Joint FAO/WHO Expert Committee on Food Additives

86th Meeting 2018
COMPRENDIUM
OF FOOD ADDITIVE
SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

86th Meeting
Geneva, 12 – 21 June 2018

Food and Agriculture Organization of the United Nations
World Health Organization
Geneva, 2018
SPECIAL NOTE

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Geneva, 12 – 21 June 2018

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 86th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Geneva, 12 – 21 June 2018. The specifications monographs are one of the outputs of JECFA’s risk assessment of food additives, and should be read in conjunction with the safety evaluation, reference to which is made in the section at the head of each specifications monograph. Further information on the meeting discussions can be found in the summary report of the meeting (see Annex 1), and in the full report which will be published in the WHO Technical Report series. Toxicological monographs of the substances considered at the meeting will be published in the WHO Food Additive Series.

Specifications monographs prepared by JECFA up to the 65th meeting, other than specifications for flavouring agents, have been published in consolidated form in the Combined Compendium of Food Additive Specifications which is the first publication in the series FAO JECFA Monographs. This publication consists of four volumes, the first three of which contain the specifications monographs on the identity and purity of the food additives and the fourth volume contains the analytical methods, test procedures and laboratory solutions required and referenced in the specifications monographs. FAO maintains an on-line searchable database of all JECFA specifications monographs from the FAO JECFA Monographs, which is available at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/. The specifications for flavourings evaluated by JECFA, and previously published in FAO Food and Nutrition Paper 52 and subsequent Addenda, are included in a database for flavourings specifications. All specifications for flavourings that have been evaluated by JECFA since its 44th meeting, including the 79th meeting, are available in the online searchable database at the JECFA website at FAO: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/en/. The databases have query pages and background information in English, French, Spanish, Arabic and Chinese. Information about analytical methods referred to in the specifications is available in the Combined Compendium of Food Additive Specifications (Volume 4), which can be accessed from the query pages.

An account of the purpose and function of specifications of identity and purity, the role of JECFA specifications in the Codex system, the link between specifications and methods of analysis, and the format of specifications, are set out in the Introduction to the Combined Compendium, which is available in shortened format online on the query page, which could be consulted for further information on the role of specifications in the risk assessment of additives.

Chemical and Technical Assessments (CTAs) for some of the food additives have been prepared as background documentation for the meeting. These documents are available online at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/technical-assessments/en/.

Contact and Feedback

More information on the work of the Committee is available from the FAO homepage of JECFA at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/. Readers are invited to address comments and questions on this publication and other topics related to the work of JECFA to:

jecfa@fao.org
SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

New (N) or revised (R) specifications monographs were prepared for 11 food additives and these are presented in this publication.

Anionic methacrylate copolymer (AMC) (N, T)
Basic methacrylate copolymer (BMC) (N)
Cassia gum (R)
Citric and fatty acid esters of glycerol (R, T)
Erythrosine (R)
Glycerol ester of wood rosin (R)
Indigotine (R)
Lutein (R)
Modified starches¹ (R, T)
Neutral methacrylate copolymer (NMC) (N, T)
Spirulina extract (N)

In the specifications monographs that have been assigned a tentative status (T), there is information on the outstanding data and a timeline by which this information should be submitted to the FAO JECFA Secretariat.

¹ Applying to all 16 modified starches: INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451
ANIONIC METHACRYLATE COPOLYMER

New specifications prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018). No ADI was established at the 86th JECFA (2018).

SYNONYMS

E 1207, INS No. 1207, acrylates copolymers, Methyl acrylate, methyl methacrylate, methacrylic acid polymer; methacrylic acid, polymer with methyl acrylate and methyl methacrylate

DEFINITION

Anionic methacrylate copolymer is a copolymer comprised of monomers, methyl acrylate, methyl methacrylate, and methacrylic acid in the molar ratio of 7:3:1. The copolymer is manufactured by emulsion polymerization of the monomers with water soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low-molecular weight substances and coagulum. The copolymer is standardized as a 30% aqueous dispersion. The copolymer dispersion may contain residual monomers (methyl acrylate, methyl methacrylate, and methacrylic acid). Anionic methacrylate copolymer is used as a coating and glazing agent for food supplements and products for special medical purposes.

Chemical name Poly (methyl acrylate-co-methylmethacrylate-co-methacrylic acid) 7:3:1

C.A.S. number 26936-24-3

Chemical formula Poly[(CH₂:CHCO₂CH₃)-co-(CH₂:C(CH₃)CO₂CH₃)-co-(CH₂:C(CH₃)COOH)]

Structural formula

![Structural formula](image-url)

The above formula is provided for illustrative purposes; in this copolymer no definitive structural unit can be defined.

Formula weight 280,000 (weight-average), 77,000 (number-average)

Assay 9.2 – 12.3 % methacrylic acid units on the dried basis
DESCRIPTION
Commercial form (30% aqueous dispersion) is a low viscosity, milky-white liquid.

FUNCTIONAL USES
Coating agent, glazing agent.

CHARACTERISTICS

IDENTIFICATION

Viscosity (Vol. 4) Not more than 20 mPa•s
Determine viscosity using Brookfield viscometer at 20° and 30 rpm using UL adapter.

pH (Vol 4) 2.0 – 3.5

Infrared absorption (Vol. 4)
The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix.
Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.

PURITY

Loss on drying (Vol. 4) 68.5 – 71.5% (110°, 5 h)

Sulfated ash (Vol. 4) Not more than 0.2%
Test 5 g of the sample (Method I)

Methanol (Vol. 4) Not more than 1,000 mg/kg

Residual monomers Methyl acrylate: Not more than 1 mg/kg
Methyl methacrylate: Not more than 3 mg/kg
Methacrylic acid: Not more than 1 mg/kg
See description under TESTS

Lead (Vol. 4) Not more than 1.0 mg/kg in the dispersion
Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

**Microbiological criteria (Vol. 4)**
- Total plate count: Less than 1,000 cfu/g
- Yeast and moulds: Less than 100 cfu/g
- Coliforms: Negative in 10 g

**TESTS**

**IDENTIFICATION TESTS**

**Residual monomers** Determined by liquid chromatography (Vol. 4)

**Standards and Reagents:**
- Acetonitrile: HPLC grade with UV absorption: $A_{max}$ of 1% at 190 nm
- Acetone, methanol, isobutanol and deionized water: HPLC grade
- Phosphoric acid solution (pH 2): Adjust phosphoric acid (85%) with an appropriate volume of deionized water to pH 2.
- Standards: methyl acrylate, methyl methacrylate and methacrylic acid (>99%)

**Preparation of mixed standard solutions:**

**Stock mixed standard solution:**
- Pipette 5 ml of isobutanol into a 50 ml volumetric flask.
- Accurately weigh approximately 10 mg of methyl acrylate, 12 mg of methyl methacrylate and 11 mg of methacrylic acid, add to isobutanol and dilute to volume with acetone.

**Intermediate mixed standard solution-1:**
- Dilute 5.0 ml of stock mixed standard solution to 50 ml with acetone in a volumetric flask.

**Intermediate mixed standard solution-2:**
- Dilute 20.0 ml of intermediate mixed standard solution-1 to 50 ml with acetone in a volumetric flask.

**Working mixed standard solution:**
- Dilute 5 ml of Intermediate mixed standard solution-2 to 25 ml with methanol:phosphoric acid-pH 2 (70:30) in a volumetric flask.

**Preparation of sample solution:**
- Accurately weigh approximately 11 g of sample, dissolve in acetone and dilute to 50 ml in a volumetric flask. Add 5.0 ml of
the solution dropwise (precipitation of the polymer should be slow to avoid entrapment of monomer in the precipitate) to 20 ml methanol and phosphoric acid- pH 2 (70:30 v/v). Centrifuge until the supernatant is clear and use the supernatant as the sample solution.

Procedure:
- Use an HPLC with diode array or UV detector at 200 nm
- Column: Octadecylsilane chemically bonded to porous silica (125 cm x 4.6 mm i.d.x 7 µm)
- Injection volume: 20 µl
- Mobile phase: Acetonitrile:Phosphoric acid-pH 2 (10:90 v/v)
- Flow rate: 2 ml/min

Inject separately 20 µl each of working mixed standard solution and sample solution. Calculate the amount of each monomer in the sample from the peak areas obtained in the chromatograms of working mixed standard solution (rR) and sample solution (rS); amount of standard (R, mg), weight of sample (W, g) and dilution factor (40).

\[
\text{Conc. monomer [µg/g]} = \frac{rS \times R \times 40}{rR \times W}
\]

Total monomers in the sample (µg/g) = Sum of monomers in the sample and correct the results for recovery.

**METHOD OF ASSAY**

Accurately weigh about 5 g sample and dissolve completely in 90 ml isopropyl alcohol and 10 ml water. Titrate with 0.5 N sodium hydroxide standard solution to a potentiometric endpoint. Perform a blank titration under the same conditions. One ml 0.5 N NaOH corresponds to 43.045 mg methacrylic acid units.

\[
\text{Methacrylic acid units (%w/w, on the dried basis)} = \frac{\text{ml of 0.5 N NaOH} \times 430.45}{\text{sample weight (g)} \times % \text{ dry substance in sample}}
\]
Appendix: Infrared spectrum of anionic methacrylate copolymer
BASIC METHACRYLATE COPOLYMER

*New specifications prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018). An ADI of “not specified” was established at 86th JECFA (2018).*

**SYNONYMS**
E 1205; INS No. 1205; basic butylated methacrylate copolymer; amino methacrylate copolymer; aminoalkyl methacrylate copolymer E; butyl methacrylate, dimethylaminoethyl methacrylate, methyl methacrylate polymer; butyl methacrylate, methyl methacrylate, dimethylaminoethyl methacrylate copolymer

**DEFINITION**
Basic Methacrylate Copolymer is a cationic copolymer comprised of the monomers dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate in the molar ratio of 1:2:1. The copolymer is manufactured by a controlled polymerization process using a free radical donor initiation system. After completion of polymerization, the viscous copolymer solution is fed into an extruder to remove solvents and volatile substances, by actively degassing through vacuum and heating. The solid granules of basic methacrylate copolymer formed in the extruder can be milled to a powder. The copolymer may contain residual monomers (dimethylaminoethyl methacrylate, butyl methacrylate, methyl methacrylate,). Basic methacrylate copolymer is used as a coating and glazing agent for food supplements and foods for special medical purposes.

**Chemical name**
Poly(butyl methacrylate-co-(2-dimethylaminoethyl)methacrylate-co-methyl methacrylate)

**C.A.S. number**
24938-16-7

**Chemical formula**
Poly[(CH₂:C(CH₃)CO₂(CH₂)₃N(CH₃)₃)-co-(CH₂:C(CH₃)CO₂CH₃)-co-(CH₂:C(CH₃)CO₂(CH₂)₃CH₃)]

**Structural formula**

![Structural formula of Basic Methacrylate Copolymer]

The above formula is provided for illustrative purposes; specific repeat units cannot be defined.

**Formula weight**
47,000 (weight-average), 22,000 (number-average)
Assay

20.8 – 25.5 % dimethylaminoethyl (DMAE) groups on the dried basis

See description under TESTS

DESCRIPTION

White powder

FUNCTIONAL USES

Coating agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Viscosity

3 - 6 mPa·s

Determine viscosity using Brookfield viscometer at 20° and 30 rpm using UL adapter.

Refractive index

$\text{n}^2_{\text{D}}$: 1.380 - 1.385

(Vol. 4)

Solubility (Vol. 4)

Freely soluble in methanol, ethanol, and 1 N aqueous hydrochloric acid

Infrared absorption

The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix.

Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.

PURITY

Loss on drying (Vol. 4)

Not more than 2.0% (110°, 3 h)

Sulfated ash (Vol. 4)

Not more than 0.1%

Test 5 g of the sample (Method I)

Particle size

< 50 μm: at least 95 %
< 20 μm: at least 50 %
< 3 μm: not more than 10 %

See description under TESTS

Residual solvents (Vol. 4)

Methanol: Not more than 50 mg/kg;

Butanol: not more than 50 mg/kg and
Propan-2-ol: not more than 100 mg/kg.

(See General Methods, Organic Components, Residual Solvents, Method 1)

**Residual monomers**

- Dimethylaminoethyl methacrylate: Not more than 500 mg/kg
- Butyl methacrylate: Not more than 100 mg/kg
- Methyl methacrylate: Not more than 50 mg/kg

See description under TESTS

**Lead (Vol. 4)**

Not more than 1.0 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 principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

**Microbiological criteria (Vol. 4)**

- Total plate count: Not more than 1,000 cfu/g
- Yeast and moulds: Not more than 100 cfu/g
- Coliforms: Negative in 10 g

**TESTS**

**PURITY TESTS**

**Particle size**

Determine using the light diffraction measurement method according to Ph. Eur. 2.9.31

(European Pharmacopeia; Particle Size Analysis by Laser Light Diffraction. 8 01/2010:0333)

**Residual monomers**

**Method for the determination of methyl methacrylate and butyl methacrylate:**

**Standards and Reagents:**

- **Phosphate buffer (0.0625 M, pH 2.0):**
  - Prepare an aqueous solution containing 8.9 g of anhydrous dibasic sodium phosphate and 8.5 g of monobasic potassium phosphate in 1 L deionized water. Adjust with phosphoric acid to pH 2.0.

- **Mobile phase:**
  - Prepare a mixture of methanol and pH 2.0 phosphate buffer (55:45).
Diluent:
Acetonitrile:Buffer (40:60)

**Preparation of standard solution**

**Stock mixed standard solution:**
Accurately weigh 20 mg of butyl methacrylate and 10 mg of methyl methacrylate, dissolve in 3 ml of n-butanol and dilute to volume to 10 ml with diluent in a volumetric flask.

**Intermediate mixed standard solution:**
Pipette 1.0 ml of stock mixed standard solution into a 10 ml volumetric flask and dilute to 10 ml with diluent.

**Working mixed standard solution:**
Pipette 1.0 ml of intermediate standard solution into a 25 ml volumetric flask and dilute to volume with diluent. This solution contains about 8 µg/ml of butylmethacrylate and 4 µg/ml of methyl methacrylate.

**Preparation of Sample solution**
Accurately weigh about 1.0 g of sample, dissolve in diluent and make up to 50 ml with diluent and mix.

**Procedure**

Chromatographic system:
The liquid chromatograph is equipped with a UV/diode array detector capable of working at 205 nm and a column (4.6 mm × 12 cm, packing material: octadecylsilane chemically bonded to porous silica or ceramic microparticles, 1.5-10 µm)). Flow rate: 2 ml/min.

