ZEAXANTHIN (SYNTHETIC) and ZEAXANTHIN-RICH EXTRACT

Chemical and Technical Assessment (CTA)

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1. Summary

Zeaxanthin, 3R,3'R-β,β-carotene-3,3'-diol, belongs to a group of pigments known as xanthophylls or oxycarotenoids which have no provitamin A activity.

Zeaxanthin (synthetic) can be produced through a process involving a Wittig reaction. Zeaxanthin-rich extract is obtained by extraction of the red flowers *of Tagetes erecta* L., followed by saponification and crystallization. The two products are of fundamentally different compositions concerning the content of zeaxanthin, by-products and impurities.

Zeaxanthin (synthetic) and zeaxanthin-rich extract can be used as nutritional supplements and colours in a wide range of foods such as baked goods, beverages, breakfast cereals, chewing gum, egg products, fats and oils, gravies and sauces, hard and soft candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soups and soup mixes in levels ranging from 0.5 to 70 mg/kg.

Zeaxanthin (synthetic) and zeaxanthin-rich extract were evaluated at the 63rd JECFA (2004); zeaxanthin (synthetic) was reevaluated at the 67th JECFA (2006). The related xanthophylls was considered at the 31st JECFA (1987). Tagetes extract (commercial xanthophylls preparation) was evaluated at the 55th JECFA (2000) and again at the 57th JECFA (2001).

Specifications for zeaxanthin (synthetic) were revised at the 67th JECFA and published in the Combined Compendium of Specifications (vol. 3, 2006). Separate specifications for zeaxanthin (synthetic) and for zeaxanthin-rich extract from *Tagetes erecta* L. (tentative), were first prepared at the 63rd JECFA (2004).

2. Description

Zeaxanthin belongs to a group of pigments known as xanthophylls, or oxygenated carotenoids, having no provitamin A activity. The chemical name for zeaxanthin is (all-E)-1,1'-(3,7,12,16-tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene-3-ol]. Synonyms are: 3R,3'R-β,β-carotene-3,3'-diol; all-trans-β-carotene-3,3'-diol; (3R,3'R)-dihydroxy-β-carotene; zeaxanthol; anchovyxanthin. Its Chemical Abstract Service (CAS) number is 144-68-3, its chemical formula is $C_{40}H_{56}O_2$, and its molecular weight is 568.87. The chemical structure is shown in figure 1.

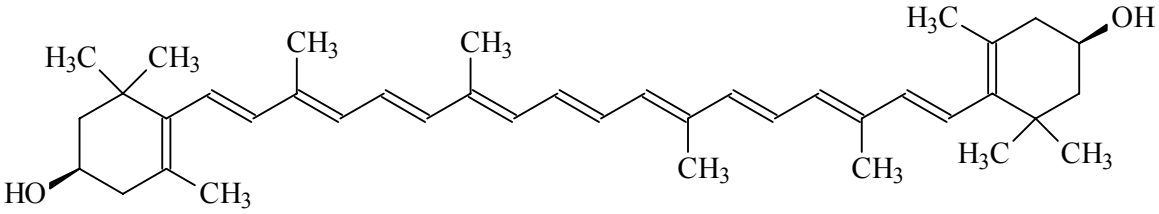

Figure 1. Zeaxanthin

Zeaxanthin (synthetic) is an orange-red crystalline powder, with little or no odour. It is practically insoluble in water and ethanol, slightly soluble in chloroform giving a clear intensive orange-red solution. Zeaxanthin (synthetic) is composed of *trans*-zeaxanthin and minor quantities of *cis*-zeaxanthin, 12'-apo-zeaxanthinal, diatoxanthin and parasiloxanthin.

Zeaxanthin-rich extract from *Tagetes erecta* contains 20 % or more of *trans*-zeaxanthin, other carotenoids in various amounts, as well as fats, oils, waxes and other organic solvent extractable, mainly as unsaponifiable compounds originally from plant material.

