spirulina and mais)



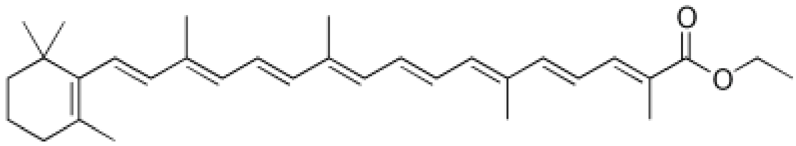
Lutein (orange – red color) in Marigold flowers
(Flora Glo by Kemin or Xangold by Cognis)



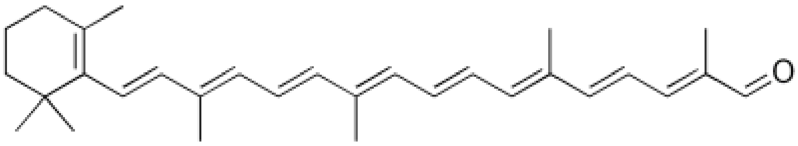
To color trout and salmon (Carophyll pink of DSM or Lucanthin pink by BASF)



Canthaxanthin
To color egg yolk (Carophyll red of DSM)



Ethyl ester of β – apo – 8'- carotenic acid (Apo Ester)
Food Orange 7
To color egg yolks (Carophyll yellow of DSM)



β – apo – 8'- carotenal
Food Orange 6
(used to produce Apo Ester)

Many of the xanthophylls have an E-number and are used as a food coloring agent.

- E 160e — Beta – apo – 8 carotenal
- E 160f — Ethyl ester of β – apo – 8'- carotenic acid made from Beta – apo – 8 carotenal (can be prepared synthetically from beta – apo – 8 – carotenal, DSM)
- E 161a — Flavoxanthin
- E 161b — Lutein
- E 161c — Cryptoxanthin
- E 161d — Rubixanthin
- E 161e — Violaxanthin
- E 161f — Rodoxanthin
- E 161g — Canthaxanthin (can be prepared synthetically, DSM)
- E 161h — Zeaxanthin
- E 161i — Citranaxanthin (can be prepared syntheticaly)
- E 161j — Astaxanthin (can be prepared synthetically, DSM