ZEAXANTHIN

Chemical and Technical Assessment (CTA)

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1 Summary

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

Zeaxanthin was not previously evaluated by JECFA and it has been recommended for priority evaluation by CCFAC. Tagetes extract (Xanthophylls) were evaluated at the 57th JECFA (2001) and specifications were published in FNP 52 Add 9 (2001), superseding tentative specifications prepared at the 55th JECFA (2000) and published in FNP 52 Add 8 (2000). Former tentative specifications for Tagetes extract were prepared at the 31st JECFA (1987) superseding, in part, the specifications for Xanthophylls published in 1984. No ADI was allocated at the 31st JECFA (1987).

Zeaxanthin can be produced synthetically by the Wittig reaction or by extraction and subsequent purification by saponification and crystallization from *Tagetes erecta* red flowers. Products obtained by these two processes are of fundamentally different compositions, concerning the content of zeaxanthin, byproducts and impurities.

Intended use of zeaxanthin is as a colour and a nutritional supplement in 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 range from 0.5 to 70 mg/kg.

Two separate specifications for Zeaxanthin (synthetic) and for Zeaxanthin rich extract are prepared at 63rd JECFA (2004) and printed in FNP 52 Add 12.

2 Description

2.1 Chemistry and nature of the product

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-beta,beta-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 148-68-3, 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.

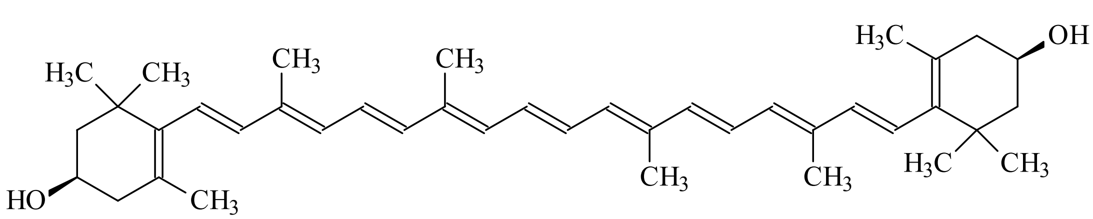

Figure 1. Structural formula of zeaxanthin

Synthetic zeaxanthin is prepared by the Wittig reaction, which yields 96 to 98 % of trans-(3R,3R)-zeaxanthin and the minor quantities of cis-zeaxanthins (max. 2.0%), 12'-apo-zeaxanthinal (max. 0.1%), diatoxanthin (max. 0.2%) and parasiloxanthin (max. 0.8%). Zeaxanthin rich extract from *Tagetes erecta* contains not less than 20 % of trans-zeaxanthin, other carotenoids in various amounts, as well as fats, oils, waxes and other organic solvent extractable, unsaponifiable and unvolatile compounds originating from plant material.

2.2 Natural vs. synthetic origin

Zeaxanthin is naturally occurring pigment present in egg yolk, in maize, marigold (*Tagetes erecta*) flowers and in fruits such as oranges, peaches, especially in Lycium fruits (*Lycium barbarum, Lycium chinese)*. It can be prepared by extraction from natural source materials (The Merck Index, 2000) or synthetically.

3 Manufacturing

3.1 Manufacturing of synthetic zeaxanthin

Manufacturing principle

Zeaxanthin is manufactured synthetically by the Wittig reaction in a multi-step process according to recognized principles of Good Manufacturing Practice. The raw materials of the synthesis are commonly used in the production of other carotenoids with application in food products. The principle of the Wittig condensation used in the production of zeaxanthin is given in the figure 2.

