Lutein from *Tagetes erecta*

Chemical and Technical Assessment (CTA)
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1 Summary

Lutein from *Tagetes erecta* L. is a purified extract obtained from marigold oleoresin, which is extracted from the petals of marigold flowers with organic solvents. The final product, after saponification, contains, as a major component, lutein and a smaller proportion of zeaxanthin. Lutein (3R,3'R,6'R-βε-carotene-3,3'-diol) is a member of a group of pigments known as xanthophylls and has no provitamin A activity.

Lutein from *Tagetes erecta* L. has not previously been evaluated by JECFA although xanthophylls, of which lutein is a member, were considered at the 31st JECFA (1987). Commercial xanthophyll preparations (Tagetes extract) were again considered at the 55th JECFA and tentative specifications were published in FNP 52 Add 8 (2000) and were superseded by specifications published in FNP 52 Add 9 (2001) following the 57th JECFA (2001).

It is used as a food colouring agent and nutrient supplement (food additive) in a wide range of baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, egg products, fats and oils, frozen dairy desserts and mixes, gravies and sauces, soft and hard candy, infant and toddler foods, milk products, processed fruits and fruit juices, soups and soup mixes in levels ranging from 2 to 330 mg/kg.

Specifications for lutein were prepared at the 63rd JECFA and published in FNP 52 Add 12 (2004).

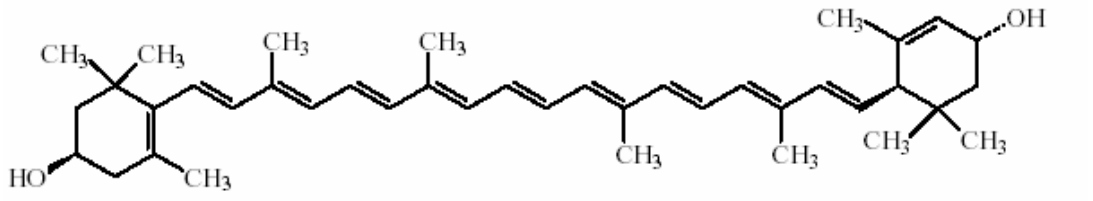
2 Description

2.1 Chemistry and nature of the product

Lutein is an oxycarotenoid, or xanthophyll, (synonyms: vegetable lutein; vegetable luteol; Bo-Xan; all-*trans*-lutein; 4',5'-didehydro-5',6'-dihydro-beta,beta-carotene-3,3'-diol) containing 2 cyclic end groups (one beta - and one alpha -ionone ring) and the basic C40 isoprenoid structure common to all carotenoids (see Figure 1). Although the polyene chain double bonds present in lutein could exist in a *cis* or *trans* conformation, giving rise to a large number of possible mono-*cis* and poly-*cis* isomers, the vast majority of carotenoids are in the all-*trans* configurations (Rice-Evans *et al.*, 1997; IOM, 2000), as depicted in Figure 1. Lutein has been assigned Chemical Abstract Service (CAS) number 127-40-2 with a chemical formula of $C_{40}H_{56}0_2$ and a molecular weight of 568.88.

Extracts of marigold flowers, fresh raw kale, corn meal, spinach, and human plasma also contain small amounts of *cis* isomers of lutein (Krinsky *et al.*, 1990; Khachik *et al.*, 1999). Lutein is commonly isolated from marigold oleoresin together with zeaxanthin.

Figure 1. Structural formula of lutein



3 Manufacturing

3.1 Manufacturing Principle

Lutein from *Tagetes erecta* L. is produced from marigold oleoresin. The marigold oleoresin is extracted from dried marigold flower petals (*Tagetes erecta* L) with hexane and contains lutein, lutein esters, other carotenoids and waxes. Purified lutein is obtained from the oleoresin by saponification and crystallisation. Other raw materials used in the manufacturing process are potassium hydroxide, methanol or propylene glycol.

3.2 Detailed Description

The Committee received details of two separate manufacturing processes. The differences in the processes are in the method of saponification and crystallization. Both approaches have been incorporated into the CTA.

The preparation of the marigold oleoresin from marigold flowers relies on the extraction of xanthophylls from the natural source material with hexane and does not include chemical synthesis. Lutein is prepared from the oleoresin by saponification and crystallization. Under saponification conditions, fatty acids and waxes are removed from lutein esters and zeaxanthin esters. This process employs mixing and heating and the reactions use potassium hydroxide, methanol or propylene glycol and water. During these processes crystals of lutein are formed. The reaction mixture from the crystallization step is then diluted with water and the resulting crystals are dried by the removal of the residual water.

4 Chemical Characterization

4.1 Composition of lutein

Lutein prepared in this manner contains more than 80% total carotenoids of which lutein is present at 70 – 78 %. Zeaxanthin (2 – 9%) and other carotenoids are also present. Waxes (14%) and fatty acids (1%), present in the unprocessed oleoresin, make up the balance of the material.

4.2 Physico-chemical properties

Lutein is a free-flowing orange-red powder. It is insoluble in water but it is soluble in hexane.

4.3 Possible Impurities (including degradation products)

Based on the information received, hexane and propylene glycol and methanol may be present at low levels following the oleoresin preparation and subsequent saponification step.