3. Manufacturing

3.1. Zeaxanthin (synthetic)

Zeaxanthin synthesis follows a sequence of reactions in which the final step is a double Wittig condensation of a symmetrical C_{10}-dialdehyde as the central building block with two equivalents of the appropriate C_{15}-phosphonium salt (Figure 2). The first step in the process is the production of an enantiopure C_9-hydroxyketone either by an enantioselective catalytic hydrogenation or by a biocatalytic process combined with a chemical reduction. The enantiopure C_9-hydroxyketone is then converted into the C_{15}-phosphonium salt employed in the Wittig condensation. The C_{10}-dialdehyde is commercially available. A similar approach is used for the synthesis of other symmetrical carotenoids, such as lycopene, β-carotene, and astaxanthin, also commercially available (Ernst, 2002, Soukup *et al*., 1996).

The industrial synthesis yields mono- and di-Z (i.e., *cis*) stereoisomers, in addition to the all-E (i.e., *trans*) product. Isomerization of minor amounts of z-isomers into the all-E product is achieved by heating, as for example by heating several hours in heptane or ethanol. Under these conditions the all-E zeaxanthin crystallizes allowing its separation or isolation from solution (Paust, 1996).

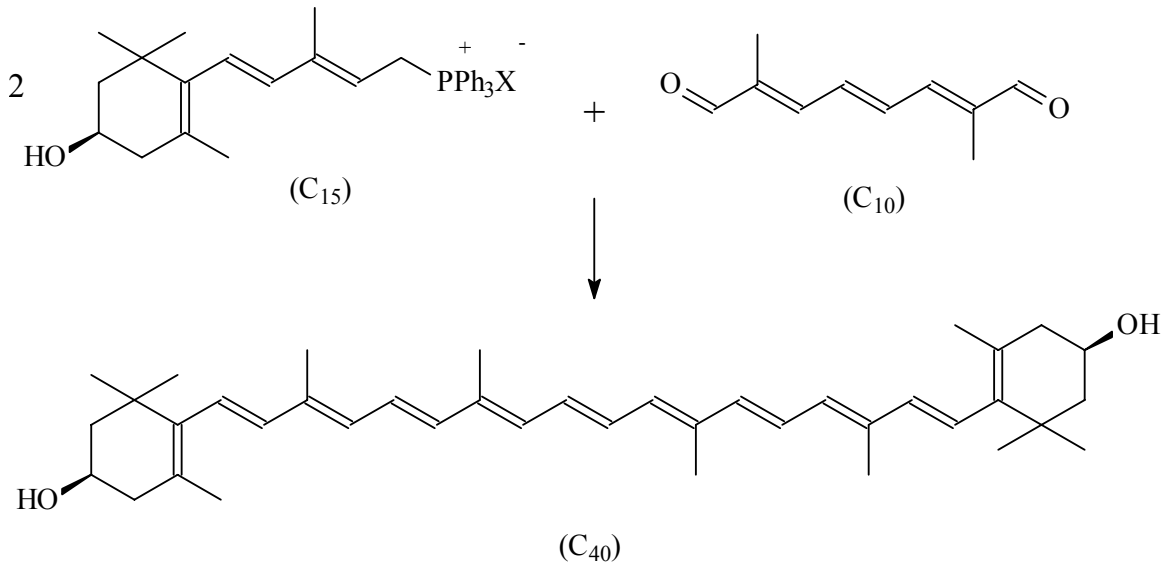

Figure 2. The Wittig reaction used in the production of zeaxanthin (synthetic)

3.2. Zeaxanthin-rich extract

A Zeaxanthin-rich extract is produced by hexane extraction of the red flowers of *Tagetes erecta* and subsequently purified by saponification and crystallization.

The red flowers *of T. erecta* (marigold) are collected and their water content reduced by treatment with a screw press. Subsequently, the flowers are hot-air dried and milled. The resultant marigold meal is pelletized and transferred to an extraction vessel. Hexane is used in the initial extraction step during which a zeaxanthin-containing oleoresin is obtained from the marigold meal.

