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LUTEIN ESTERS FROM *TAGETES ERECTA*

Chemical and Technical Assessment (CTA)

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1. Summary

Lutein esters from *Tagetes erecta* L. is a purified extract derived from the dried petals of yellow marigold flowers with organic solvents. It contains, as a major component, lutein esters of which helenien (lutein dipalmitate) accounts for the major part. A smaller proportion of zeaxanthin esters is also present. Helenien (β,ε-carotene-3,3'-diol dipalmitate) is member of a group of pigments known as xanthophylls and have no provitamin A activity.

Lutein esters are used as a food colour and nutrient supplement 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.

2. Introduction

Lutein esters from *Tagetes erecta* L. were evaluated by JECFA at the 79th meeting (2014). The Committee prepared new specifications different from the Tagetes Extract (xanthophylls) (of which lutein esters are members), that were considered at the 31st JECFA (1987) but in view of the limited information provided for the identity of the extract, designated them as tentative. Commercial xanthophyll preparations (Tagetes extract) had been again considered at the 55th JECFA and tentative specifications were published in FNP 52 Add 8 (2000) and were superseded by specifications published in FNP 52 Add 9 (2001) following the 57th JECFA (2001). Specifications for lutein (the saponified product of lutein esters were prepared at the 63rd JECFA and published in FNP 52 Add 12 (2004).

3. Description

Lutein esters from *Tagetes erecta* is a dark orange-brown solid. It is insoluble in water and soluble in hexane. A solution in n-hexane shows an absorption maximum at about 444 nm.

4. Manufacturing process

Lutein esters from *Tagetes erecta* L. is produced from the marigold flower petals. The marigold oleoresin is extracted from dried milled marigold flower petals (*Tagetes erecta* L) using food grade solvents. Following filtration and solvent removal under vacuum, the material is purified using low molecular weight alcohol and concentrated and vacuum dried to form a granular solid. This preparation contains lutein esters, other carotenoids and waxes. Commercial preparations may be standardized by the addition of solubilizing agents.

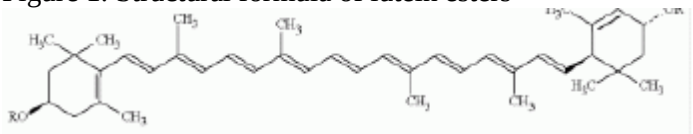
5. Chemical characterization

5.1 Composition

Lutein esters consists of esters of oxycarotenoids, or xanthophylls and long chain fatty acids. The major component is lutein dipalmitate (helenien) (3R, 3´S, 6´S-beta,epsilon-carotene-3,3'-diol dipalmitate). Helenien contains 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 esters 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 esters have been assigned Chemical Abstract Service (CAS) number 547-17-1 with a chemical formula of $C_{72}H_{116}0_4$ and a molecular weight of 1045.71 g/mol.

Extracts of marigold flowers, fresh raw kale, corn meal, spinach, and human plasma also contain small amounts of *cis* isomers of lutein esters (Krinsky *et al.*, 1990; Khachik *et al.*, 1999). Lutein esters are commonly isolated from marigold extracts together with small amounts of zeaxanthin esters. (See the pattern of carotenoids and long chain fatty acids in appendix 1). Other constituents of the extract are alkanes (ca. 15%), and esters of fatty acids with long chain alcohols (ca. 5%).

Figure 1. Structural formula of lutein esters



Lutein: R = H
Helenien: R = $CH_3(CH_2)_{14}CO$

Lutein esters contains more than 75% total carotenoids of which lutein esters is present at more than 90% of the total carotenoid esters. Zeaxanthin esters (<10%) are also present. Waxes (25%), namely alkanes and esters of fatty acids with long chain alcohols, make up the balance of the material..

5.2(Possible) Impurities
Based on the information received, organic solvents (methanol, ethanol, propan-2-ol, hexane, acetone, methyl ethyl ketone) may be present in the final preparation.

5.3 Stability

Stability testing performed on the lutein esters in commerce indicated that they are stable at room temperature for a period of 12 months.

5.4 Analytical methods

The analytical methods employed are found in Vol. 4, the standards of other respected organizations, journal references or were developed in-house.

