

LUTEIN ESTERS FROM *TAGETES ERECTA* (TENTATIVE)

New tentative specifications prepared at the 79th JECFA (2014) and published in FAO Monographs 16 (2014). A temporary ADI "not specified" was established at the 79th JECFA (2014).

Information required:

- *Details of the manufacturing process including purification steps*
- *Detailed analytical data on the full composition of at least five different batches of commercially available product to support the specifications*
- *Method of analysis to determine carotenoid composition*
- *Method of analysis to determine the composition of the non-carotenoid lipidic fraction*

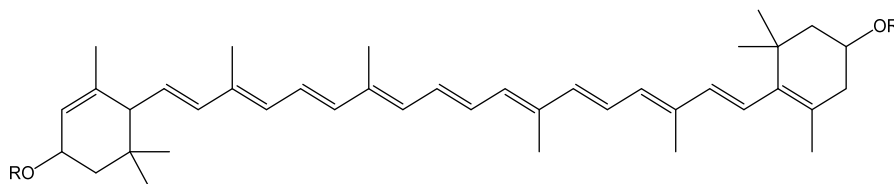
SYNONYMS

Xanthophylls

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. Lutein esters accounts for the major part and a smaller proportion of zeaxanthin esters is also present. Diesters constitute a major component though monoesters are 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 and fatty acid-containing moieties 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. Products of commerce are normally further formulated e.g. in order to standardize colour content or to obtain water soluble/dispersible products.

Structural formula



Lutein esters: R = CH₃(CH₂)₁₂CO, CH₃(CH₂)₁₄CO or CH₃(CH₂)₁₆CO

Assay

Not less than 60% total carotenoid esters (as lutein esters)

DESCRIPTION

Dark yellow-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.

<u>Melting range</u> (Vol. 4)	53 - 55°
<u>Test for carotenoids</u> (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.

PURITY

<u>Ash</u> (Vol. 4)	Not more than 1%							
<u>Zeaxanthin</u>	Not more than 10% of total carotenoids. See description under TESTS.							
<u>Residual solvent</u> (Vol. 4)	<table> <tr> <td>Hexane</td> <td rowspan="6">} Not more than 50 mg/kg, singly or in combination</td> </tr> <tr> <td>Methanol</td> </tr> <tr> <td>Ethanol</td> </tr> <tr> <td>2-propanol</td> </tr> <tr> <td>Acetone</td> </tr> <tr> <td>Methyl ethyl ketone</td> </tr> </table>	Hexane	} Not more than 50 mg/kg, singly or in combination	Methanol	Ethanol	2-propanol	Acetone	Methyl ethyl ketone
Hexane	} Not more than 50 mg/kg, singly or in combination							
Methanol								
Ethanol								
2-propanol								
Acetone								
Methyl ethyl ketone								
<u>Waxes</u>	Not more than 25%. See description under TESTS.							

<u>Glycerides and free fatty acids</u>	Information required
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<u>Lead</u> (Vol. 4)	Not more than 2 mg/kg. Determine using an AAS (electrothermal atomization) 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").
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TESTS

PURITY TESTS

<u>Zeaxanthin</u>	Information required
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<u>Waxes</u>	Determine by gas chromatography using the following conditions:
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Apparatus

GC equipped with an autosampler, a splitless injection system, flame ionization detector (FID), programmable column and detector flow rates.

GC column	DB5 (30 m x 0.25 mm ID with a 0.25 µm film thickness) or equivalent
GC injector temperature:	280°
FID temperature:	300°
GC column initial temperature:	50° (held for 2 min)
GC oven temperature increase rate:	13°/min
GC column final temperature:	300° (held for 8 min)
Carrier gas (Helium) flow rate:	1.0 ml/min
Injection mode:	splitless
Injection volume:	1.0 µl
Approximate run time:	30 min

Internal standard pentacosane (C25)

Standard curves are prepared through the addition of absolute hydrocarbon standards to methylene chloride to provide hydrocarbon concentrations of 2.0, 10, 25, 50, 75, and 100 mg/kg.

Sample Preparation

Accurately weigh 200 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. Transfer 40 µl into 2 ml autosampler vial that contains 1.6 ml of methylene chloride and 20 µl of (5000 mg/kg) pentacosane for a final concentration of 50 mg/kg.

Sample Analysis

Using an autosampler, sequentially inject a 1.0 µl aliquot of each of the calibration standards solution onto the GC column and record the peak areas. Inject a 1.0 µl aliquot of the sample.

Results

The approximate retention according to GC/FID times of C29, C30, C31, C32, C33, C34, C35, and the internal standard pentacosane (C25) are 18.6, 19.1, 19.6, 20.0, 20.5, 20.9, 21.4, and 16.3 minutes, respectively.

Construct standard curve using the peak areas from analysis of the standard solutions and use it to calculate total and individual wax content in the sample.

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, isobestic point of all lutein isomers) using hexane as blank.

Calculation:

$$\text{Total carotenoid ester content (\% w/w)} = \frac{\text{Abs} \times d \times 100}{A_{\text{isobestic}}^{1\%} \times W}$$

Where:

Abs = measured absorbance

d = dilution factor

$A_{\text{isobestic}}^{1\%}$ (specific absorbance of lutein ester at the wavelength of the isobestic point) = 898

W = weight of sample (g)