Chromatograph the working standard solution, and record the peak responses. The resolution, R, between butyl methacrylate and methyl methacrylate is not less than 10; and the relative standard deviation for replicate injections is not more than 3.0%.

Separately inject 50 µl each of the working standard solution and sample solution and record the peak areas of the monomers.

Calculate the quantity of each monomer in the sample using the formula:

\[
\text{Monomer concentration (µg/g)} = \frac{rU}{rS} \times \frac{CS}{CU} \times F
\]

- \(rU\) = Peak area for the monomer in the sample chromatogram
- \(rS\) = Peak area for the monomer in the working standard chromatogram
- \(CS\) = Concentration of monomer in the working standard solution (µg/ml)
- \(CU\) = Concentration of polymer in the sample solution (mg/ml)
- \(F\) = Conversion factor (10³ mg/g)
Method for the determination of 2-Dimethylaminoethyl methacrylate

Standards and Reagents:
Monobasic potassium phosphate buffer solution (0.025M):
Prepare an aqueous solution containing 3.4 g of monobasic potassium phosphate per litre.

Mobile phase:
Tetrahydrofuran:monobasic potassium phosphate buffer solution (75:25).

Preparation of standard solution
Stock standard solution (200 µg/ml):
Accurately weigh about 20 mg of (2-dimethylaminoethyl) methacrylate, dissolve in tetrahydrofuran, make up to volume in a 10 ml volumetric flask with tetrahydrofuran and mix.

Working standard solution (8 µg/ml):
Dilute 2.0 ml of the stock standard solution to 50 ml in a volumetric flask with tetrahydrofuran and mix.

Preparation of Sample solution
Accurately weigh about 1.0 g of sample, dissolve in tetrahydrofuran, dilute to 50 ml with tetrahydrofuran in a volumetric flask and mix.

Procedure
Chromatographic system:
The liquid chromatograph is equipped with a UV/diode array detector capable of working at 215 nm and a column (4.6 mm × 12 cm, packing material: an essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5-10 µm) . Flow rate: 2 ml/min.

Chromatograph the working standard solution, and record the peak area. The relative standard deviation for replicate injections is not more than 2.0%.

Separately inject 50 µl of the working standard solution and the sample solution and record the peak areas.

Calculate the quantity of each monomer in the sample using the formula:

\[
2-\text{Dimethylaminoethyl methacrylate, (µg/g)} = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times F
\]

\[
r_U = \text{Peak area for the monomer in the sample chromatogram}
\]
\[
r_S = \text{Peak area for the monomer in the standard chromatogram}
\]
CS = Concentration of monomer in the working standard solution (µg/ml)
CU = Concentration of polymer in the sample solution (mg/ml)
F = Conversion factor (10³ mg/g)

**METHOD OF ASSAY**

Determine the percentage of Dimethylaminoethyl (DMAE) groups using a potentiometric titration.

Dissolve 200 mg of dried sample in 4 ml water and 96 ml of glacial acetic acid. Titrate with 0.1 N standard perchloric acid solution to a potentiometric end point. Perform a blank determination.

\[
DMAE \text{ groups}\% (\text{w/w}, \text{on the dried basis}) = \frac{(VS - VB) \times N \times F}{W} \times 100
\]

VS = titrant volume consumed by the sample (ml)
VB = titrant volume consumed by the blank (ml)
N = actual normality of the titrant (mEq/ml)
F = equivalency factor, 72.1 mg/mEq
W = dried sample weight (mg)

Appendix: Infrared spectrum of basic methacrylate copolymer
CASSIA GUM

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018), superseding tentative specifications prepared at the 82nd JECFA (2016) and published in FAO JECFA Monographs 19 (2016). An ADI “not specified” was established at the 71st JECFA (2009)

SYNONYMS

INS 427

DEFINITION

Cassia gum is obtained from the ground purified endosperm of the seeds of Cassia tora and Cassia obtusifolia (Fam. Leguminosae) containing less than 0.05% of Cassia occidentalis. It consists mainly of high molecular weight (approximately 200,000-300,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 5:1. The seeds are dehusked and degermed by thermal and mechanical treatment followed by milling and screening of the endosperm. The ground endosperm is purified by extraction with isopropanol.

Assay

Not less than 75% of galactomannans

DESCRIPTION

Pale yellow to off-white, odourless free-flowing powder. Forms colloidal solutions in cold water.

FUNCTIONAL USES

Thickener, emulsifier, foam stabilizer, moisture retention agent, and texturizing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility

Insoluble in ethanol

Gel formation with borate

Add sodium borate TS to an aqueous dispersion of the sample to raise the pH above 9; a gel is formed.
Gel formation with xanthan gum

Passes test
See description under TESTS

Gum constituents (Vol. 4)
Proceed as directed under ‘Gum Constituents Identification’ using 100 mg of sample (instead of 200 mg) and 1-10 µl of the hydrolysate (instead of 1-5 µl). Use galactose and mannose as reference standards. These constituents should be present.

Viscosity
Less than 500 mPa × s
See description under TESTS

pH (Vol. 4)
5.5-8.0 (1% solution)

PURITY

Loss on drying (Vol. 4)
Not more than 12% (105º, 5 h)

Total ash (Vol. 4)
Not more than 1.2%

Acid-insoluble matter (Vol. 4)
Not more than 2.0%

Protein (Vol. 4)
Not more than 7.0%
Multiply percent nitrogen by 6.25.

Crude fat
Not more than 1%
See description under TESTS

Starch
To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.

Anthraquinones
Not more than 0.5 mg/kg
See description under TESTS

Residual solvents (Vol. 4)
Isopropanol: Not more than 1.0%
See description under TESTS

Lead (Vol. 4)
Not more than 1 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").

**Microbiological criteria (Vol. 4)**

- Total plate count: Not more than 5,000 cfu/g
- Yeast and mould: Not more than 100 cfu/g
- *E. coli*: Negative in 1 g
- *Salmonella*: Negative in 25 g

**TESTS**

**IDENTIFICATION TESTS**

**Gel formation with xanthan gum**

Weigh 1.5 g of sample, 1.5 g of xanthan gum and blend them. Add this blend (with rapid stirring) to 300 ml water at 80º in a 400 ml beaker. Stir until the mixture is dissolved and continue stirring for an extra 30 min after dissolution (maintain the temperature above 60º during the stirring period). Discontinue stirring and allow the mixture to cool to room temperature for at least 2 h.

A firm, viscoelastic gel forms after the temperature drops below 40º, but no such gel forms in a 1% control solution of cassia gum or xanthan gum alone prepared in a similar manner.

**Viscosity**

Add 495 ml of deionized water into a 1L beaker, insert a magnetic stir bar and place the beaker on a magnetic stirrer equipped with a heater. Adjust the stirrer speed to about 750 rpm. Weigh 5 g of sample and quickly add to the beaker. Switch on the heater and heat the beaker to reach 90º and keep it at 90º for 15 min. Cool the solution to room temperature (25º ±1.5º) in a water bath. Measure the viscosity at 25º, after 2 h, using a RVT Brookfield Spindle 1 and 20 rpm speed. Repeat the procedure with a sample of 5 g of carob (locust) bean gum. The viscosity of the cassia gum (150 – 500 mPa × s) must be less than 50% that of carob bean gum (2000 - 3500 mPa × s)

**PURITY TESTS**

**Residual solvents**

Determine residual solvents using headspace gas chromatography (Vol. 4; Method I) under the following conditions.

Internal standard solution
Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 µl of 3-methyl-2-pentanone through the septum and reweigh the vial to within 0.01 mg.

Standard solution
Add 50.0 ml water to a 50 ml vial and seal weigh accurately. Inject 15 µl isopropanol and reweigh the vial.

Blank solution:
Add 5.0 ml of water and pipette 1.0 ml of the internal standard solution into a headspace vial. Seal the vial and mix the contents using a vortex mixer.

Calibration solution:
Add 4.0 ml of water into the headspace vial. Pipette 1.0 ml each of the internal standard solution and the standard solution. Seal the vial and mix the contents using a vortex mixer.

Preparation of sample:
Pipette 5 ml of water and 1 ml internal standard solution into a headspace vial. Accurately weigh 0.500 ± 0.001 g of sample in a small weighing boat and add the sample carefully to prevent clumping of sample at the bottom of the vial. Seal the vial and mix the contents using a vortex mixer. Do not shake the sample vial.

Follow the procedure described in Vol. 4 for the determination of residual solvents.

Crude fat

Apparatus
The apparatus consisting of a Butt-type extractor, as shown below, having a standard-taper 34/45 female joint at the upper end, to which is attached a Friedrichs- or Hopkins-type condenser, and a 24/40 male joint at the lower end, to which is attached a 125-ml Erlenmeyer flask.

Procedure
Transfer about 10 g of the sample, previously ground to 20-mesh or finer and accurately weighed, to a cellulose thimble or a 15-cm filter paper (roll the paper tightly around the sample), and place it in a suitable extraction shell. Plug the top of the thimble or the extraction shell with cotton previously extracted with hexane, and place it in the extractor. Attach the extractor to a dry 125-ml Erlenmeyer flask containing about 50 ml of hexane and to a water-cooled condenser, apply heat to the flask to produce 150 to 200 drops of condensed solvent per min, and extract for 16 h. Disconnect the flask, and filter the extract to remove any insoluble residue. Rinse the flask and filter with a few ml of hexane, combine the washings and filtrate in a tared flask, and evaporate on a steam bath until no odour of solvent remains. Dry in a vacuum for 1 h at 100°, cool in a desiccator, and weigh.
Anthraquinones

**Principle**

Anthraquinones are extracted with chloroform and determined by High Performance Liquid Chromatography (Vol.4) using the conditions below.

**NOTE:** Anthraquinones are photosensitive. Samples and standards shall be protected from light and all manipulations shall be carried out under the subdued light.

**Standards and Reagents:**

- Emodin, Aloe-emodin, Physcion (1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone), Rhein and Chrysophanic acid (>99%).
- Internal standard: Danthrone (1,8-dihydroxy-anthraquinone, >99%)
- Methanol, acetonitrile, deionized water, chloroform, trifluoroacetic acid, sulfuric acid and sodium hydrogen carbonate

**Individual stock standard and internal standard solutions (100 µg/ml)**

Accurately weigh about 10 mg of the standards and internal standard, transfer to 100 ml volumetric flasks with about 5 ml of methanol, sonicate for 15 min and dilute to volume with methanol.

Store these solutions in amber coloured bottles at 4° (the solutions are stable for 2 weeks under these conditions).
Internal spike standard solution (20 µg/ml)
Dilute 2 ml of internal standard stock solution to 10 ml with methanol.

Mixed standard solution (10 µg/ml)
Transfer 1 ml of each of the anthraquinones stock standard solution into a 10 ml volumetric flask and dilute to volume with methanol.

Working standard solutions
To each of five 10 ml volumetric flasks transfer 0, 0.5, 1, 2 and 5 ml respectively of the mixed standard solution, and 1 ml of the internal spike standard solution (20 µg/ml), dilute to volume with methanol and mix.

Sample preparation:
Accurately weigh about 4.0 g of the sample into a 250 ml Erlenmeyer flask. Add 80 µl of internal standard solution (100 µg/ml), and 100 ml 2N H₂SO₄ to the flask. Stopper the flask using a PTFE stopper and heat at 103º for 3.5 hours in an oven. After cooling to room temperature, add 100 ml of chloroform and shake well. Allow phase separation. Evaporate 50 ml of the chloroform layer to dryness in a rotary evaporator at 68º. Dissolve the residue in 2 ml of methanol. Filter the solution through a PTFE membrane syringe filter.

Chromatographic conditions:
Column: Hypersil ODS C₁₈ (250 mm x 4.6 mm ID, 5 µm)
Mobile phase:
(A) 0.1 % trifluoroacetic acid in water
(B) Acetonitrile
Injection volume: 20 µl

Gradient:

<table>
<thead>
<tr>
<th>Time, min</th>
<th>% (A)</th>
<th>% (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>86</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>86</td>
<td>14</td>
</tr>
<tr>
<td>15</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
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</tr>
<tr>
<td>55</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>66</td>
<td>86</td>
<td>14</td>
</tr>
</tbody>
</table>

Flow rate: 1 ml/min
Detector: Photodiode Array/UV Detector operated at 435 nm.
Procedure
Inject individual standard solutions and internal standard solution (dilute, if required) and record retention times.

Construction of standard curves
Inject 20 µl of each working standard solution. Construct the standard curves by plotting the ratios of the peak areas of each of the specific anthraquinone / internal standard against the concentrations of each working standard solution (µg/ml).

Inject 20 µl of the Sample solution. Calculate the ratios of the peak areas of each anthraquinone / internal standard, and obtain the amount (A) of each anthraquinone from the respective standard curve.

Concentration of anthraquinone in the sample (µg/g) = \( \frac{A \times 4}{W} \)

Where
- A = the amount of each anthraquinone (µg) obtained from the standard curve
- W = Mass of sample (g)
- 4 = Dilution factor for sample

**METHOD OF ASSAY**

\[
\%	ext{ Galactomannans} = 100 - (L + A + I + P + F)
\]

- L  % Loss on Drying
- A  % Total Ash
- I  % Acid-Insoluble Matter
- P  % Protein
- F  % Crude Fat
CITRIC AND FATTY ACID ESTERS OF GLYCEROL (TENTATIVE)

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monographs 22 (2018), superseding specifications prepared at the 82nd JECFA (2016), and published in FAO JECFA Monographs 19 (2016). An ADI 'not limited' was established at the 17th JECFA (1973)

Information required:
- A validated method for the determination of total citric acid content
- Performance characteristics (method validation data) of the citric acid determination method
- Data on the total citric acid content, in at least five batches of products currently available in commerce, determined using the above method.

SYNONYMS
Citric acid esters of mono- and di-glycerides, citroglycerides, CITREM; INS No. 472c

DEFINITION
Citric and fatty acid esters of glycerol (CITREM) consists of mixed esters of citric acid and edible fatty acids with glycerol. It may contain free fatty acids, glycerol, citric acid and mono- and diglycerides, in minor quantities. The mono- and di- glycerides may include either one or two edible fatty acids from C12:0 to C18:0, mainly the saturated palmitic (C16:0) and stearic (C18:0) acids. It may also contain minor amounts of other fatty acids such as myristic (C14:0), oleic (C18:1), linoleic (C18:2) and arachidic acid (C20:0). CITREM is obtained by esterification of glycerol with citric acid and edible fatty acids, or by reaction of a mixture of mono- and diglycerides of edible fatty acids, with citric acid. CITREM may be partially or wholly neutralized with sodium hydroxide or potassium hydroxide.