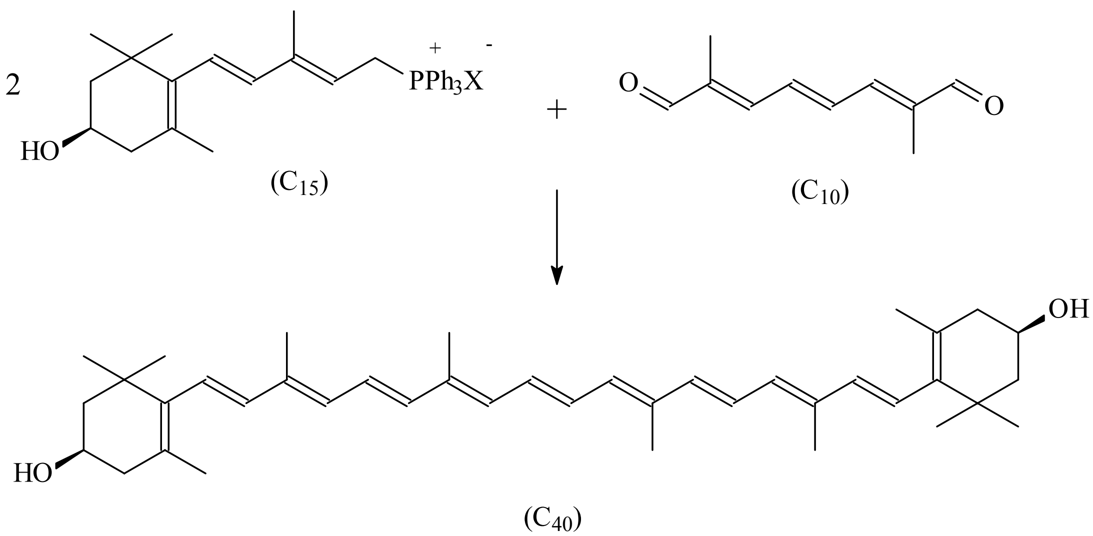

Figure 2. The principle of the Witting reaction used in the production of zeaxanthin

3.2 Manufacturing of zeaxanthin from Tagetes erecta

Manufacturing principle

Zeaxanthin rich extract is produced by hexane extraction of *Tagetes erecta* red flowers and subsequent purification of obtained oleoresin by saponification and crystallization.

Detailed description

Tagetes erecta (marigold) red flowers 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 marigold meal.

The extraction process is completed by subjecting the marigold oleoresin to saponification and purifying the resultant zeaxanthin crystals. Fully formed zeaxanthin crystals are separated out by filtration and washed alternatively in hexane and methanol to achieve desired purity. The final crystalline zeaxanthin product is hot-air dried.

4 Chemical characterization

4.1 Composition of zeaxanthin

Zeaxanthin belongs to a group of pigments known as xanthophylls, or oxygenated carotenoids, which has no provitamin A activity. A synonym for zeaxanthin is 3R,3'R-beta,beta-carotene-3,3'-diol.

Synthetic zeaxanthin is composed of trans-zeaxanthin 96.0 to 98.0 % and the minor quantities of cis-zeaxanthin max. 2.0 %, 12'-apo-zeaxanthinal max. 0.1%, diatoxanthin max. 0.2% and parasiloxanthin max. 0.8%.

Zeaxanthin from *Tagetes erecta* containes not less than 20 % of trans-zeaxanthin, other carotenoids in various amounts, as well as fats, oils, waxes and other organic solvent extractable, mainly unsaponifiable compounds originating from plant material.

4.2 Physico-chemical properties

Zeaxanthin is an orange-red crystalline powder, with little or no odour. It is practically insoluble in water, sparingly soluble in ethanol and soluble in chloroform giving a clear intensive orange-red solution. Zeaxanthin occurring naturally and by chemically synthesis is exclusively the 3R,3’R-zeaxanthin and none of its optical stereoisomers (3R,3’S- or 3S,3’S-zeaxanthin) occur naturally or synthetically. In principal, 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 practice, the vast majority of carotenoids are in the all-trans configurations (Rice-Evans et al., 1997). This may be due to the fact that the trans configuration, which is globally linear, imposes fewer steric constraints than the cis configuration, and is therefore generally more stable. Additionally, the presence of hydroxyl groups on two of the outermost carbon atoms makes xanthophylls, such as zeaxanthin, more water-soluble than other, very hydrophobic carotenoids.

4.3 Possible impurities (including degradation products)

Synthetic zeaxanthin is >96% pure and is equivalent to natural zeaxanthin from typical human food in all aspects including the distribution of geometric isomers (Goralczyk et al. 2002, Wolz et al. 2002). Several byproducts, including diatoxathin, parasiloxanthin and C25-zeanylaldehyde, as well as the zeaxanthin cis-isomers, have been identified as resulting from the production process of zeaxanthin. All by-products and the cis-isomers are separated from trans-zeaxanthin by the high-pressure liquid chromatography (HPLC) method.

Small amounts of cis isomers of zeaxanthin, accompanied by their respective all-trans configuration, have been isolated and purified from extracts of marigold flowers, fresh raw kale, corn meal, spinach, and human plasma (Krinsky et al., 1990; Khachik et al., 1999). Cis-zeaxanthin, which occurs naturally in food, is present in synthetic zeaxanthin at a level of max. 2% by weight of zeaxanthin, resulting in an exposure to 20 µg 13-cis-zeaxanthin/mg zeaxanthin. Structurally, the cis isomers are very closely related to zeaxanthin. Parasiloxanthin and diatoxanthin occur naturally in fish and shellfish. Two further constituents, 12’-apo zeaxanthinal and triphenyl phosphine oxide (TPPO), are also present in the final material at very low levels.

Zeaxanthin from *Tagetes erecta* contains at least 20 % of trans-zeaxanthin. The rest up to 80 % of the commercial product is not clearly defined neither qualitatively nor quantitatively. It consists of other carotenoids and mainly unsaponifiable fats, waxes and other hexane extractable nonvolatile matters from plant material in variable proportions. The limits and methods for determination of these components are not proposed by the producer.