The marigold oleoresin is subjected to saponification and further purified by crystallization. Fully formed zeaxanthin crystals are then separated by filtration and alternately washing in hexane and methanol until the desired purity is achieved. The final crystalline zeaxanthin product is hot-air dried.

4. Chemical characterization

Carotenoids occur in nature predominantly in the all-*trans* configuration (Schieber and Carle, 2005). Thus naturally occurring zeaxanthin is constituted mostly by the 3R,3'R- isomer. In principle, the polyene chain double bonds present in zeaxanthin could exist in a *cis* or *trans* conformation, giving rise to a large number of possible mono-cis and poly-cis isomers. However, in nature, the vast majority of carotenoids have the all-*trans* configuration. The natural preference for the more thermodynamically stable all-*trans* isomer can be understood in terms of the globally linear geometry, which imposes fewer steric constraints than the *cis* configuration. Small amounts of *cis*-isomers of zeaxanthin have been identified in kale, spinach, green beans, broccoli, romaine lettuce, mandarine oranges, nectarines, canned peas and lima beans, canned corn, corn meal,

wheat, pasta, extracts of marigold flowers, and human plasma (Humphries and Khachik, 2003; Krinsky *et al*., 1990; Khachik *et al*., 1999).

Zeaxanthin (synthetic), i.e., all-*trans*-zeaxanthin, can be obtained with purity equal to or greater than 96%. Several by-products, including diatoxanthin, parasiloxanthin and 12'apo-zeaxanthinal, as well as the zeaxanthin cis-isomers, have been identified as resulting from the production process. All by-products and the cis-isomers can be separated from trans-zeaxanthin by high-performance liquid chromatography (HPLC). Parasiloxanthin and diatoxanthin occur naturally in fish and shellfish. Diatoxanthin has been reported in Korean fresh-water fish at levels ranging from 2 – 5 mg/kg (Seung *et al*., 1999). Parasiloxanthin and diatoxanthin have been found in fish as minor components of the carotenoid fraction (total carotenoid content ranging from 0.7 to 6.1 mg/kg) by Polish researchers (Czeczuga and Czeczuga-Semeniuk, 1999). Triphenyl phosphine oxide (TPPO) and 12'apo-zeaxanthinal are also present in the final material at very low levels. The presence of triphenyl phosphine oxide is a consequence of the use of the Wittig reaction.

Solvent residues in zeaxanthin are present at very low levels, <5 mg/kg and <17.4 mg/kg for hexane and methanol, respectively, according to data on all batches reported to the 63rd JECFA, except for one which did not meet the specifications limits. The content of lead in the material of commerce is lower than the general JECFA maximum limit (2 mg/kg).

Zeaxanthin-rich extract from *T. erecta* contains at least 20 % of *trans*-zeaxanthin. The remainder, up to 80% of the commercial product, is not clearly defined either qualitatively or quantitatively. It consists of other carotenoids and mainly unsaponifiable fats, waxes and other hexane-extractable non-volatile matters from plant material in variable proportions.

5. Analytical methods

Most of the analytical methods for the proposed specifications of zeaxanthin (synthetic) and zeaxanthin-rich extract are based on general tests for identity and purity published in the FAO Combined Compendium of Specifications, Vol.4, 2006 (Solubility, spectrophotometry, test for carotenoid, loss on drying, and lead). For zeaxanthin (synthetic) a validated HPLC method of assay was developed in order to quantitate trans-zeaxanthin by separating it from the by-products formed during its synthesis (the *cis*-zeaxanthins, 12'apo-zeaxanthinal, diatoxanthin and parasiloxanthin). HPLC is also used for the determination of TPPO levels in the final product.

The combined spectrophotometry-HPLC method of assay for zeaxanthin-rich extract from *T. erecta* is based on a published HPLC method (Bailey and Chen, 1988). The test for residual solvents is published in the FAO Combined Compendium of Specifications, Vol.4, 2006.

Standards for all *trans*-zeaxanthin, 12'apo-zeaxanthinal, and parasiloxanthin are available from DSM[1]. All-*trans*-zeaxanthin is also available from Fluka[2]. TTPO is available from Fluka and Supelco[3].