Structural formula

\[
\begin{align*}
\text{CH}_2 & - \text{OR}_1 \\
\text{CH} & - \text{OR}_2 \\
\text{CH}_2 & - \text{OR}_3 
\end{align*}
\]

Where at least one of R1, R2 or R3 represents a citric acid moiety, one represents a fatty acid moiety and the remainder may represent citric acid, fatty acid or hydrogen.

DESCRIPTION
White to ivory coloured, oily to waxy material.

FUNCTIONAL USES
Stabilizer, emulsifier, dough conditioner, antioxidant synergist
CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)  Insoluble in water; soluble in oils and fats; insoluble in ethanol

Test for fatty acids  (Vol. 4)  Passes test

Test for citric acid  Information required

Test for glycerol  (Vol. 4)  Passes test

PURITY

Sulfated ash (Vol. 4)  Non-neutralized products: not more than 0.5%
Partially or wholly neutralized products: not more than 10%; test 2 g of the sample (Method I)

Free glycerol (Vol. 4)  Not more than 4%

Total glycerol  8-33%

See description under TESTS

Total citric acid  13-50%

(Information required)

Total fatty acid  37-81%

See description under TESTS

Lead (Vol. 4)  Not more than 2 mg/kg.
(Not more than 0.1 mg/kg for use in infant formula and formula for special medical purposes intended for infants)

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 methods described in Volume 4 (under “General Methods, Metallic Impurities”).
TESTS

PURITY TESTS

**Total glycerol**  
CITREM is hydrolyzed, glycerol in the aqueous phase is oxidized using known excess of sodium periodate in a strongly acid medium and the unreacted periodate is back titrated using standard sodium thiosulfate solution.

Procedure:
Accurately weigh about 2 g of the sample into a saponification flask, add 50 ml of 0.5 M ethanolic potassium hydroxide, and reflux for 30 min.

To a 1-L volumetric flask add 99 ml ± 0.2 ml of chloroform using a burette and add 25 ml of glacial acetic acid using a graduated cylinder. Quantitatively transfer the content of the saponification flask to the volumetric flask, using three 25 ml portions of water. Add about 500 ml of water further, and shake vigorously for about 1 min. Dilute to volume with water, stopper, mix thoroughly and set aside for separation of layers.

Pipet 50 ml of acetic periodic acid TS into a series of 400 ml beakers. Prepare two blanks by adding 50 ml of water to each. Pipet 50 ml of the aqueous layer into one of the 400 ml beakers containing 50 ml of acetic periodic acid TS; shake gently to mix; cover with watch glass, and allow to stand 30 min but not longer than 1.5 h. Add 20 ml of 15% potassium iodide solution, shake gently to mix, and allow to stand at least 1 min. but not more than 5 min. Do not allow to stand in bright or direct sunlight. Add 200 ml of water and titrate with 0.1 N sodium thiosulfate. Use a variable speed electric stirrer to keep the solution thoroughly mixed. Continue the titration to the disappearance of the brown iodine colour from the aqueous layer. Add 2 ml of starch TS and continue the titration to the disappearance of iodine from the tiny chloroform layer separated during titration and the disappearance of the blue iodine-starch complex colour from the aqueous layer. Read the burette to the nearest 0.01 ml. Treat the blanks in the same way as the sample.

**Calculation**

\[
\% \text{ total glycerol} = \frac{(B - S) \times N \times 2.302 \times 900}{W \times 50}
\]

where

- \(B\) volume of 0.1 N sodium thiosulfate used for the blank, ml
- \(S\) volume of 0.1 N sodium thiosulfate used for the sample, ml
- \(N\) exact normality of 0.1 N sodium thiosulfate
- \(W\) mass of sample, g

**Total citric acid**  
*Information required*
**Total fatty acid**

**Principle:** This method measures total fatty acids by extracting with diethyl ether.

**Procedure**

Weigh accurately 5 g of the sample into a 250-ml round-bottomed flask, add 50 ml of potassium hydroxide, ethanolic, TS, and reflux for 1 h on a boiling water bath.

Quantitatively transfer the contents of the saponification flask to a 1,000 ml separating funnel, using three 25 ml portions of water, and add 5 drops of methyl orange indicator solution.

Cautiously add 50% hydrochloric acid until the colour of solution changes to orange red. Add 1 ml of excess acid. Shake well to mix the contents and separate the fatty acids.

Cool to room temperature and extract the separated fatty acids with three 100 ml portions of diethyl ether. Combine the extracts, and wash with 50 ml portions of 10% sodium chloride solution until the washed sodium chloride solution becomes neutral.

Dry the ether solution with anhydrous sodium sulfate. Then evaporate off ether on a steam bath, leave additional 10 min on the steam bath, and weigh the residue. This is the weight of the total fatty acids.

**Calculation:**

\[
\text{Total Fatty acids \%} = \frac{\text{mass of fatty acids g} \times 100}{\text{mass of sample g}}
\]
ERYTHROSINE


SYNONYMS

INS No. 127, CI Food Red 14, CI (1975) No. 45430, Food Red No. 3, FD&C Red No. 3

DEFINITION

Erythrosine consists of the disodium salt of 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Erythrosine is manufactured by iodination of fluorescein, the condensation product of resorcinol and phthalic anhydride.

Erythrosine may be converted to the corresponding aluminium lake in which case only the requirements in the General Specifications for Aluminium Lakes of Colouring Matters apply.

Chemical names

Disodium 2-(2,4,5,7-tetraiodo-6-oxido-3-oxoxanthen-9-yl)benzoate monohydrate;

Disodium;2',4',5',7'-tetraiodofluorescein monohydrate

Disodium 2',4',5',7'-tetraiodofluorescein monohydrate

C.A.S. number 16423-68-0

Chemical formula C_{20}H_{14}Na_{2}O_{5} \cdot H_{2}O

Structural formula

\[
\begin{array}{c}
\text{\includegraphics[width=0.3\textwidth]{erythrosine_structural_formula.png}}
\end{array}
\]

Formula weight 879.86

Assay Not less than 87% total colouring matters
**DESCRIPTION**
Red powder or granules

**FUNCTIONAL USES**
Colour

**CHARACTERISTICS**

**IDENTIFICATION**

<table>
<thead>
<tr>
<th><strong>Solubility</strong> (Vol. 4)</th>
<th>Soluble in water, slightly soluble in ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spectrophotometry</strong> (Vol. 4)</td>
<td>Maximum wavelength approximately 527 nm</td>
</tr>
<tr>
<td></td>
<td>Determine the UV-visible absorption spectrum of the sample dissolved in water.</td>
</tr>
</tbody>
</table>

**PURITY**

<table>
<thead>
<tr>
<th><strong>Loss on drying, chloride and sulfate as sodium salts</strong> (Vol. 4)</th>
<th>Not more than 13%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determine chloride as sodium chloride, sulfate as sodium sulfate, and loss on drying (135°, 6 h) as described in Volume 4 (under “Specific Methods, Food Colours”).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Inorganic iodides</strong></th>
<th>Not more than 0.1% calculated as sodium iodide</th>
</tr>
</thead>
<tbody>
<tr>
<td>See description under TESTS</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Water insoluble matter</strong> (Vol. 4)</th>
<th>Not more than 0.2%</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Zinc</strong> (Vol. 4)</th>
<th>Not more than 50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>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”).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Lead</strong> (Vol. 4)</th>
<th>Not more than 2 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>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”).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Subsidiary colouring matters</strong></th>
<th>Not more than 4% (except fluorescein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>See description under TESTS</td>
<td></td>
</tr>
</tbody>
</table>
Note: Do not allow the sample and standard solutions to be exposed to direct sunlight.

**Fluorescein**

Not more than 20 mg/kg

See description under TESTS

**Organic compounds other than colouring matters**

Triiodoresorcinol: Not more than 0.2%

2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid: Not more than 0.2%

See description under TESTS

**Ether extractable matter (Vol. 4)**

From a solution of pH not less than 7, not more than 0.2%

**Hydrochloric acid-insoluble matter in Erythrosine Lake**

Not more than 0.5%

See description under TESTS

### TESTS

#### PURITY TESTS

**Inorganic iodides**

Weigh 1.0 g of the sample into a 100-ml beaker. Add 75 ml distilled water and a magnetic stirrer. Stir to dissolve. Immerse an iodide specific electrode and a reference electrode in the solution and use a suitable millivoltmeter to read the potential of the system in millivolts.

Add 0.001 M silver nitrate solution from a burette initially in 0.5 ml aliquots, reducing these to 0.1 ml as the end-point approaches as indicated by an increasing change in potential for each addition. After allowing time for the reading to stabilize, record the millivolt readings after each addition. Continue the titration until further additions make little change in the potential.

Plot the millivolt readings against the volume of silver nitrate solution added. The equivalence point is the volume corresponding to the maximum slope of the curve.

The percentage of sodium iodide in the sample = Titre × 0.015%

where
Titre

ml-equivalent of silver nitrate solution

0.015% 0.001 mol/l \times 10^{-3} l/ml \times 149.89 g sodium iodide/mol \times 1 mol/equivalent \times 1/1.0 g (sample weight) \times 100.

Subsidiary colouring matters

Determine subsidiary colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C8 (250 mm x 4.6 mm i.d., 5 µm particle size)
- Eluent A: 0.1 M ammonium acetate in water
- Eluent B: methanol
- Injection volume: 20 µl
- Column temperature: ambient
- Detector: UV-visible/diode array at 514 nm
- Flow rate: 1.0 ml/min

Gradient:

<table>
<thead>
<tr>
<th>Elution time (min)</th>
<th>Eluent A (%)</th>
<th>Eluent B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>20</td>
<td>34</td>
<td>66</td>
</tr>
<tr>
<td>21.1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>25.5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>26.0</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>40.0</td>
<td>55</td>
<td>45</td>
</tr>
</tbody>
</table>

Reagents: HPLC grade

Standards:

- 2',4',5'-Triiodofluorescein (C.A.S. 56254-06-9) – synthesized material (see Appendix)
- 2',4',7'-Triiodofluorescein (C.A.S. 83498-90-2) – synthesized material (see Appendix)
- 4',5'-Diiodofluorescein, disodium salt (C.A.S. 33239-19-9) – Alfa Aesar, Cat. No. A15626 or equivalent
- 2'-Monoiodofluorescein, disodium salt (C.A.S. 52010-85-2) – synthesized material (see Appendix)
- 4'-Monoiodofluorescein, disodium salt (C.A.S. 52010-86-3) – synthesized material (see Appendix)
- Erythrosine (C.A.S. 16423-68-0) – TCI, >95.0% disodium 2',4',5',7'-tetraiodofluorescein, Cat. No. F0139 or equivalent (use if subsidiary colouring matter standards are not available)

Prepare standard solutions as required.
Sample preparation:
Weigh accurately 200±2 mg sample and dissolve in 100 ml of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component in order to improve their resolution.

Calculations:
Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 514 nm. If Erythrosine is used as a standard, calculate the ratio of the sum of all peaks not corresponding to Erythrosine to the sum of all peaks.

**Fluorescein**
Determine fluorescein by the test for subsidiary colouring matters content except use the following conditions:
- Injection volume: 50 μl
- Detector: UV-visible/diode array at 492 nm

Standard: Fluorescein, disodium salt (C.A.S. 518-47-8) – TCI, Cat. No. F0096 or equivalent

Sample preparation:
Weigh accurately 2.00±0.05 g sample and dissolve in 10 ml of water.

**Organic compounds other than colouring matters**
Determine organic compounds other than colouring matters by reversed-phase HPLC (Vol. 4) using the following conditions:
- Column: C18 (150 mm x 2.1 mm i.d., 5 μm particle size)
- Eluent A: 0.05 M sodium dihydrogen phosphate in 95/5 water/methanol, pH 4.0
- Eluent B: methanol
- Injection volume: 5 μl
- Column temperature: 27°
- Detector: UV-visible/diode array at 223 nm
- Flow rate: 0.5 ml/min

Gradient:

<table>
<thead>
<tr>
<th>Elution time (min)</th>
<th>Eluent A (%)</th>
<th>Eluent B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>13</td>
<td>35</td>
<td>65</td>
</tr>
</tbody>
</table>
Reagents: HPLC grade

Standards:

- 2,4,6-Triiodoresorcinol (C.A.S. 19403-92-0) – Alfa Chemistry, Cat. No. ACM19403920 or equivalent
- 2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic acid (C.A.S. 3480-21-5) – Wako, Cat. No. 043-32981 or equivalent

Prepare standard solutions as required. Dissolve the standards in methanol. Use an amber glass volumetric flask for 2,4,6-triiodoresorcinol and prepare the standard and calibration solutions immediately before use.

Sample preparation:

Weigh accurately 100±5 mg sample and dissolve in 10 ml of methanol.

Calculations:

Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 223 nm.

Hydrochloric acid-insoluble matter in Erythrosine Lake

Reagents

- Concentrated hydrochloric acid
- Hydrochloric acid, 0.5% v/v
- Dilute ammonium hydroxide solution (dilute 10 ml of 14.5 M ammonium hydroxide to 100 ml with water).

Procedure

Accurately weigh approximately 5 g of the lake into a 500-ml beaker. Add 250 ml water and 60 ml concentrated hydrochloric acid. Boil to dissolve the alumina while the Erythrosine converts to its "free acid" form, which is insoluble in acid. Filter through a tared No. 4 sintered glass crucible. Wash the crucible with a small amount of hot 0.5% hydrochloric acid and then with some hot distilled water. Remove the acid filtrate from the filter flask, replace the crucible, and wash with hot dilute ammonium hydroxide solution until the washings are colourless. Dry the crucible to constant weight at 135°. Express the residue as a percentage of the weight taken.
METHOD OF ASSAY

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and an appropriate solvent.

Using water as the solvent:
- absorptivity $(a) = 110 \text{ l/(g} \times \text{cm)}$
- wavelength of maximum absorbance = 527 nm.
GLYCEROL ESTER OF WOOD ROSIN

Prepared at the 86th JECFA and published in FAO JECFA Monographs 22 (2018), superseding specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI of 0-25 mg/kg bw for glycerol ester of wood rosin was established at the 77th JECFA (2013).