The residual solvents hexane and methanol are present at very low levels, <5 mg/kg and <17.4 mg/kg respectively in all investigated different batches except in one which does not frame the specifications limits that had significantly higher levels of residual solvents (291 mg/kg for hexane and 80.6 mg/kg for methanol). Hexane as extraction solvent was assessed at 14th JECFA (1970) with ADI limited by GMP. Present specifications are prepared at 51st JECFA and published in FNP 52 Add 6. Specifications for methanol as extraction solvent are prepared at 28th JECFA and published in FNP 52 (1992). The low levels of residual solvents in the final material would result in a neglect exposure following dietary intake of zeaxanthin in proposed use levels.

The content of lead in material of commerce is lower than general JECFA limit (2 mg/kg).

4.4 Analytical methods

The analytical methods for the proposed specifications of zeaxanthin are based on general tests for identity and purity published in the Food and Nutrition Paper 5, Rev 2 (FAO, 1991) (Solubility, spectrophotometry, test for carotenoid, loss on drying and lead), as well as a new validated HPLC method that was developed in order to separate all byproducts formed during the synthesis of zeaxanthin (12'-apo-zeaxanthinal, diaxanthin and parasiloxanthin), as well as the cis and trans isomers of zeaxanthin. Zeaxanthin cis-isomers and parasiloxanthin are not available as reference material and according to ICH GL Q2B these substances are calculated as trans-zeaxanthin. HPLC also is used for the determination of TPPO levels in the final product.

The combined spectrophotometry-HPLC mehod of assay for zeaxanthin from *Tagetes erecta* is based on the published HPLC method (Bailey and Chen, 1988). Test for residual solvents is published in FNP 52 Add 11, section E.

4.5 Rationale for proposed specifications

The specifications for synthetic zeaxanthin are based on the manufacturing process and raw materials and to define the composition of the crystalline material. The parameters tested include the identified components of zeaxanthin. The purity assay is designed to identify the levels of zeaxanthin within the final product. Batches containing less than 96.0 % would not meet specification. Levels of specific impurities are also included in the specification to ensure that these levels remain at a minimum and to ensure that the article of commerce is identical to that evaluated in the toxicological tests. Lead limit is included in the specification for safety purposes and it meets the general limit adopted by the Committee. In addition, analytical data for 3 different manufacturing lots of zeaxanthin indicate that the method of production produces a consistent product. The data also supports the proposed specifications, and suggests that the finished product produced by the described manufacturing process comply with the specifications.

On the other hand the specifications for zeaxanthin from *Tagetes erecta* based on the manufacturing process and used plant material, beside the minimal content of zeaxanthin, limits for residual solvents and lead 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, vaxes, fats, protein and other contained byproducts will be helpful to define up to 80 % of the final product. The limit for lead meets the general limit adopted by the Committee. The limits for residual solvents are included in specifications for safety reasons and comply with the usual limits in JECFA specifications for extacted carotenoids and other natural extracts. While three of four investigated batches (with zeaxanthin content about 23 %) meets these limits, the fourth batch (with zeaxanthin content 40.5 %) have significantly higher levels of residual solvents (291 mg/kg for hexane and 80,6 mg/kg for methanol) and does not comply with the specifications showing some inconsistency of the commercial material.

5 Functional use

5.1 Technological function

Zeaxanthin is intended for use in foods and beverages as a colour and as a nutrient supplement.

5.2 Food Categories and use levels

Zeaxanthin is intended for use as a colour and as a nutrient supplement 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 presented in Appendix 1.

6 Reactions and Fate in Foods

The stability of crystalline zeaxanthin has been determined under various temperature conditions (5, 25, and 35°C) while stored in airtight containers, sealed under inert gas and protected from light. After 30 months of storage under the recommended conditions (cool, protected from oxygen and light), zeaxanthin remained stable, and conformed to the specifications. Stability testing performed on zeaxanthin products in commerce indicated that the addition of such antioxidants as sodium ascorbate is required to maintain stability. Food products with added zeaxanthin and lutein, such as fruit juices, soy drinks, yoghurt, ice cream, biscuits, cereals and cereal bars, margarine, and soft candies, remained stable for periods of up to 12 months (Appendix 2). Only the extruded cereals lost nearly 80% of the xanthophylls during the storage time. Zeaxanthin is not anticipated to react with other components of the foods, or with environmental constituents.

7 References

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The Merck Index on CD-Rom version 12:3, 2000, Merck & Co. Inc, Whitehouse Station, NY, USA

Annex 1

INTENDED FOOD USES AND USE-LEVELS FOR ZEAXANTHIN

Food Cat. No.[1]	Food Category[1]	Use Levels (mg/kg)[2]
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] Food categorization system for the General Standard for Food Additives (GSFA)

[2] 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

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