5.6 Rationale for Proposed Specifications

The specifications are based on the manufacturing process and raw materials to define the composition of the material. Furthermore, the specifications have been produced to define this material. The specifications include some of the parameters of lutein esters tested and identified above. The purity assay is designed to identify the levels of lutein esters and total carotenoids within the final product. Batches containing less than 75% lutein esters would not meet specifications. Furthermore, the extraction solvents methanol, ethanol, propan-2-ol, hexane, acetone, methyl ethyl ketone are included in the specifications to ensure residual levels of these are kept to a minimum. Ash and waxes are included since they form the major non-carotenoid

portion of the final lutein esters 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 lots of lutein esters 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 process described is well within the product specifications.

6. Functional uses

6.1 Technological function

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

6.2 Food categories and use levels

Lutein esters 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 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 (2.0 –330 mg/kg) are presented in Appendix 2.

6. Regulatory status

Lutein esters from *Tagetes erecta* L has GRAS status in the USA since 2003.

Lutein from *Tagetes erecta* was evaluated by the 63rd meeting of JECFA in 2004. It was stated that xanthophylls may be ingested in either free or esterified forms, although non-esterified lutein was subject to the evaluation. As lutein from lutein esters is bioavailable to an extent similar to the bioavailability of lutein itself (EFSA 2011) it should be included in the group ADI of 0 – 2 mg/kg bw which was established by JECFA (2004). Specifications for Tagetes extract have been prepared at the 57th JECFA (2001). The INS No. 161b(ii) was introduced when separate specifications were established for lutein from *Tagetes erecta* (INS No. 161b(i)) . In the European Union the lutein esters are authorized as a food color (E 161b) and were reevaluated by the European Food Safety Authority (EFSA) in 2010, 2011 and 2012.

7. References

Alam, A.U.; Crouch, J.R.; Creger, C.R. (1968) Fatty acid composition of Xanthophyll esters of *Tagetes erecta* petals. Lipids 3: 184-184.

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 ofDRIs, 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 esters from Marigold flowers, kale, and human plasma. J AgricFood Chem 47:455-481.

Krinsky, N.I.; Russett, M.D.; Handelman, G.J.; Snodderly, D.M. (1990) Structural andgeometrical 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 expectcarotenoids to be anioxidants *in vivo*? Free Rad Res 26:381-398.

Appendix 1: Patterns of carotenoids and long chain fatty acids

Table 1: Carotenoid pattern of five batches after saponification.

	Content (area %)				
Carotenoids	**Batch1**	**Batch2**	**Batch3**	**Batch4**	**Batch 5**
di *Z* Lutein	1.95	2.02	1.71	1.92	1.85
all E Lutein	68.77	68.56	69.44	68.98	68.19
all E Zeaxanthin	3.00	3.06	2.78	2.85	2.91
9 *Z* Lutein	2.49	3.45	2.30	3.17	3.03
13 *Z* Lutein	18.24	17.48	17.82	18.28	18.83
15 *Z* Lutein	2.80	2.44	2.81	1.43	2.22
13 *Z* Zeaxanthin	0.43	0.29	0.48	0.40	0.30
Lutein (total)	**94.25**	**93.95**	**94.08**	**93.78**	**94.12**
Zeaxanthin (total)	**3.43**	**3.35**	**3.26**	**3.25**	**3.21**
Others *)	**2.31**	**2.58**	**2.48**	**2.97**	**2.41**

*) Other colouring matters are identified as carotenes and mono-hydroxy carotenoids.

Table 2: Average content of main and side components in lutein ester concentrate The main components are always the diesters of all-trans lutein. Side components could differ with respect to the position of the double bond (zeaxanthin vs lutein1) or the geometry of one of the conjugateddouble bonds (E/Z).

Lutein/carotenoid ester	**Fatty acid length**	**Average content (% area) main component / *plus side components***
Lauric / Myristic	C12/C14	1.42 / *1.90*
Myristic / Myristic	C14/C14	9.05 / *11.35*
Myristic / Palmitic	C14/C16	22.34 / *27.79*
Palmitic / Palmitic	C16/C16	32.28 / *38.60*
Palmitic / Stearic	C16/C18	11.80 / *14.13*
Stearic / Stearic	C18/C18	2.40 / *2.90*

Appendix 2: Intended Food-Uses and Use-Levels for Lutein esters.

Food Category	GSFA Food Categorization and Food-Use[1]	Use-Levels for Lutein esters 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 Analogues	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 (Does not include infant formula)	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.