SYNONYMS
INS No. 445(iii)

DEFINITION
Glycerol ester of wood rosin (GEWR) is a complex mixture of glycerol di- and tri-esters of resin acids from wood rosin, with a residual fraction of glycerol monoesters. In addition, neutrals (non-acidic saponifiable and unsaponifiable substances) and residual free resin acids are present. Wood rosin is obtained by the solvent extraction of aged pine stumps, followed by a liquid-liquid solvent refining process. Refined wood rosin is composed of approximately 90% resin acids and approximately 10% neutrals. The resin acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula C₂₀H₃₀O₂, of which the main components are dehydroabietic and abietic acids. GEWR is produced by esterifying the resin acids with food grade glycerol. The product is then purified by steam stripping or by direct countercurrent steam distillation.

These specifications do not cover substances derived from gum rosin, an exudate of living pine trees, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing.

C.A.S. number 8050-30-4

DESCRIPTION
Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES
Emulsifier, density adjustment agent (flavouring oils in beverages), stabilizer, plasticizer (in chewing gum bases).

CHARACTERISTICS

IDENTIFICATION

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

Infrared absorption (Vol. 4) The infrared spectrum of a thin film of the sample (potassium bromide disc) corresponds with the typical infrared spectrum below
Sulfur test

Negative

Weigh 40-50 mg of sample into a test tube and add 1-2 drops of a 20% (w/v) solution of sodium formate. Place a strip of lead acetate test paper over the mouth of the test tube. Heat the tube until fumes are formed that contact the test paper. Continue heating for 2-5 min. The formation of a black spot of lead sulfide indicates the presence of sulfur-containing compounds. (Detection Limit: 50 mg/kg sulfur)

Gas chromatography of resin acids and glycerol

Passes test

See description under TESTS

PURITY

Specific gravity (Vol. 4)

d (20, 25): Not less than 0.935 (50% solution in d-limonene)

Ring and ball softening point (Vol. 4)

Not less than 82° (see “Specific Methods, Glycerol Esters of Rosins”)

Acid value (Vol. 4)

Between 3 and 9 (see “Specific Methods, Fats, Oils, and Hydrocarbons”)

Lead (Vol. 4)

Not more than 1 mg/kg

Determine using 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

IDENTIFICATION TESTS

Gas chromatography of resin acids

The ester groups in the glycerol esters of wood rosin are reduced with a metal hydride to form a mixture of corresponding resin alcohols and glycerol which are analyzed by gas chromatography (Vol. 4). The characteristic chromatogram shows predominant peaks for abietic and dehydroabietic alcohols.

Apparatus

- Gas Chromatograph equipped with a flame ionization detector.
- Centrifuge: table top, capable of achieving 3200 rpm
Standards and reagents
- Internal Standard (1,4-Butanediol: >99%)
- Toluene
- Sodium Vitride Reagent [(Sodium bis(2-methoxyethoxy) aluminium dihydride], 70% in toluene: (~3.5 mol/l)

Sodium Vitride solution:
Pipet 10.0 ml of sodium vitride reagent into a 100 ml volumetric flask dilute to volume with toluene and mix thoroughly.

Hydrolysis solution:
Slowly add 50 ml of concentrated sulfuric acid, reagent grade, to 200 ml distilled water while stirring in an ice bath. Cool to room temperature.

Procedure

Sample preparation
Weigh 250-300 mg sample into a 25 ml Erlenmeyer flask containing a Teflon coated stirrer bar. Pipet 5.0 ml toluene into the flask and stir until sample is dissolved. Pipet 5.0 ml of sodium vitride solution into the flask, stopper the flask and stir for 30 min. While stirring, pipet 3.0 ml of hydrolysis solution into the flask. Continue stirring for 3 min. Transfer contents of flask to centrifuge tube (15 ml), stopper, and shake vigorously. Vent and centrifuge at 2800-3200 rpm for 5 min. Inject 0.5 µl of the toluene layer into the gas chromatograph operating under the following conditions and record the chromatogram. Compare with the chromatogram shown below to verify the approximate retention order of the resin alcohols.

Chromatographic conditions
- Column: DB-1 methyl silicone (bonded and crosslinked) wide-bore capillary (15 m x 0.53 mm i.d., 1.5 µm).
- Injector: Flash vaporization injector
- Flow rates: Carrier Gas (He): 30 ml/min at 63 psi, Hydrogen: 30 ml/min and Air: 240 ml/min
- Temperatures: Column: Isothermal, 190º; Injector: 250º, and Detector: 250º

Gas chromatography of glycerol
Standards and reagents
- Glycerol: >99%
- 1,4-Butanediol (Internal standard): >99%

Internal Standard Solution:
Weigh 0.1 g of 1,4-butanediol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly.
Glycerol solution:
Weigh 0.1 g of 1,4-butanediol and 0.1 g glycerol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly

Phenolphthalein Solution: 1% in ethanol.

Sodium Hydroxide Solution:
Dissolve 16 g of reagent grade NaOH in 70-80 ml of distilled water and cool to room temperature. Dilute to 100 ml with distilled water and mix thoroughly. Store in a polyethylene bottle.

Procedure
Sample preparation
Proceed as in the sample preparation for the analysis of resin acids until the centrifugation step. Using a pipet or syringe, remove the toluene layer and part of the aqueous layer leaving approximately 2 ml of the aqueous layer in the centrifuge tube. Add 1 drop of phenolphthalein solution to the remaining aqueous layer in the centrifuge, and neutralize with the sodium hydroxide solution (aluminium salts will precipitate). Pipet 5 ml of the internal standard solution into the tube, dilute to 15 ml with distilled water, stopper, shake, and then centrifuge at 2800-3200 rpm for 5 min. Inject 1 µl of the clear supernatant liquid into the gas chromatograph operating under the following conditions and record the chromatogram. Inject 1 µl of the glycerol solution and record the chromatogram. Measure the retention times of any observed peaks relative to 1,4-butanediol. Compare retention times to that of glycerol standard.

Chromatographic conditions
- Column: DB-WAX polyethylene glycol (bonded and cross-linked), wide bore capillary (15 m x 0.53 mm i.d., 1.0 µm)
- Flow rates: Carrier Gas (He): 30 ml/min at 60 psi, Hydrogen: 30 ml/min and
- Air: 240 ml/min
- Temperatures: Column: Programmed, 120 to 200º at 6º /min; Injector: 250º,
  - and Detector: 250º

Gas chromatography of resin acids in GEWR (determined as alcohols)
Typical GC-FID chromatogram of a GEWR sample. Retention times correspond to pimaric (18.8 min), isopimaric (22.4 min), palustric (23.1 min), dehydroabietic (25.9 min), abietic (29.6 min), and neoabietic (35.2 min) alcohols. This is a product
derived from a plant-based source which can demonstrate significant variability and relative intensities.

Gas chromatogram

FTIR Spectrum of Glycerol esters of wood rosin
INDIGOTINE


SYNONYMSS

INS No. 132, CI Food Blue 1, CI (1975) No. 73015, Indigo Carmine, Food Blue No. 2, FD&C Blue No. 2

DEFINITION

Indigotine consists of a mixture of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate and disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate and subsidiary colouring matters. Sodium chloride and/or sodium sulfate are the principal uncoloured components. Indigotine is manufactured by heating indigo in the presence of sulfuric acid. The indigo (or indigo paste) is manufactured by the fusion of N-phenylglycine (prepared from aniline and formaldehyde) in a molten mixture of sodamide and sodium and potassium hydroxides under ammonia pressure. It is isolated and subjected to purification procedures prior to sulfonation.

Indigotine may be converted to the corresponding aluminium lake in which case only the requirements in the General Specifications for Aluminium Lakes of Colouring Matters apply.

Chemical names

Disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,5'-disulfonate
Disodium (2E)-3-oxo-2-(3-oxo-5-sulfonato-2,3-dihydro-1H-indol-2-ylidene)-2,3-dihydro-1H-indole-5-sulfonate
Disodium;(2E)-3-oxo-2-(3-oxo-5-sulfonato-1H-indol-2-ylidene)-1H-indole-5-sulfonate

C.A.S. number
860-22-0 (5,5' isomer)

Chemical formula
C_{16}H_{8}N_{2}Na_{2}O_{8}S_{2}

Structural formula

Formula weight
466.36
**Assay**
- Not less than 85% total colouring matters
- Not more than 18% of disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate

**DESCRIPTION**
Blue powder or granules

**FUNCTIONAL USES**
Colour

**CHARACTERISTICS**

**IDENTIFICATION**

**Solubility (Vol. 4)**
Soluble in water, sparingly soluble in ethanol

**Spectrophotometry (Vol. 4)**
Maximum wavelength approximately 610 nm

Determine the UV-visible absorption spectrum of the sample dissolved in water.

**PURITY**

**Loss on drying, chloride and sulfate as sodium salts (Vol. 4)**
Not more than 15%

Determine chloride as sodium chloride, sulfate as sodium sulfate, and loss on drying (135°, 6 h) as described in Volume 4 (under “Specific Methods, Food Colours”).

**Water insoluble matter (Vol. 4)**
Not more than 0.2%

**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”).

**Subsidiary colouring matters**
Not more than 18% disodium 3,3'-dioxo-[delta2,2'-biindoline]-5,7'-disulfonate (isomeric subsidiary colouring matter)

Not more than 1% other subsidiary colouring matters

See description under TESTS

**Organic compounds other**
Not more than 0.5% of sum of isatin-5-sulfonic acid, 5-
than colouring matters  sulfoanthranilic acid, and anthranilic acid

See description under TESTS

Unsulfonated primary aromatic amines (Vol. 4)

Not more than 0.01% calculated as aniline

Ether extractable matter (Vol. 4)

Not more than 0.2%

Weigh accurately about 2 g sample instead of the 5 g stated in the general methods

TESTS

PURITY TESTS

Subsidiary colouring matters

Determine subsidiary colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:

- Column: C18 (250 mm x 4 mm i.d., 5 µm particle size)
- Eluent A: 0.2 M ammonium acetate in water
- Eluent B: acetonitrile
- Injection volume: 20 µl
- Column temperature: ambient
- Detector: UV-visible/diode array at 610 nm
- Flow rate: 1.0 ml/min

Gradient:

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<tr>
<th>Elution time (min)</th>
<th>Eluent A (%)</th>
<th>Eluent B (%)</th>
</tr>
</thead>
<tbody>
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</tr>
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<tr>
<td>40.0</td>
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</table>

Reagents: HPLC grade

 Standards:

- Indigotine disodium salt (5,7' isomer) (isomeric subsidiary colour) (C.A.S. 27414-68-2) – Angene Chemical, Cat. No. AGN-PC-0R372R or equivalent
- Sodium indigo sulfonate monosodium salt (monosulfonated subsidiary colour) (C.A.S. 27414-69-3) – Atomax Chemicals Co., Ltd., Cat No. AM27414693 or equivalent
- Trisodium indigo-5,5',7'-trisulfonate
- (trisulfonated subsidiary colour), potassium salt (C.A.S. 67627-18-3) – Sigma-Aldrich, Cat. No. 234087 or equivalent
- Indigotine (C.A.S. No. 860-22-0) – TCI, Cat. No. F0148 or equivalent (use if subsidiary colouring matter standards are not available)

Prepare standard solutions as required.

Sample preparation:
Weigh accurately 100±2 mg sample and dissolve in 100 ml of water. Dilute the solution, if required, to separate subsidiary colours from the primary colour component in order to improve their resolution. Analyze immediately after preparation.

Calculations:
Construct the relevant standard curves. Integrate all peaks of the chromatogram obtained at 610 nm. If Indigotine is used as a standard, calculate the ratio of the sum of all peaks not corresponding to Indigotine to the sum of all peaks.

Determine organic compounds other than colouring matters content by reversed-phase HPLC (Vol. 4) using the following conditions:
- Column: Luna C18 (250 mm x 4.6 mm i.d., 5 µm particle size) or equivalent
- Eluent A: 0.1% trifluoroacetic acid in water
- Eluent B: acetonitrile
- Injection volume: 20 µl
- Column temperature: 25°
- Detector: UV-visible/diode array at 244 nm
- Flow rate: 1.0 ml/min

Gradient:

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<th>Elution time (min)</th>
<th>Eluent A (%)</th>
<th>Eluent B (%)</th>
</tr>
</thead>
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<td>0</td>
</tr>
<tr>
<td>32</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Reagents: HPLC grade
Standards:
- Isatin-5-sulfonic acid sodium salt dihydrate (C.A.S. 207399-16-4) – Sigma-Aldrich Cat. No. 58245 or equivalent
- 5-Sulfoanthranilic acid (2-amino-5-sulfobenzoic acid) (C.A.S. 3577-63-7) – TCI Cat No. S0802 or equivalent
- Anthranilic acid (C.A.S. 118-92-3) – Sigma-Aldrich Cat No. A89855 or equivalent

Prepare standard solutions as required.

Sample preparation:
Weigh accurately 100±2 mg sample and dissolve in 100 ml of water. Analyse immediately after preparation.

Calculations:
Construct the relevant standard curves. Integrate the chromatogram peaks obtained at 244 nm.

**METHOD OF ASSAY**

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and an appropriate solvent. Analyse immediately after preparation.

Using water as the solvent:
absorptivity \(a = 48.0 \text{ l/(g} \times \text{cm)}\)
wavelength of maximum absorbance = 610 nm.

Determine isomer content by HPLC using the test for subsidiary colouring matters.
LUTEIN FROM TAGETES ERECTA

Prepared at the 86th JECFA (2018) and published in FAO JECFA Monograph 22 (2018), superseding specifications prepared at the 63rd JECFA (2004) and published in FNP52 Add 12 (2004). A group ADI of “not specified” was established for Tagetes extract, Lutein from Tagetes erecta, Lutein esters from Tagetes erecta, Zeaxanthin (synthetic), and meso-zeaxanthin at the 86th JECFA (2018) superseding the group ADI of 0 - 2 mg/kg bw for lutein from T. erecta L. and synthetic zeaxanthin established at the 63rd JECFA (2004).

SYNONYMS
INS No. 161b(i), Vegetable lutein; vegetable luteol; Bo-Xan, luteine

DEFINITION
Lutein from Tagetes erecta is a purified extract of xanthophylls obtained from oleoresin in marigold. The oleoresin is prepared from hexane extracts of Tagetes erecta L. flowers, saponified with potassium hydroxide in either methanol or propylene glycol. The resulting reaction mixture is diluted with water and dried. The crystalline product contains lutein along with minor components that include other carotenoids and waxes.