4.4 Analytical Methods

The analytical methods employed are found in FNP 5, the standards of other respected organizations, journal references and were developed in-house.

4.5 Rationale for Proposed Specifications

The specifications are based on the manufacturing process and raw materials to define the composition of the crystalline material. Furthermore, the specifications have been produced to differentiate this material from the specification for *Tagetes* extract (FNP 52 Add 9 (2001). These specifications include some of the parameters of lutein tested and identified above. The purity assay is designed to identify the levels of lutein and total carotenoids within the final product. Batches containing less than 70.0% lutein would not meet specifications. Furthermore, the extraction solvents hexane, methanol and propylene glycol are included in the specifications to ensure residual levels of these are kept to a minimum. Ash, moisture and waxes are included since they form the major non-carotenoid portion of the final crystalline lutein product. Lead is included in the specification for safety purposes, since high levels of the metal could have toxicity

implications. In addition, analytical data from several different manufacturing lots of lutein indicate that the methods of production give rise to a consistent product. The data supplied also support the proposed specifications, and suggest that the finished product produced by the manufacturing processes described is well within the product specifications.

5 Functional Use

5.1 Technological Function

Lutein is intended for use as a colouring agent and a nutrient supplement.

5.2 Food Categories and Use Levels

Lutein is used as a colouring agent in foods such as baked goods and baking mixes, beverages and beverage bases, breakfast cereals, chewing gum, dairy product analogs, 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 (2.0 –330 mg/kg) are presented in Appendix 1.

6 Reactions and Fate in Foods

Stability testing performed on the lutein products in commerce indicated that they are stable at room temperature for a period of 12 months. Stability testing also was performed on lutein in various food products, including pasta sauce, cereal bars and baked, ready-to-eat cereal, which showed that they are stable at room temperature for a period of 12 months. Lutein is not anticipated to react with other components of the food matrix or with environmental constituents.

7 References

FAO (1991) Guide to Specifications. Food and Agriculture Organization of the United Nations (FAO); Rome. FAO Food and Nutrition Paper, No. 5, Rev. 2.

IOM (2000) β-carotene and other carotenoids. In: IOM. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of DRIs, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (IOM). National Academy Press (NAP); Washington, DC, pp. 325-382.

Khachik, F.; Steck, A.; Pfander, H. (1999) Isolation and structural elucidation of (13Z,13'Z,3R,3'R,6'R)-lutein from Marigold flowers, kale, and human plasma. J Agric Food Chem 47:455-481.

Krinsky, N.I.; Russett, M.D.; Handelman, G.J.; Snodderly, D.M. (1990) Structural and geometrical isomers of carotenoids in human plasma. J Nutr 120:1654-1662.

Rice-Evans, C.A.; Sampson, J.; Bramley, P.M.; Holloway, D.E. (1997) Why do we expect carotenoids to be anioxidants *in vivo*? Free Rad Res 26:381-398.

Appendix 1.

Intended Food-Uses and Use-Levels for Lutein

Food Category	GSFA Food Categorization and Food-Use[1]	Use-Levels for Lutein mg/kg[2]
Baked Goods and Baking Mixes	15.1 Cereal and Energy Bars	50
	07.1.2 Crackers and Crispbreads	67
Beverages and Beverage Bases	14.1.1.1 Bottled Water	2.1
	14.1.4.1 Carbonated Beverages	8.3
	13.4 Meal Replacements	8.3
	14.1.5 Tea, Ready-to-Drink	2.6
Breakfast Cereals	06.5 Instant and Regular Hot Cereals	8.3
	06.3 Ready-to-Eat Cereals	36 - 130
Chewing Gum	05.3 Chewing Gum	330
Dairy Product Analogs	01.3.3 Imitation Milks	8.3
	01.5.2 Soy Milks	6.3
Egg Products	10.2 Liquid, Frozen, or Dried Egg Substitutes	40
Fats and Oils	02.2.1.2 Margarine-like Spreads	100
	12.6.1 Salad Dressings	50 – 100
Frozen Dairy Desserts and Mixes	01.7 Frozen Yogurt	8.3
Gravies and Sauces	12.6.2 Tomato-Based Sauces	2.6
Hard Candy	05.2 Hard Candy	67
Infant and Toddler Foods*	13.2 Junior, Strained, and Toddler Type Baby Foods	5.9 - 140
Milk Products	01.5 Dry Milk	13
	01.2.1 Fermented Milk Beverages	2.6
	01.1.2 Flavoured Milk and Milk Drinks	13
	13.4 Milk-Based Meal Replacements	13
	01.7 Yogurt	13
Processed Fruits and Fruit Juices	14.1.4 Energy, Sport, and Isotonic Drinks	8.3
	14.1.4.2 Fruit-Flavoured Drinks	8.3
	14.1.2.1 Fruit Juice	8.3
	14.1.3 Nectars	8.3
	14.1.2.2 Vegetable Juice	8.3
Soft Candy	05.2 Chewy and Nougat Candy	25

	05.2 Fruit Snacks	25
Soups and Soup Mixes	12.5.1 Canned Soups	2.6

[1] Food categorization system for the General Standard for Food Additives

[2] When a range of use-levels (mg/kg) 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.