Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 82\textsuperscript{nd} meeting 2016

**Lutein Esters from *Tagetes Erecta***

This monograph was also published in: *Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 82\textsuperscript{nd} meeting 2016. FAO JECFA Monographs 19*
LUTEIN ESTERS FROM *TAGETES ERECTA*


**SYNONYMS**

Xanthophyll esters

**DEFINITION**

Lutein esters from *Tagetes erecta* is obtained by solvent extraction of dried petals of *Tagetes erecta* L., further purification and subsequent removal of solvents under vacuum. Lutein diesters account for the major part and a smaller proportion of zeaxanthin diesters is also present. The esters contain saturated long chain fatty acids, such as myristic, palmitic and stearic acid in various proportions with palmitic acid being a major component. Waxes naturally occurring in the source material may also be present. Only the following solvents may be used in the production: methanol, ethanol, 2-propanol, hexane, acetone, methyl ethyl ketone and carbon dioxide. Usually food grade antioxidants are added to stabilize the product.

Products of commerce are normally further formulated e.g. in order to standardize colour content or to obtain water soluble/dispersible products.

**Chemical formula**

Lutein esters: 

\[
R = \text{CH}_3(\text{CH}_2)_{10}\text{CO, CH}_3(\text{CH}_2)_{12}\text{CO, CH}_3(\text{CH}_2)_{14}\text{CO or CH}_3(\text{CH}_2)_{16}\text{CO}
\]

**Structural formula**

\[
\text{\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structural_formula.png}
\end{figure}}
\]

**Formula weight**

496.43

**Assay**

Not less than 75% total carotenoid esters (as lutein esters)

**DESCRIPTION**

Dark orange brown solid

**FUNCTIONAL USES**

Colour, nutrient

**CHARACTERISTICS**

**IDENTIFICATION**

Solubility (Vol. 4) Insoluble in water, soluble in hexane

Spectrophotometry (Vol. 4)

A hexane solution of the sample shows a maximum absorption at about 444 nm

Test for carotenoids (Vol. 4)

The colour of a solution of the sample in acetone disappears after successive addition of a 5% solution of sodium nitrite in 0.5 M sulfuric acid.

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PURITY

Ash (Vol. 4) Not more than 1%
Zeaxanthin (Vol. 4) Not more than 10% of total carotenoids.
See description under TESTS.

Residual solvents (Vol. 4) Determine using method (I)
Hexane
Methanol Not more than 50 mg/kg, singly or in combination
Ethanol
2-Propanol
Acetone
Methyl ethyl ketone

Waxes Not more than 25%.
See description under TESTS

Lead (Vol. 4) Not more than 2 mg/kg.
Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Zeaxanthin Principle
The saponified sample is analysed by reversed phase liquid chromatography (HPLC).

Reagents
40% Methanolic potassium hydroxide: Dissolve 40 g of KOH in about 50 ml methanol. Dilute to 100 ml with methanol.
10% Sodium sulfate solution: Dissolve 10 g of sodium sulfate in 100 ml water.
Hexane, HPLC-grade
Ethyl acetate, HPLC-grade

Chromatography
Column: 250 mm x 4.6 mm i.d. beta-cyclodextrin (Cyclobond I or equivalent)
Mobile phase: Hexane/Ethyl acetate (75/25)
Filter through a 0.45μm membrane
Flow rate: 1.0 ml/min
Detector wavelength: UV/Vis or PDA at 445nm
Injection volume: 10 μl
Procedure
Weigh about 10 mg of sample and transfer into a 100 ml round bottom flask. Dissolve in 30 ml of extraction solvent. Add 2 ml methanolic potassium hydroxide solution. Attach a reflux condenser to the flask to prevent loss of solvent. Place flask in a 56° water bath for 20 min. Cool sample and transfer to a 100 ml volumetric flask using a small volume of extraction solvent. Let stand in the dark for 1 h. Add 30 ml of hexane, swirl for 1 min. Dilute with sodium sulfate solution up to the 100 ml mark. Shake 1 min. Let stand in the dark until organic layer (top layer) is clear (about 1 h). Transfer 1 ml of organic layer to a scintillation vial. Evaporate to almost dry under vacuum at 45°. Dissolve residue with 1 ml mobile phase. Inject 10 µl of this solution into the chromatograph.