Chemical names
3R,3'R,6'R-β,ε-carotene-3,3'-diol; all-trans-lutein; 4',5'-didehydro-5',6'-dihydro-beta,beta-carotene-3,3'-diol

C.A.S. number
127-40-2

Chemical formula
C_{40}H_{56}O_{2}

Structural formula

Formula weight
568.88

Assay
Not less than 80% total carotenoids, not less than 70% lutein

DESCRIPTION
A free-flowing, orange-red powder

FUNCTIONAL USES
Colour, nutrient supplement
CHARACTERISTICS

IDENTIFICATION

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

Spectrophotometry (Vol. 4) A 2 mg/l solution in acetone shows maximum absorbance at approximately 446 nm.

Test for carotenoids (Vol. 4) The colour of 2 ml of a 2 – 4 mg/l solution of the sample in acetone immediately disappears after successive addition of about 0.5 ml of 5% sodium nitrite and about 0.5 ml of 0.5 M sulfuric acid.

PURITY

Moisture (Vol. 4) Not more than 1.0%

Ash (Vol. 4) Not more than 1.0%

Zeaxanthin Not more than 9.0%

See description under METHOD OF ASSAY

Lead (Vol. 4) Not more than 3 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 methods described in Vol. 4 (under “General Methods, Metallic Impurities”).

Hexane (Vol. 4) Not more than 50 mg/kg

Methanol (Vol. 4) Not more than 10 mg/kg

Propylene glycol Not more than 1000 mg/kg

See description under TESTS

Waxes Not more than 14.0%

See description under TESTS
TESTS

PURITY TESTS

Propylene glycol

Determine by gas chromatography (Vol. 4) under the following conditions.

Internal standard solution
Prepare a 500 μg/ml solution of ethylene glycol in tetrahydrofuran.

Standard solutions
Prepare a range of standard solutions containing 1, 5, 10, 25 and 50 μg/ml of propylene glycol and 5 μg/ml of ethylene glycol in tetrahydrofuran.

Chromatography conditions
- Column: Polydimethylsiloxane (30 m x 0.32 mm i.d. with 0.25 μm film)
- Carrier gas: Helium
- Flow rate: 1.5 ml/min (Constant flow)
- Detector: FID
- Temperatures: injection port: 230°
- Column Temperature: Hold for 3 min at 40°, then 40-250° at 20°/min, hold for 5 min at 250°
- Detector Temperature: 270°

The retention times of ethylene glycol and propylene glycol derivatives under the above conditions are approx. 7.6 min and 7.8 min, respectively.

Procedure
Weigh accurately 1 g of the sample into a 10-ml volumetric flask, and add 100 μl of the internal standard solution. Dissolve and make up to volume with tetrahydrofuran. Take 0.5 ml of the sample solution in a centrifugation tube, and add 0.25 ml of 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and 0.1 ml of trimethylchlorosilane (TMCS). After sealing the tube, shake it vigorously, let stand for 30 min at room temperature, then centrifuge. Inject 1.0 μl of this centrifugal supernatant into the chromatograph.

Standard curve
Prepare following the same procedure using 0.5 ml of the standard solutions in place of the sample solution.

Calculate the concentration of propylene glycol in mg/kg (C_{PG}) from:
where

\[ CPG (mg/kg) = \frac{C \times 10}{W} \]

where

\( C \) is polyethylene glycol concentration determined (µg/ml); and
\( W \) is weight of sample (g)

**Waxes**

Determine by gas chromatography (Vol. 4) using the following conditions:

- 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: Hexatriancontane (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 hexatriancontane 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) hexatriancontane 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:

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

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 carotenoid content and the content of lutein and zeaxanthin by UV spectrophotometry and HPLC using the following conditions:

**Reagents:**
- Hexane (HPLC grade)
- Ethyl acetate (HPLC grade)
- Acetone
- Dehydrated ethyl alcohol (absolute alcohol)
- Toluene

**System Suitability Solution for HPLC:**
150 µg/ml of lutein standard in solvent mixture (use USP Lutein RS available from U. S. Pharmacopeia, or equivalent standard)

**Apparatus**
- UV/Vis spectrophotometer; 1-cm cuvettes
- HPLC system with suitable diode array detector, autosampler, column oven, signal processor and degasser.
- Analytical column: 3 µm silica, 4.6 mm x 250 mm

**Instrument Conditions**
- Temperature: ambient
- Mobile Phase: 70:30 (v:v) hexane/ethyl acetate (isocratic elution)
- Flow Rate: 1.5 ml/min
- Injection: 10 µl
- Detection: UV/Vis 446 nm
- Run Time: approximately 40 min

**Concentrated Sample Preparation**
For the UV/Vis spectrophotometry weigh sample (30 mg) into a glass weighing funnel. Using the solvent mixture, wash crystals into a 100 ml volumetric flask, dilute to the mark with the solvent mixture and stir for 10 min.

**Sample Preparation**
Pipette 1 ml of concentrated sample preparation into a
100 ml volumetric flask. Dilute up to the mark with dehydrated ethyl alcohol, mix by inversion for 20 seconds. Read samples in a spectrophotometer at 446 nm using dehydrated ethyl alcohol as the blank.

For HPLC, evaporate 1 ml of the concentrated sample preparation to dryness using a stream of nitrogen, dissolve solids in 1 ml 70:30 hexane:ethyl acetate, and add 0.5 ml to HPLC vials. Analyze this sample and the system suitability solution for HPLC using the HPLC conditions above.

Results

Compare the results of the chromatogram from the system suitability solution for HPLC to identify the lutein and zeaxanthin peaks at a resolution of not less than 3.

Calculation

Using the results obtained from the UV/Vis spectrophotometry calculate the % Total carotenoids

\[
\text{% Total carotenoids} = \frac{\text{Absorbance at 446 nm} \times 10000 \times 100}{\text{sample weight in g} \times 2550}
\]

Note: The factors 10000 and 2550 are the dilution factor and extinction value for a 1% solution, respectively.

Using the chromatogram of the sample, calculate the concentration of lutein and zeaxanthin.

\[
\text{Lutein} (%) = \frac{\text{Peak Area LUTEIN}}{\text{Peak Area TOTAL}} \times \text{% Total carotenoids}
\]

\[
\text{Zeaxanthin} (%) = \frac{\text{Peak Area ZEAXANTHIN}}{\text{Peak Area TOTAL}} \times \text{% Total carotenoids}
\]
MODIFIED STARCHES


MODULAR MONOGRAPH consisting of "GENERAL SPECIFICATIONS"\(^{(a)}\) that contains common specifications to all modified starches(INN 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and 8 ANNEXES that contain specifications related to the chemical treatments of native starches:

- ANNEX 1\(^{(a)}\) – Fragmentation.
- ANNEX 2\(^{(a)}\) – Bleaching.
- ANNEX 3\(^{(a)}\) – Esterification and/or crosslinking with phosphorous containing compounds.
- ANNEX 4\(^{(a)}\) – Acetylation.
- ANNEX 5\(^{(a)}\) – Oxidation.
- ANNEX 6\(^{(a)}\) – Esterification with octenyl succinic anhydride.
- ANNEX 7\(^{(a)}\) – Etherification with propylene epoxide.
- ANNEX 8\(^{(a)}\) – Esterification and crosslinking with adipic anhydride.

The General specifications are applicable to the following modified starches, each of which should additionally fulfil the specifications of the ANNEXES as follows:

<table>
<thead>
<tr>
<th>Modified Starch</th>
<th>INS</th>
<th>Annex</th>
<th>ADI</th>
<th>STATUS</th>
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Should any of the modified starches be subjected to additional chemical treatment, the appropriate specifications outlined in the respective ANNEX should be met. Consequently, for all fragmented and/or bleached starches the specifications of ANNEXES 1 and/or 2 respectively should be met.


\(^{(1)}\)An ADI “not specified” was established at the 26th JECFA (1982).

\(^{(2)}\)An ADI “not specified” was established at the 57th JECFA (2001).

\(^{(3)}\)T: TENTATIVE
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GENERAL SPECIFICATIONS FOR MODIFIED STARCHES

(version 2018 - tentative)

Information required:
- Suitable microbiological acceptance criteria and supporting data

**DEFINITION**

Starch consists mainly of amylose and amylopectin. Amylose is a linear molecule of α-D-glucopyranosyl units linked by (1-4)-α-linkages. Amylopectin is a highly-branched polymer of α-D-glucopyranosyl units linked by (1-4)-α-linkages and by(1-6)-α-linkages that constitute the branch points. Each glucose unit possesses a maximum of three hydroxyls that can undergo chemical substitution.

Native starches can be physically (pre-gelatinized starches) and/or chemically modified for improved functionality. The most common sources of native starch used in these modifications are various roots, tubers, cereals and legumes. Modified starches are used in applications requiring special properties not attainable by native starches.

Chemical modifications of native starches are often performed, in an aqueous suspension under controlled conditions of pH, time and temperature, unless otherwise indicated in the description of the respective annex. After sufficient reaction time, the modified starch is recovered by filtration or centrifugation, washed with water, dried and packaged. The relevant modification reactions can be, separately or in combination, fragmentations (hydrolysis, oxidation, enzymatic), bleaching, oxidation, esterification, etherification or phosphorylation of one or more of the hydroxyl groups of the α-D-glucopyranosyl units or crosslinking using polyfunctional agents.

See the appropriate Annex or Annexes for the treatment that is applicable to individual modified starch products.

**C.A.S numbers** See ANNEXES

**DESCRIPTION**

White or nearly white powder or granules or (if pre-gelatinized) flakes, or amorphous powder or coarse particles.

**FUNCTIONAL USES**

Thickener, stabilizer, binder, emulsifier
CHARACTERISTICS

IDENTIFICATION

**Solubility (Vol. 4)**

Insoluble in cold water (if not pre-gelatinised); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

**Microscopy**

Passes test

See description under TESTS

**Iodine stain**

Passes test

See description under TESTS

**Copper reduction**

Passes test

See description under TESTS

PURITY

**General Requirements:**

**pH**

3.0 – 9.0

See description under TESTS

**Loss on drying (Vol 4)**

Cereal starch: not more than 15.0%

Potato starch: not more than 21.0%

Other starches: not more than 18.0%

Conditions: 120°, 4 h, vacuum not exceeding 100 mm Hg

**Lead (Vol. 4)**

Not more than 0.2 mg/kg on the dried basis

Not more than 0.1 mg/kg on the dried basis for Starch sodium octenylsuccinate (INS 1450) for use in infant formula and formula for special medical purposes intended for infants (see Annex 6)

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

**Microbiological Criteria** *(Vol 4)*

Aerobic plate count: Not more than 1000 CFU/g

Yeast and moulds: Not more than 1000 CFU/g

Total coliforms: Not more than 10 CFU/g

Information required

**Sulphur dioxide** *(Vol. 4)*

Not more than 50 mg/kg on the dried basis for modified cereal starches

Not more than 10 mg/kg on the dried basis for other modified starches

**TESTS**

**IDENTIFICATION TESTS**

**Microscopy**

Each modified starch, which has not been pre-gelatinized, retains its granular structure and can be identified as a starch by microscopic observation. The typical polarization cross is observed when sample is examined with a polarizing microscope, in polarized light under crossed Nicol prisms.

Corn starch: Polygonal, rounded or spherical granules up to 35 µm diameter having a circular or several-rayed central cleft.

Potato starch: Irregular shaped, ovoid, pear-shaped granules (30-100 µm diameter, occasionally >100 µm); both, the ovoid, the pear-shaped granules and the rounded granules have an eccentric hilum. All granules show clearly visible concentric striations.

Tapioca starch: Spherical granules with one truncated side (5-35 µm diameter) usually having a circular or several-rayed central cleft.

Wheat starch: large and small granules (10-60 µm diameter). The central hilum and striations are visible and barely visible.

**Iodine stain**

Add a few drops of 0.1 N potassium triiodide to an aqueous suspension of the sample. The modified starch stains with iodine in the same way as native starches. The colour can range from dark blue to red.

**Copper reduction**

Place about 2.5 g of the sample previously washed with water, in a boiling flask; add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water; mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced.
PURITY TESTS

pH (Vol. 4)  
Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (in the case of pregelatinised starches, 3 g should be suspended in 97 ml of water).
ANNEX 1: ADDITIONAL SPECIFICATIONS FOR STARCHES MODIFIED BY FRAGMENTATION

(VERSION 2018 - TENTATIVE)

Information is required on:

- A suitable method for dispersion and a method for reducing sugars and data on at least 5 representative batches using the method(s) from each of the fragmentation processes.

APPLIES TO

- Dextrin roasted starch (INS No. 1400)
- Acid treated starch (INS No. 1401)
- Alkaline treated starch (INS No. 1402)
- Enzyme-treated starch (INS No. 1405)
- All modified starches that are fragmented

SYNONYMS

Modified starch by fragmentation, converted starch, hydrolysed starch.

TREATMENT

The fragmentation of native starch results in products containing polymers with a lower average molecular weight and reduced viscosity. The manufacturing details for the various modified starches by fragmentation in this monograph are described as below:

- **Dextrin roasted starch, INS. 1400**: is manufactured by dry heating or roasting of native starch with hydrochloric acid or ortho-phosphoric acid in heated and/or agitated vessels. The final dextrin roasted starch is obtained by drying.

- **Acid treated starch, INS. 1401**: is obtained by treating a slurry or a suspension of native food starch with dilute hydrochloric acid, ortho-phosphoric acid, or sulphuric acid.

- **Alkaline treated starch, INS. 1402**: is obtained by treating a suspended solution of native food starches with sodium hydroxide or potassium hydroxide.

- **Enzyme-treated starch, INS 1405**: is obtained by treating a suspension of native food starch with one or more food-grade amylolytic-enzymes (e.g., α-amylase (E.C. 3.2.1.1), β-amylase (3.2.1.2), glucoamylase (3.2.1.3), isoamylase (3.2.1.68), pullulanase (E.C. 3.2.1.41)).
The properties of the modified starches by fragmentation vary depending on the source of native starch, reaction conditions (pH, reaction time, reaction temperature, fragmenting reagent etc.) The alteration of native starch allows for applications that require reduced viscosity in hot solutions and/or typically utilise high levels of modified starches.

C.A.S number

9004-53-9 (Dextrins)
65996-63-6 (Acid-hydrolysed starch)
68909-37-5 (Acid-hydrolysed amylopectin)
9005-84-9 (Starch soluble)
65996-64-7 (Enzyme-hydrolysed starch)
1001439-91-3 (Enzyme-treated amylopectin).