6. Rationale for proposed specifications

[1] DSM Nutritional Products Ltd, Kaiseraugst, Switzerland
[2] Fluka, Buchs Saint Gallen, Switzerland
[3] Supelco, Bellefonte, Pennsylvania, U.S.A.

The specifications for zeaxanthin (synthetic) published in the Combined Compendium of Specifications (vol. 3, 2006) are based on the characteristics of the manufacturing process and raw materials used. The purity assay is designed to identify the levels of zeaxanthin in the final product. Batches containing less than 96 % would not meet specification. Maximum limits for specific impurities (*cis*-zeaxanthins, 12'-apo-zeaxanthinal, parasiloxanthin, and diatoxanthin) are also included in the specification to ensure that their concentrations are held to a minimum and to ensure that the article of commerce is equivalent to that evaluated in the toxicological tests. A limit for lead equal to the general limit adopted by the Committee is included in the specification for safety purposes. In addition, analytical data presented to the 63rd JECFA for three different manufacturing lots of zeaxanthin indicate that the method of production produces a consistent product. The data also support the proposed specifications, and suggest that the finished product conforms to the specifications.

The specifications for zeaxanthin-rich extract from *T. erecta* are based on the manufacturing process and plant material used. They state a minimal content for zeaxanthin and limits for residual solvents and lead, but do not clearly define other components of the material of commerce. These are usual parameters in specifications for extracted carotenoids, but some limits and tests for other carotenoids, waxes, fats, protein and other by-products possibly existing in the extract will be helpful to define up to 80 % of the final product. The limit for lead is set equal to the general limit adopted by the Committee. The limits for residual solvents are included in the specifications at levels conforming to the limits in JECFA specifications for extracted carotenoids and other natural extracts. The data submitted by the sponsor showed that three of four investigated batches (with zeaxanthin content about 23 %) met these limits.

7. Functional uses

Zeaxanthin (synthetic) and zeaxanthin-rich extract are intended for use as colours and as nutrient supplements in foods such as baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogues, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, hard candy, infant and toddler foods (other than infant formula), milk products, processed fruits and fruit juices, soft candy, and soups and soup mixes. The intended food uses and use-levels are the same as for carotenes from vegetable origin (Annex 1) using the Food Category System of the Codex General Standard for Food Additives (Codex Stan 192-1995).

8. Reactions and fate in foods

The thermal stability of crystalline zeaxanthin has been determined at 5, 25, and 35°, while stored in airtight containers, sealed under inert gas and protected from light. After 30 months at the recommended storage conditions (cool, protected from oxygen and light), the zeaxanthin still conformed to specifications. Stability testing performed on zeaxanthin-containing products in commerce indicated that the addition of such antioxidants as sodium ascorbate is required to maintain stability. Food products, such as fruit juices, soy drinks, yoghurt, ice cream, biscuits, cereals and cereal bars, margarine, and soft candies with added zeaxanthin (and lutein), remained stable for periods of up to 12 months. Also, products stored for up to six months showed no significant loss of zeaxanthin. "Jelly bears" and extruded cereals showed losses of zeaxanthin of 25% and 78% after 9 and 12 months, respectively (Annex 2) (Koening-Grillo, 2002).

Carotenoid activity in food systems has been modeled using studies of peroxidation of methyl ethers in a heterogeneous lipid/water system, which demonstrated that astaxanthin, *beta*-carotene, canthaxanthin, and zeaxanthin protected methyl esters against oxidation. The antioxidative effect increased with increasing carotenoid concentration and decreasing oxygen partial pressure and showed little dependence on carotenoid structure (Jorgensen and Skibsted, 1993).

Processing of foods may cause *all*-trans carotenoids to change into *cis*-isomers. *Cis*-isomers differ from *trans*-isomers in terms of bioavailability and anti-oxidant capacity (Schieber and Carle, 2005). *Cis*-zeaxanthins are formed by *trans*-*cis* isomerization during thermal processing of corn. The increase in the presence of *cis*-isomers was 17% according to one group of researchers and 25% according to another group (Updike and Schwartz, 2003; Aman *et al*., 2005). During canning and sterilization of sweet corn, total zeaxanthin content decreased by 29% and *cis*-isomers increased from 7 to 25% (Aman *et al*., 2005).