Results
A typical chromatograph should look as follows.

Integrate the area under peaks at ca:
18 min (di-cis lutein)
19.5 min (trans lutein)
22 min (trans zeaxanthin)
32 min (9-cis lutein)
37.6 min (13-cis lutein)
40.3 min (15-cis lutein)
59.8 min (13-cis zeaxanthin)

Calculate the percentage of zeaxanthin from the sum of the areas of the trans-zeaxanthin peak (RT ~ 20 – 22 min) and cis-zeaxanthin peak (RT ~ 55 - 65 min) vs the sum of the areas of all the lutein and zeaxanthin peaks.
Waxes

Determine by gas chromatography using the following conditions:

**Apparatus**

Gas chromatograph (GC) equipped with an autosampler, a splitless injection system, flame ionization detector (FID), programmable column and detector

- GC column DB-5 (30 m x 0.25 mm ID with a 0.25 μm film thickness) or equivalent
- GC injector temperature: 280°
- FID temperature: 350°
- GC temperature program: 50° (2 min) 13°/min to 340° and hold for 8 min
- Carrier gas (Helium) flow rate: 1.0 ml/min
- Injection mode: splitless
- Injection volume: 1.0 μl
Standards:
Hydrocarbons mixed standard: C25 to C46

Internal standard:
Hexatriacontane (C36)

Standard solutions: Prepare standard solutions by addition of hydrocarbon standards to methylene chloride to get hydrocarbon concentrations of 2.0, 5.0, 10, 25, 50, mg/l respectively. Add required quantity of hexatriacontane- internal standard to get a final concentration 50 mg/l in all standard solutions.

Sample Preparation
Accurately weigh 100 mg of sample into a centrifuge tube and dissolve in exactly 20 ml of methylene chloride. Sonication or vortex mixing may be required to completely dissolve the product. Centrifuge sample at 2500 rpm for 5 min, if the sample appears turbid. Add 1.6 ml of methylene chloride and 20 μl of (5000 mg/l) hexatriacontane solution (to a final concentration of 50 mg/l) into 2 ml volumetric flask. Transfer 40 μl of sample solution and dilute with methylene chloride to the 2 ml. Transfer the solution into a 2 ml autosampler vial.

Analysis
Inject 1.0 μl of each of the standards solutions. Record the peak areas. Construct standard curves using the peak ratios of each hydrocarbon to the internal standard against the concentration of the hydrocarbon. Inject 1.0 μl of the sample solution and determine individual wax in the sample(mg/l) from the respective standard curve. Add the concentration of individual waxes to get the total wax concentration in the sample solution (mg/l)

Calculation: \[ \text{Waxes \% w/w} = \frac{C \text{ (mg/l)} \times 2 \text{ ml} \times 20 \text{ ml} \times 100}{1000 \text{ (ml/l)} \times W \text{ (mg)} \times 0.04 \text{ ml}} = \frac{(100 \times C)}{W} \]

Where: C is the total concentration of waxes, mg/l in the sample
W is the weight of sample, mg

METHOD OF ASSAY
Determine the total content of carotenoid esters as follows:

Apparatus:
UV/VIS spectrophotometer
1-cm cuvettes

Sample analysis:
Accurately weigh about 1.0 g of the sample into a 100 ml volumetric flask. Add about 80 ml hexane and 5 ml 2-propanol. Place the
volumetric flask into an ultrasonic bath for 5 min to achieve complete dissolution. Let cool to room temperature. Adjust to the 100 ml volume mark with hexane. Mix well. Make serial dilutions with hexane such that the absorbance at 428 nm falls between 0.2 and 0.8. Measure absorbance of the sample at 428 nm (inflection point of the curve, isosbestic point of all lutein isomers) using hexane as blank.

**Calculation:**

Total carotenoid ester content (% w/w) = \( \frac{\text{Abs x d x 100}}{A_{1\text{cm isosbestic}} x W} \)

Where:

Abs is the measured absorbance

d is the dilution factor

\( A_{1\text{cm isosbestic}} \) (specific absorbance of lutein ester at the wavelength of the isosbestic point) = 898

W is the weight of sample (g)