CHARACTERISTICS

IDENTITY

Dispersion identity Information required.
Reducing sugars Information required

TESTS

IDENTIFICATION TESTS

Dispersion test Information required
Reducing sugars Information required
ANNEX 2: ADDITIONAL SPECIFICATIONS FOR BLEACHED STARCHES

(VERSION 2018 - TENTATIVE)

Information is required on:
- Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s).

APPLIES TO

Bleached starch INS No. 1403
All modified starches that are bleached

TREATMENT

Peracetic acid and/or hydrogen peroxide, or sodium hypochlorite, sodium chlorite, sulfur dioxide, alternative permitted forms of sulfites, potassium permanganate, or ammonium persulfate

Bleaching is performed to improve physical attributes such as colour due to oxidation of traces of pigments such as carotenoids and xanthophylls. The change is essentially in the colour only. Residual reagents are either removed or limited to technically unavoidable levels.

C.A.S number

977075-42-5
and all other modified starches submitted to bleaching

CHARACTERISTICS

PURITY

Manganese (Vol. 4)
Not more than 50 mg/kg on the dried basis

Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Residual oxidising substances
Information required

Carboxyl groups (Vol. 4)
Not more than 0.1% on the dried basis applying the correction for phosphate content as outlined in Note 6 of the method for starches esterified with phosphorus containing compounds.
ANNEX 3: ADDITIONAL SPECIFICATIONS FOR STARCHES ESTERIFIED AND/OR CROSSLINKED WITH PHOSPHORUS CONTAINING COMPOUNDS

(VERSION 2018 - TENTATIVE)

Information required on: A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches.

APPLIES TO

Monostarch phosphate (INS No. 1410)
Distarch phosphate (INS No. 1412)
Phosphated distarch phosphate (INS No. 1413)
Acetylated distarch phosphate (INS No. 1414)
Hydroxypropyl distarch phosphate (INS No. 1442)

TREATMENT

The phosphorus containing compounds ortho-phosphoric acid, sodium or potassium ortho-phosphate and sodium tripolyphosphate, can be used for esterification and the sodium trimetaphosphate or phosphorus oxychloride for crosslinking.

- **Monostarch phosphate (INS 1410)** is obtained by esterification/crosslinking of unmodified food starch with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate

- **Distarch phosphate (INS 1412)** is obtained by crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride

- **Phosphated distarch phosphate (INS 1413)** is obtained by esterification/crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate

- **Acetylated distarch phosphate (INS 1414)** is obtained by esterification/crosslinking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate

- **Hydroxypropyl distarch phosphate (INS 1442)** is obtained by esterification of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide

Phosphorylation results in partial substitution of the 2, 3- or 6- position of the anhydro glucose unit unless the 6-position is occupied for branching. In the case of cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains, the structure can be
represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure.

C.A.S numbers

Monostarch phosphate (INS 1410)
11120-02-8 (Modified starch)
63055-37-8 (Modified amylopectin)

Distarch phosphate (INS No. 1412)
55963-33-2 (Modified starch)
63055-37-8 (Modified amylopectin)

Phosphated distarch phosphate (INS No. 1413)
11120-02-8 (Modified starch)
63055-37-8 (Modified amylopectin)

Acetylated distarch phosphate (INS No. 1414)
9067-33-8 (Modified starch)
68130-14-3 (Modified starch)
113894-91-0 (Modified amylopectin)

Hydroxypropyl distarch phosphate (INS No. 1442)
53124-00-8 (Modified starch)
113894-92-1 (Modified amylopectin)

CHARACTERISTICS

PURITY

Phosphate (calculated as phosphorus) (Vol. 4)

For monostarch phosphate (INS No. 1410), distarch phosphate (INS No. 1412), and phosphate distarch phosphate (INS No. 1413)

Not more than 0.5% on the dried basis for potato or wheat starches

Not more than 0.4% on the dried basis for other starches

For acetylated distarch phosphate (INS No. 1414) and hydroxypropyl distarch phosphate (INS No. 1442)

Not more than 0.14% on the dried basis for potato and wheat starch

Not more than 0.04% on the dried basis for other starches

IDENTITY

Crosslinking Information Required
ANNEX 4: ADDITIONAL SPECIFICATIONS FOR ACETYLATED STARCHES

Version 2018

APPLIES TO
Acetylated distarch phosphate (INS No. 1414)
Starch acetate (INS No. 1420)
Acetylated distarch adipate (INS No. 1422)
Acetylated oxidized starch (INS No. 1451)

TREATMENT
This type of modified starch is obtained by esterification with acetic anhydride or vinyl acetate. Acetylation results in substitution of hydroxyl groups with acetyl esters.

- **Acetylated distarch phosphate (INS 1414)** is obtained by esterification/cross-linking of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate.
- **Starch acetate (INS 1420)** is obtained by esterification of food starches with acetic anhydride or vinyl acetate
- **Acetylated distarch adipate (INS 1422)** is obtained by esterification of unmodified food starch with acetic anhydride and esterification/cross-linking with adipic anhydride
- **Acetylated oxidized starch (INS 1451)** is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride

C.A.S numbers

- Acetylated distarch phosphate (INS No. 1414)
  9067-33-8 (Modified starch)
  68130-14-3 (Modified starch)
  113894-91-0 (Modified amylopectin)
- Starch acetate (INS No. 1420)
  9045-28-7 (Modified starch)
- Acetylated distarch adipate (INS No. 1422)
  63798-35-6 (Modified starch)
  63055-36-7 (Modified amylopectin)
- Acetylated oxidized starch (INS No. 1451)
  68187-08-6 (Modified starch)
CHARACTERISTICS

IDENTIFICATION

Specific reaction for Acetyl groups
PASSES TEST
See description under TESTS

Ester groups
PASSES TEST
See description under TESTS

PURITY

Acetyl groups
Not more than 2.5% on the dried basis
See description under TESTS

Vinyl acetate
Not more than 0.1 mg/kg
See description under TESTS

TEST

IDENTIFICATION TESTS

Specific reaction for acetyl groups
Principle
Acetate is liberated upon saponification of acetylated starch and converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure
Suspend about 10 g of the sample in 25 ml water. Add 20 ml of 0.4 M NaOH. After shaking for 1 h filter the starch off and evaporate the filtrate in an oven at 110°. Dissolve the residue in a few drops of water and transfer to a test tube. Add calcium hydroxide and heat the tube. If the sample is acetylated starch, acetone vapours are produced. These produce a blue colour on a paper strip soaked in a fresh saturated solution of o-nitrobenzaldehyde in 2 M NaOH. The blue colour is more distinct when the original yellow colour of the reagents is removed with 1 drop of a 1 in 10 solution of hydrochloric acid.

Ester groups
The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm\(^{-1}\) which is an indication
for ester groups. The limit of detection is about 0.5% acetyl groups in the product.

**PURITY TESTS**

**Acetyl groups**

Accurately weigh about 5 g of the sample and transfer into a 250 ml conical flask. Suspend in 50 ml of water, add a few drops of phenolphthalein TS, and titrate with 0.1 M sodium hydroxide to a permanent pink end-point. Add 25.0 ml of 0.45 M sodium hydroxide, stopper the flask, and shake vigorously for 30 min, preferably with a mechanical shaker. (NOTE: the temperature should not exceed 30° as some starches may gelatinise). Remove the stopper, wash the stopper and sides of the flask with a few ml of water, and titrate the excess alkali with 0.2 M hydrochloric acid to the disappearance of the pink colour. Record the volume, in ml of 0.2 M hydrochloric acid required as S.

Perform a blank titration on 25.0 ml of 0.45 M sodium hydroxide, and record the volume, in ml, of 0.2 M hydrochloric acid required as B.

\[
\text{Acetyl groups } \% = \frac{(B - S) \times M \times 0.043 \times 100}{W}
\]

where

- \( M \) is the molarity of hydrochloric acid solution; and
- \( W \) is the weight of sample, in grams.
ANNEX 5: ADDITIONAL SPECIFICATIONS FOR STARCHES SUBJECTED TO OXIDATION

(version 2018 - tentative)

Information is required on:
- A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method.

APPLIES TO

Oxidized starch (INS No. 1404)
Acetylated oxidized starch (INS No. 1451)

TREATMENT

Sodium hypochlorite is used for oxidation.

- Oxidized starch (INS 1404) is obtained by treatment of food starch with sodium hypochlorite.
- Acetylated oxidized starch (INS 1451) is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride.

Oxidation involves the deliberate production of carboxyl groups.

C.A.S number

Oxidised starch (INS No. 1404)
65996-62-5 (modified starch)
113894-86-3 (modified amylopectin)

Acetylated oxidised starch (INS No. 1451)
68187-08-6

CHARACTERISTICS

IDENTIFICATION

Test for hypochlorite oxidized starch Passes test
See description under TESTS

PURITY

Carboxyl groups (Vol. 4) Not more than 1.3% on the dried basis

Residual hypochlorite Information required
TESTS

IDENTIFICATION TESTS

Test for hypochlorite-oxidized starch

Principle
Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue. The test is not suitable for slightly oxidized potato starch due to the presence of phosphate groups.

Procedure
50 mg of the sample are kept in suspension for 5-10 min in 25 ml of a 1% aqueous dye solution and stirred occasionally. After decantation of the excess solution, the starch is washed with distilled water. Microscopic inspection clearly shows colouring, if the sample is hypochlorite-oxidized starch. By this test hypochlorite-oxidized starch is distinguished from native and acid modified starch of the same botanical origin.
ANNEX 6: ADDITIONAL SPECIFICATIONS FOR STARCHEES ESTERIFIED WITH OCTENYLSUCCINIC ANHYDRIDE

Version 2018

APPLIES TO
Starch sodium octenylsuccinate (INS No. 1450)

TREATMENT
Octenylsuccinic anhydride can be used for the esterification and either sodium hydroxide or sodium carbonate as a pH buffer for neutralisation.

C.A.S numbers
Starch sodium octenylsuccinate
66829-29-6(Modified starch)
52906-93-1(Modified starch)
125109-81-1 (Modified amylopectin)

CHARACTERISTICS

PURITY

Octenylsuccinyl groups
Not more than 3% on the dried basis

Residual free octenylsuccinic acid
Not more than 0.3% on the dried basis

PURITY TEST

Octenylsuccinate groups and residual free octenylsuccinic acid in Starch sodium octenyl succinate

Principle
Residual free octenylsuccinic acid in the sample is extracted and determined by HPLC/UV. Total octenylsuccinic content is determined using the same method after hydrolysis of the sample. Octenylsuccinate ester groups on the modified starch are calculated by subtraction of the residual free octenylsuccinic acid from the total.

Standard and Reagents

Octenylsuccinic anhydride:
2-Octen-1-ylsuccinic anhydride, mixture of cis and trans (>97%) (CAS: 42482-06-4)

0.1 N potassium hydroxide:
Weigh 1.4 g of potassium hydroxide, dissolve in water and dilute to 250 ml.
0.073 mol/l phosphoric acid:
Dilute 1 ml of phosphoric acid (85%, density 1.686g/cm³) to 200 ml with water.

Preparation of standard solutions
Accurately weigh about 20 mg of octenylsuccinic anhydride, add 10 ml of 0.1N potassium hydroxide, stopper and heat at 80°C for 3 hours. After cooling, add 8 ml of 0.073 mol/l phosphoric acid and dilute with water to 20 ml. Pipette 2 ml of this solution into a 20 ml volumetric flask and dilute with water. Pipette 1 ml, 2 ml, 5 ml, and 10 ml of the resulting solution into four separate 20-ml volumetric flasks, and dilute each to volume with water to prepare standards of 5 μg/ml, 10 μg/ml, 25 μg/ml and 50 μg/ml respectively.

Preparation of test solution A (for residual octenylsuccinic acid):
Accurately weigh about 0.1 g of sample, add 20 ml of methanol, and shake for 18 hours or more. Centrifuge the mixture at about 3000 rpm for 5 minutes, pipette 10 ml of the supernatant, and evaporate to dryness under vacuum at 40°C. Dissolve the residue and dilute with water in a 5 ml volumetric flask.

Preparation of test solution B (for total octenylsuccinic acid):
Accurately weigh about 20 mg of sample, dissolve in 10 mL of 0.1N potassium hydroxide, stopper and heat at 80°C for 3 hours. After cooling, add 8 ml of 0.05 mol/l phosphoric acid, dilute with water to 20 ml.

Procedure
HPLC operating conditions
- Column: A octadecylsilanized silica gel column (250 mm x 4.6 mm, 5µm) (L-Column ODS-V CERI or equivalent)
- Column temperature: 40°C
- Detector: UV at 205 nm
- Mobile phase: A 1:1 mixture of 0.1% (v/v) phosphoric acid solution / acetonitrile
- Injection volume: 20μl
- Flow rate: Adjust the retention time of the main peak to about 9 minutes.

Inject the test solution A and B and the standard solutions into an HPLC under the same conditions.

Measure the sum of the peak areas of two main peaks of cis- and trans-2-octenylsuccinic acid for each standard solution, and prepare a standard curve for octenylsuccinic anhydride from the sums obtained and the concentrations of octenylsuccinic anhydride in the standard solutions. Measure the sum of the peak areas of two main peaks for the test solutions A and B. Determine the concentration of
octenylsuccinic acid (µg/ml) in the test solutions A and B from
the standard curve, and calculate residual and total
octenylsuccinic acid, respectively. The value of
octenylsuccinate groups in the sample is calculated by the
following formula:

Calculation:

\[
Residual\ free\ octenylsuccinic\ acid\ % = \frac{C_{os} \times 1.086}{W_r} \times 100
\]

\[
Total\ octenylsuccinic\ acid\ % = \frac{C_{os} \times 1.086}{W_s} \times 500
\]

Where
- 1.086 is the molecular weight of octenylsuccinic acid
divided by the molecular weight of octenylsuccinic
anhydride
- \( C_{os} \) is the octenylsuccinic anhydride concentration
(µg/ml);
- \( W_r \) or \( W_s \) is the dry-basis weight of the sample (g).

Content (%) of octenylsuccinyl groups =
Content of total octenyl succinic acid – Content of
residual octenylsuccinic acid.
ANNEX 7: ADDITIONAL SPECIFICATIONS FOR STARCHES ETHERIFIED WITH PROPYLENE OXIDE

(Version 2018 - TENTATIVE)

Information is required on:
- A suitable method for the determination of propylene chlorohydrin with detection limit lower than 0.1 mg/kg and data on at least 5 representative batches of Hydroxypropyl starch using the method.