Currently, encapsulation of carotenoids with lipids, dextrins, and polyvinylpyrrolidone is one approach being tried to reduce carotenoid degradation in foods. Decrease in degradation during storage has been observed for encapsulated carotenoids (Basu and Del Vecchio, 2001; Rodriguez-Hueso *et al*., 2004, Salim *et al*., 2000).

9. References

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Annex 1

INTENDED FOOD USES AND USE-LEVELS FOR ZEAXANTHIN[1]

Food Cat. No[2]	Food Category[2]	Use Levels (mg/kg)[3]
01.1.2	Flavoured milk and milk drinks	2.6
01.2.1	Fermented milk beverages	0.5
01.3.3	Imitation milks	1.7
01.5	Dry milk	2.6
01.5.2	Soy milks	1.2
01.7	Yoghurt	2.6
01.7	Frozen Yoghurt	1.7
02.2.1.2	Margarine-like spreads	20.0
05.2	Chewy and nougat candy	5.0
05.2	Fruit Snacks	5.0
05.2	Hard candy	13.3
05.3	Chewing gum	66.7
06.3	Ready-to-eat cereals	7.3 - 26.7
06.5	Instant and regular hot cereals	1.7
07.1.2	Crackers and crispbreads	13.3
10.2	Liquid, frozen, or dried egg substitutes	8.0
12.5.1	Canned Soups	0.5
12.6.1	Salad dressings	10.0 - 20.0
12.6.2	Tomato-based sauces	0.5
13.2	Junior, strained, and toddler type baby foods*	1.2 - 28.6
13.4	Milk-based meal replacements	2.6
13.4	Meal replacements	1.7
14.1.1.1	Bottled water	0.4
14.1.2.1	Fruit juice	1.7
14.1.2.2	Vegetable juice	1.7
14.1.3	Nectars	1.7
14.1.4	Energy, sport, and isotonic drinks	1.7
14.1.4.1	Carbonated beverages	1.7
14.1.4.2	Fruit-flavoured drinks	1.7
14.1.5	Tea, ready-to-drink	0.5
15.1	Cereal and energy bars	10.0

[1] Prepared from data submitted by DSM Nutritional Products.

[2] Food categorization system for the Codex General Standard for Food Additives (GSFA; Codex Stan 192-1995). Zeaxanthin has not yet been included in the GSFA.

[3] When a range of use-levels is reported for a proposed food-use, particular foods within that food-use may differ with respect to their serving size.

* Does not include infant formula.

Annex 2

STABILITY OF ZEAXANTHIN AND LUTEIN IN FOODS

Product	Storage time	Results		
		Physical and visual stability	Retention of lutein	Retention of zeaxanthin
Soft drinks without Juice	6 month	Physically stable without Pectin; Not stable with Pectin and 10 ppm of lutein	No chemical stability evaluated	
Juice drinks	6 month	No visible influence	86%	92%
Health eye drink	3 month	No visible influence	100%	100%
Yoghurt	Processing	No visible influence	96%	100%
Yoghurt	3 weeks	No visible influence	98%	100%
Ice Cream	Processing	No visible influence	94%	94%
Ice Cream	6 month	No visible influence	100%	100%
Soy drink	Processing	No visible influence	95%	92%
Soy drink	6 month: ambient temp.	Tiny yellow ring, flaky sediment from juice pulp	95%	92%
Soy drink	6 month: 5°C	No ring, flaky sediment from juice pulp	100%	100%
Biscuits	6 month	No visible influence	100%	96%
Extruded Cereals	12 month	No visible influence	21%	22%
Cereal Bars	6 month	No visible influence	99%	78%
Margarine	6 month	No visible influence	93%	Not tested
Jelly bears	9 month	No visible influence	70%	75%

Koenig-Grillo, 2002