APPLIES TO
- Hydroxypropyl starch (INS No. 1440)
- Hydroxypropyl distarch phosphate (INS No. 1442)

TREATMENT
Propylene oxide is used for etherification.
- Hydroxypropyl starch (INS No. 1440) is obtained by etherification of unmodified food starch with propylene oxide.
- Hydroxypropyl distarch phosphate (INS No. 1442) is obtained by esterification of unmodified food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide.

Hydroxypropylation results in substitution of hydroxyl groups with 2-hydroxypropyl ether.

C.A.S numbers
- Hydroxypropyl starch (INS No. 1440)
  - 9049-76-7 (Modified starch)
  - 74315-67-6 (Modified amylopectin)
- Hydroxypropyl distarch phosphate (INS No. 1442)
  - 53124-00-8 (Modified starch)
  - 113894-92-1 (Modified amylopectin)

CHARACTERISTICS

IDENTIFICATION
- Hydroxypropyl ether groups: Passes test
  See description under TESTS

PURITY
- Hydroxypropyl groups: Not more than 7.0% on the dried basis
  See description under TESTS
Propylene chlorohydrins
Not more than 1 mg/kg
See description under TESTS

TESTS

IDENTIFICATION

TESTS

Hydroxypropyl ether groups
Ninhydrin reagent
A 3% solution of 1,2,3-triketohydrindene crystals in 5% aqueous sodium bisulfite solution.

Procedure
Weigh 100 mg of the sample into a 100-ml volumetric flask and add 12.5 ml of 2 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake).

A violet colour develops only in the modified sample within 5 min due to the presence of hydroxypropyl groups (starch ether). For all other non-hydroxypropyl treated starches a light pink colour is observed.

PURITY TEST

Hydroxypropyl groups
Ninhydrin reagent
A 3% solution of 1,2,3-triketohydrindene crystals in 5% aqueous sodium bisulfite solution.

Procedure
Accurately weigh 50 - 100 mg of the sample into a 100-ml volumetric flask and add 25 ml of 1 N sulfuric acid. Prepare a sample of unmodified starch of the

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2 USP29-NF34: U.S. Pharmacopeial Convention, Hydroxypropyl corn starch monograph, 2015. Reproduced from the USP-NF with permission from The U.S. Pharmacopeial Convention (USP)
same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. If necessary, dilute the sample further to assure the presence of no more than 4 mg of hydroxypropyl group per 100 ml, and then dilute the blank starch in the same proportion. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake). Immediately transfer portions of the solutions to 1-cm cells and after exactly 5 min, measure the absorption (A) at 590 nm, using the starch blank as the reference. Prepare a calibration curve with 1-ml aliquots of standard aqueous solutions, containing 10, 20, 30, 40 and 50 µg of propylene glycol per ml.

Calculation

\[ \text{Hydroxypropyl groups (\%)} = \frac{C \times 0.7763 \times 10 \times F}{W} \]

where

- C is the amount of propylene glycol in the sample solution read from the calibration curve (µg/ml);
- F is the dilution factor (if a further dilution has been necessary); and
- W is the weight of sample (mg).

**Propylene chlorohydrins**

**Principle**

Propylene chlorohydrins (1-chloro-2-propanol and 2-chloro-1-propanol) in sample are determined by capillary gas chromatography.

**Standards and Reagents**

propylene chlorohydrins

**Preparation of standard addition calibration curve**

Accurately weigh about 50 mg propylene chlorohydrins, and dilute with water to 100 ml. Dilute 10 ml of this solution to 100 ml with water to make a standard stock solution (50 µg/ml). Take four Erlenmeyer flasks and weigh 50.0 g of sample in each one. Add 125 ml of 1 M sulfuric acid in each one. Add 0.5 ml, 1 ml, 2 ml, or 5 ml of standard stock solution, to the 1st, 2nd, etc flasks respectively. Proceed as directed for the test solution, beginning
with “and swirl the flask to disperse the contents,” to prepare the standard solutions of added concentration 5, 10, 20 and 50 µg/ml respectively.

Preparation of test solution
Accurately weigh 50 g of sample into an Erlenmeyer flask, add 125 ml of 1 M sulfuric acid, and swirl the flask to disperse the contents. Stopper loosely, heat in a water bath at 100°C for 10 min, mix the contents well, and heat for an additional 30 min. For starches that are not easy to hydrolyze, such as wheat starch, heating time should be longer (90 min). Cool to room temperature, adjust the pH to 7 with 25% sodium hydroxide solution, and filter with suction through a glass-fiber filter paper. Wash the flask and the residue on the filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, stir for 5–10 min to dissolve, and transfer the solution into a separating funnel. Wash the flask with 25ml of water, and add the washings to the funnel. If precipitate remains, stir well with a small amount of water to dissolve it completely, and add the solution to the funnel. Extract five times with five 50ml portions of diethyl ether. Combine the diethyl ether extracts, add 3 g of anhydrous sodium sulfate, let it stand for a few minutes and filter through a filter paper. Wash the flask and the filter paper with 25 ml of diethyl ether, and combine the washings with the filtrate. Evaporate to 4 ml in a water bath at about 40°C under atmospheric pressure, cool, transfer to a 5 ml volumetric flask and add diethyl ether to the exact volume.

Procedure

GC operating conditions
- GC equipped with a flame ionization detector (FID).
- Column: A fused silica column coated with polyethylene glycol (30 m x 0.25 mm i.d., 0.25 µm) (Inert Cap WAXGL Sciencesor equivalent)
- Carrier gas: N₂ or He
- Flow rate: Adjust the retention time of 1-chloro-2-propanol to about 15 min
- Column temperature: 40°C— for 2 min; heat at 5°C/min to 80°C, keep for 8 min, heat at 25°C/min to 230°C, keep for 5 min
- Injector temperature: 150°C
- Detector temperature: 230°C
- Split-less (purge start: 1 min after injection)
Analyse 1-µl portions of the test solution and the standard solutions by gas chromatography, using the operating conditions given above. Prepare a standard addition curve: Plot in the y axis the sum of the peak areas corresponding to 1-chloro-2-propanol and 2-chloro-1-propanol in the chromatographs and in the x-axis the added concentration of propylene chlorohydrins in the standard solution. For the test solution the added concentration is equal to 0. A linear calibration curve should be obtained. Extrapolate to 0 in the y axis. The concentration (µg/ml) of propylene chlorohydrins in the test solution is equal to the absolute value of the concentration at the point where the curve intercepts the x axis (C_t). Determine the content of propylene chlorohydrins in the sample using the following formula:

Calculation

Content (mg/kg) of Propylene chlorohydrins = C_t × 5 / W

where
- C_t: amount of propylene chlorohydrins in test solution (µg/mL);
- W: mass of sample (g, on the dried weight basis)
ANNEX 8: ADDITIONAL SPECIFICATIONS FOR STARCHES CROSSLINKED WITH ADIPIC ANHYDRIDE

(Version 2018 – Tentative)

Information is required on:
- A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches
- Levels of free adipic acid in at least 5 representative batches

APPLIES TO
Acetylated distarch adipate (INS No. 1422)

TREATMENT
Adipic anhydride can be used for esterification and crosslinking. In cases of cross-linking, where adipic anhydride connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = CO-(CH₂)₄-CO and starch refers to the linear and/or branched structure.

C.A.S numbers
Acetylated distarch adipate
63798-35-6 (Modified starch)
63055-36-7 (modified amylopectin)

CHARACTERISTICS

PURITY

Adipate groups
Not more than 0.135% on the dried basis
See description under TESTS

Free adipic acid
Information Required
See description under TESTS

PURITY TEST

Adipate groups and free adipic acid
Determine by gas chromatography after derivatization

Principle
Free adipic acid in the sample is extracted and determined by capillary gas chromatography after trimethylsilyl-derivatization. Total adipic acid is determined using the same method after hydrolysis of the sample and adipate groups are calculated by subtraction of the free adipic acid from the total.
Standards and Reagents
Adipic acid (>99%)
Glutaric acid (>99%)
Starch, unmodified (of the same botanical origin as the sample)
Sodium hydroxide solution (4N): weigh 40g of NaOH, dissolve in water and dilute to 250 ml.
Concentrated HCl (36%)
Ethyl acetate
Sodium sulphate, anhydrous
N,O-Bis(trimethylsilyl)trifluoroacetamide
Pyridine

Internal standard solution (1 mg/ml)
Accurately weigh 0.1 g of glutaric acid, dissolve in water and dilute to 100 ml.

Standard stock solution (1 mg/ml)
Accurately weigh 0.1 g of adipic acid, dissolve in 90 ml of warm water, cool to room temperature, dilute to 100 ml and mix.

Working standard solutions (0.02, 0.1, 0.2 and 0.4 mg/ml)
Pipette 1, 5, 10, and 20 ml of the standard stock solution in four separate 50 ml volumetric flasks, and dilute with water.

Procedure
Preparation standard curve solutions
Weigh 1.0 g of starch into each of four Erlenmeyer flasks, add 50 ml of water and 1 ml of internal standard solution. Add 5 ml each of the four working standard solutions, respectively. Stopper the flask and shake them well to disperse the starch, add 50 ml of 4N sodium hydroxide solution, and shake for 5 min. Place the flasks in a water bath, at room temperature, and add cautiously 20 ml of conc. hydrochloric acid. Cool, and quantitatively separately transfer the contents of the flasks into four separation funnels with a little amount of water. Extract three times with 100 ml of ethyl acetate each time. Collect the ethyl acetate layers separately in four dry Erlenmeyer flasks, add 20 g of anhydrous sodium sulphate, allow to stand for 10 min with occasional shaking, and filter into a rotary evaporator flask. Wash the Erlenmeyer flask and the residue on the filter paper twice with a small quantity of ethyl acetate, and combine the washings with the filtrate. Evaporate the ethyl acetate under a reduced pressure of 6.7 kPa at a temperature below 40°. Remove the remaining ethyl acetate completely by nitrogen stream. The evaporation of ethyl acetate should be effected as quickly as possible. Successively add 2 ml of pyridine and 1 ml of N,O-bis(trimethylsilyl)trifluoroacetamide to the residue and stopper the flask. Allow the solution to stand for 1 hour, transfer 2 ml of it into a GC vial, and immediately stopper tightly. Use these solutions to construct standard curve (Internal standard 1 mg/g starch, standards 0.1, 0.5, 1 and 2 mg/g starch respectively)
Preparation of test solution A (for residual free adipic acid)
Weigh accurately about 5 g of sample into an Erlenmeyer flask, add 100 ml of water and 1 ml of the internal standard solution. Shake well for 1 hour, and filter through a 0.45 \( \mu \)m membrane filter. To the filtrate, add exactly 1 ml of hydrochloric acid (in the case of pre-gelatinized starch or water-soluble starch, directly add 1 ml of hydrochloric acid to the resulting suspension without filtering), and transfer into a separation funnel. Proceed as directed for the preparation of standard solutions, beginning with “…and wash the inside of the flask with a little amount of water into the funnel.” Use this solution for the determination of residual free adipic acid (Internal standard 1 mg/5 g starch).

Preparation of test solution B (for total adipic acid)
Weigh accurately about 1 g of sample into an Erlenmeyer flask, add 50 ml of water and exactly 1 ml of the internal standard solution. Shake the mixture well to disperse the starch, add 50 ml of 4N sodium hydroxide solution and shake well for 5 minutes. Place the flask in a water bath at room temperature, and add cautiously 20 ml of concentrated hydrochloric acid. After cooling, transfer the contents in the flask into a separation funnel. Proceed as directed for the preparation of standard solution, beginning with “…and wash the inside of the flask with a little amount of water into the funnel.” Use this solution for the determination of total adipic acid (Internal standard 1 mg/g starch).

Procedure
GC operating conditions
- GC equipped with a flame ionization detector (FID)
- Column: A fused silica column coated with a mixture of 50% diphenyl and 50% dimethylpolysiloxane (15 m x 0.25 mm i.d., 0.25 \( \mu \)m)
- Carrier gas: He
- Column flow: 1.0 ml/min.
- Column temperature: 120\(^\circ\)C-5\(^\circ\)/min-150\(^\circ\)C
  (Glutaric and adipic acids elute at about 5 min and 8 min respectively)
- Injector temperature: 250\(^\circ\)C
- Detector temperature: 250\(^\circ\)C
- Injection volume: 1\( \mu \)l
- Split ratio: 30:1

Inject standard curve solutions into the capillary GC under the conditions indicated and construct a standard curve using the peak area ratios of adipic acid and glutaric acid against the amounts of adipic acid in the standard solutions (in g). Inject the test solution A and B and obtain the peak area ratio of adipic acid to glutaric acid for each of the test solutions A and B.
Determine the amount of adipic acid in each test solution from the standard curve and calculate the percent of adipate groups using the following formula:

Free adipic acid, %w/w = \([\frac{CF}{MF}] \times 100\)

Adipate groups. %w/w = \([\frac{CT}{MT} - \frac{CF}{MF}] \times 100\)

where

- CT = amount of the total adipic acid in the test solution B (g)
- CF = amount of the free adipic acid in the test solution A (g)
- MT = mass of sample in the test solution for the determination of total adipic acid (g, on the dried weight basis)
- MF = mass of the sample in the test solution for the determination of free adipic acid (g, on the dried weight basis)
NEUTRAL METHACRYLATE COPOLYMER (TENTATIVE)


Information required on:
- A validated method for the assay of neutral methacrylate copolymer (e.g., quantitative IR)
- Performance characteristics (method validation data) of the assay method
- Assay and monomers data on at least five batches of products currently available in commerce

SYNONYMS
E 1206, INS No. 1206, Ethyl acrylate methyl methacrylate polymer, Ethyl acrylate methyl methacrylate polymer; Ethyl acrylate polymer with methyl methacrylate, Methyl methacrylate ethyl acrylate polymer, Methyl methacrylate polymer with ethyl acrylate.

DEFINITION
Neutral Methacrylate Copolymer is a copolymer comprised of the monomers ethyl acrylate and methyl methacrylate in the molar ratio of 2:1. The copolymer is manufactured by emulsion polymerization of the monomers with water-soluble radical initiators. The product is purified by water vapour distillation and filtration to remove residual monomers, excess water, other volatile low-molecular weight substances and coagulum. The copolymer is standardized as a 30% aqueous dispersion with polyethylene glycol monostearil ether. The copolymer dispersion may contain the residual monomers (methyl methacrylate and ethyl acrylate). Neutral methacrylate copolymer is used as a coating and glazing agent for food supplements and foods for special medical purposes.

Chemical name Poly(ethyl acrylate-co-methylmethacrylate)

C.A.S. number 9010-88-2

Chemical formula Poly[(CH₂:CHCO₂CH₂CH₃)-co-(CH₂:C(CH₃)CO₂CH₃)]
The above formula is provided for illustrative purposes; in this copolymer no definitive structural unit can be defined.

Formula weight 600,000 (weight-average), 220,000 (number-average)

Assay Information required

DESCRIPTION Commercial form (30% aqueous dispersion) is a low viscosity milky-white liquid

FUNCTIONAL USES Coating agent, binding agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Viscosity (Vol. 4) Not more than 50 mPa.s

Determine viscosity using Brookfield viscometer at 20° and 300 pm using UL adapter.

pH (Vol 4) 5.5 – 8.6

Infrared absorption The infrared absorption spectrum of a dry film of sample corresponds to the infrared spectrum in the Appendix.

Apply one drop of sample to a glass plate, cover with a water-resistant crystal disc (AgCl, KRS 5), press lightly, remove the crystal disc and dry for about 15 minutes at 60°.

PURITY

Loss on drying (Vol 4) 68.5 – 71.5% (110°, 3 h)

Sulfated ash (Vol. 4) Not more than 0.4%
Test 5 g of the sample (Method I)

**Residual solvents**

(Meth. 4)

Methanol: Not more than 100 mg/kg

**Residual monomers**

Ethanol: Not more than 1,000 mg/kg

Methyl methacrylate: Not more than 50 mg/kg

Ethyl acrylate: Not more than 20 mg/kg

See description under TESTS

**Lead** (Vol. 4)

Not more than 1.0 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 principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

**Microbiological criteria**

(Vol. 4)

Total plate count: Not more than 1,000 cfu/g

Yeast and moulds: Not more than 100 cfu/g

Coliforms: Negative in 10 g

**TESTS**

**PURITY TESTS**

**Residual monomers**

Determined by liquid chromatography (Vol. 4)

**Standards and Reagents:**

- Acetonitrile: UV absorption: $A_{max}$ of 1% at 190 nm
- Tetrahydrofuran and deionized water:
- Sodium perchlorate (35g/l)
- Standards: Ethyl acrylate and methyl methacrylate (>99%)

**Preparation of mixed standard solutions**

Stock mixed standard solution (200 µg/ml):

Accurately weigh about 10 mg each of ethyl acrylate and methyl methacrylate, dissolve in tetrahydrofuran and make up to 50 ml with tetrahydrofuran in a volumetric flask.

Intermediate mixed standard solution-1 (20 µg/ml):

Dilute 1.0 ml of stock mixed standard solution to 10 ml with tetrahydrofuran in a volumetric flask.
Intermediate mixed standard solution-2 (2 µg/ml):
Dilute 1.0 ml of intermediate mixed standard solution-1 to 10 ml with tetrahydrofuran in a volumetric flask.

Working mixed standard solution (0.67 µg/ml):
To 10 ml of Intermediate mixed standard solution-2 add 5 ml of sodium perchlorate and mix. Dilute 5 ml of this mixture to 10 ml with deionized water.

Preparation Sample Solution
Accurately weigh approximately 1.0 g of sample, dissolve in tetrahydrofuran and dilute to 50.0 ml in a volumetric flask. To 5 ml of sodium perchlorate solution, add 10 ml of sample solution drop wise, whilst stirring continuously. Centrifuge and filter the clear supernatant. Dilute 5 ml of this mixture to 10 ml with deionized water.

Procedure
Use a HPLC with diode array/UV detector at 205 nm
Column: Octadecylsilic silica gel (12 cm x 4.6 mm i.d., 5-10 µm.)
Injection volume: 50 µl
Mobile phase: Acetonitrile:Water (15:85)
Flow rate: 2 ml/min

Inject separately 50 µl each of working mixed standard solution and sample solution. Calculate the amount of each monomer in the sample from the peak areas obtained in the chromatograms of working mixed standard solution (rR) and sample solution (rS); amount of standard in the injected solution (R, µg) and weight of sample in injected sample solution (W, g)

\[
\text{Amount of each monomer (µg/g)} = \frac{rS \times R}{rR \times W}
\]

Total monomers in the sample (µg/g) = Sum of monomers in the sample

**METHOD OF ASSAY** Information Required
Appendix: Infrared spectrum of neutral methacrylate copolymer
SPIRULINA EXTRACT (TENTATIVE)


Information Required on:
- Full compositional characterization of commercial products in both liquid and powder forms.
- Full compositional characterization of the aqueous extract before formulation/standardization.
- Validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data).
- Validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).

SYNONYMS
INS 134; Spirulina colour

DEFINITION
Spirulina extract is obtained by aqueous extraction of the biomass of Arthrospira platensis, an edible cyanobacterium. The organism is cultivated and harvested under conditions that prevent the growth of other cyanobacteria and the production of microcystins. The material extracted from the biomass is further treated by steps that may include pH adjustment, centrifugation, filtration, concentration, sterilization, drying, and dilution to the desired degree of pigment concentration. The main colouring principles are two phycobiliproteins, C-phycocyanin and allophycocyanin, which are water-soluble pigment-protein complexes where the chromophore is covalently bonded to the protein. Extracts may also contain trace amounts of chlorophyll, beta-carotene, and other carotenoids. Spirulina extract may contain peptides, other proteins, carbohydrates and minerals. Commercial products are formulated in liquid and powder forms.

C.A.S. number
20298-86-6
(Phycocyanobilin; 3-[(2Z,5E)-2-[[3-(2-carboxyethyl)-5-[(Z)-[(3E,4R)-3-ethylidene-4-methyl-5-oxopyrrolidin-2-yldene]methyl]-4-methyl-1H-pyrrol-2-yl]methylidene]-5-[(4-ethyl-3-methyl-5-oxopyrrol-2-yl)methylidene]-4-methylpyrrol-3-yl]propanoic acid)

Chemical formula
C_{33}H_{38}N_{4}O_{6} (Phycocyanobilin)
Structural formula

![Phycocyanobilin]

Formula weight 586.68 (Phycocyanobilin)

Assay Total phycocyanins as the sum of C-phycocyanin and allophycocyanin not less than declared.

See description under TESTS

DESCRIPTION Clear blue liquid or blue powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water. Insoluble in ethanol.

Colour Value Not less than declared (15 to 300 for powdered products on the dried basis and 10 to 70 for liquid products).

See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 6% for the powdered product (105°, 4h)

Arsenic (Vol. 4) Not more than 1 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 methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
Cadmium (Vol. 4)  Not more than 1 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 methods described in Vol. 4 (under “General Methods, Metallic Impurities”).

Lead (Vol. 4)  Not more than 1 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 methods described in Vol. 4 (under “General Methods, Metallic Impurities”).

Mercury (Vol. 4)  Not more than 1 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 methods described in Vol. 4 (under “General Methods, Metallic Impurities”).

Microbiological criteria (Vol. 4)  Total (aerobic) plate count: less than 1000 CFU/g
Yeast and moulds: less than 100 CFU/g
Coliforms: absent in 10 g
Salmonella spp.: absent in 25 g
S. aureus: absent in 10 g

Microcystins  Less than 0.5 µg/g as microcystin-LR (dried basis)
See description under TESTS

TESTS

PURITY TESTS

Microcystins  Principle
Determine microcystins by enzyme linked immunoassay (ELISA) under the following conditions:

Reagent
Methanol/water (75:25, v/v)
**Equipment**

Use a commercially available ELISA kit with cross reactivity for microcystin-LR and other microcystins.

**Sample preparation**

In the absence of other instructions provided by the ELISA kit manufacturer, follow the procedure presented here.

Dry an appropriate amount of spirulina extract. Homogenize 3.0 g of the dried material in 20.0 ml of the methanol/water reagent for 20 minutes. Centrifuge the resulting suspension at 4500 rpm for 10 minutes. Transfer the supernatant into a glass flask. Add 10.0 ml of the methanol/water reagent to the homogenizer and homogenize the residue for 30 seconds. Centrifuge the resulting suspension at 4500 rpm for 10 minutes. Combine the supernatants and dilute with water to a concentration within the range indicated by the ELISA kit manufacturer.

**Procedure**

Follow the instructions provided by the ELISA kit manufacturer.

**Colour Value**

For the purpose of this specification, Colour Value is based on the absorbance of a buffered solution at 618 nm.

**Reagent**

_Sodium phosphate buffer (100 mM, pH 6.0):_ Transfer 14.04 g of sodium phosphate monobasic dihydrate and 1.75 g of sodium phosphate dibasic anhydrous into a 1000 ml volumetric flask and dilute to volume with water containing 0.05% sodium azide. Adjust the pH to 6.0 with a few drops of phosphoric acid or 1 M NaOH if needed.

**Procedure**

Transfer 330 mg of spirulina extract into a 100 ml volumetric flask and dilute to volume with water. Transfer 10 ml of the solution into a second 100 ml volumetric flask and dilute to volume with the sodium phosphate buffer (100 mM, pH 6.0). Determine the absorbance ($A_{618}$) of the solution in a 1-cm cell at 618 nm with a suitable spectrophotometer using sodium phosphate buffer (100 mM, pH 6.0) as the reference.

Calculate the Colour Value of the spirulina extract as follows:

$$\text{Colour Value} = \frac{A_{618} \times 100}{W_1}$$

Where

$W_1$ is the weight of spirulina extract taken, in g
METHOD OF ASSAY

Principle

Determine total phycocyanins as the sum of C-phycocyanin and allophycocyanin under the following conditions:

Reagent

**Sodium phosphate buffer** (100 mM, pH 6.0): Transfer 14.04 g of sodium phosphate monobasic dihydrate and 1.75 g of sodium phosphate dibasic anhydrous into a 1000 ml volumetric flask and dilute to volume with water containing 0.05% sodium azide. Adjust the pH to 6.0 with a few drops of phosphoric acid or 1 M NaOH if needed.

Procedure

Transfer 100 mg of spirulina extract into a 25 ml volumetric flask and dilute to volume with sodium phosphate buffer (100 mM, pH 6.0). Sonicate the mixture for 30 minutes maintaining the temperature at 8°. Incubate at 30° for 8 h, shaking manually every hour. Mix the contents of the flask and transfer to a centrifugation tube; centrifuge at 3500 rpm for 4 minutes. Determine the absorbance of the supernatant in a 1-cm cell at 620 nm (A$_{620}$) and 650 nm (A$_{650}$) with a suitable spectrophotometer using sodium phosphate buffer (100 mM, pH 6.0) as the reference. The dilution should be adjusted with additional buffer, if needed, to obtain absorbance values of 0.2 to 0.6 at 620 nm.

Calculate the C-phycocyanin content of the spirulina extract (% w/w) as follows:

$$T_{cPC} = (0.162 \times A_{620}) - (0.098 \times A_{650}) \times V_1 \times 100 / W_1$$

Where

- $W_1$ is the weight of spirulina extract taken, in mg
- $V_1$ is the volume of the volumetric flask used to prepare the sample solution, in mL

Calculate the allophycocyanin content of the spirulina extract (% w/w) as follows:

$$T_{aPC} = (0.180 \times A_{620}) - (0.042 \times A_{650}) \times V_1 \times 100 / W_1$$

Where

- $W_1$ is the weight of spirulina extract taken, in mg
- $V_1$ is the volume of the volumetric flask used to prepare the sample solution, in mL

Calculate the total phycocyanin content of the spirulina extract as follows:

$$T_{PC} = T_{cPC} + T_{aPC}$$
SPECIFICATIONS FOR CERTAIN FLAVOURING AGENTS

At the 86th meeting, the Committee prepared specifications of identity and purity of 69 flavourings in 8 sub-categories for the following numbers: 380.1, 380.2, 427, 433, 619, 973-975, 980-982, 1480, 1491-1526, 2103-2105, 2123, 2235-2244, 2246-2255.

Information on specifications for flavouring agents is given in the tables, most of which are self-explanatory: Name; Chemical name (Systematic name, normally IUPAC name); Synonyms; Flavour and Extract Manufacturers’ Association of the United States (FEMA) No; FLAVIS (FL) No; Council of Europe (COE) No; Chemical Abstract Service Registry (CAS) No; Chemical formula (Formula); Molecular weight (MW); Physical form/Odour; Solubility; Solubility in ethanol; Boiling point (B.P. °C – for information only); Identification test (ID) referring to type of test (NMR: Nuclear Magnetic Resonance spectrometry; IR: Infrared spectrometry; MS: Mass spectrometry); Assay min % (Gas chromatographic (GC) assay of flavouring agents); Acid value max; Refractive index (R.I.) (at 20°, if not otherwise stated); Specific gravity (S.G) (at 25°, if not otherwise stated). The field called "Other requirements" contains four types of entry:

1. Items that are additional requirements, such as further purity criteria or other tests.
2. Items provided for information, for example the typical isomer composition of the flavouring agent. These are not considered to be requirements.
3. Substances which are listed as Secondary Constituents (SC) which have been taken into account in the safety evaluation of the named flavouring agent. If the commercial product contains less than 95% of the named compound, it is a requirement that the major part of the product (i.e. not less than 95%) is accounted for by the sum of the named compound and one or more of the secondary constituents.
4. Information on the status of the safety evaluation.

The fields named Session/Status contain the number of the meeting at which the specifications were prepared and the status of the specification. All specifications prepared at the 86th meeting were assigned full status.

The flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents and a list for conclusions in alphabetical order is given in Annex I.
<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS</th>
<th>M.W.</th>
<th>Solubility</th>
<th>mp</th>
<th>%</th>
<th>B.P. °</th>
<th>Acid Value</th>
<th>Assay min %</th>
<th>Odour</th>
<th>Physical Form</th>
<th>Solubility in</th>
<th>M.W.</th>
<th>Synonyms</th>
<th>Status</th>
<th>Chemical Name</th>
<th>FEMA</th>
<th>JECFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridine, 2-(((3-(2,3-dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)thio)methyl)</td>
<td>4802</td>
<td>C₁₆H₁₆N₄O₂S</td>
<td>Soluble</td>
<td>NA</td>
<td>&gt;98 %</td>
<td>328.39</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>153-159°C</td>
<td>90.0</td>
<td>90.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-1-((3-(4-amino-2,2-dioxiido-1H-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one</td>
<td>2236</td>
<td>C₁₈H₂₆N₄O₄S</td>
<td>Soluble</td>
<td>NA</td>
<td>&gt;95 %</td>
<td>394.49</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>236-238°C</td>
<td>&lt;5%</td>
<td>236-238°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-1-((3-(4-amino-2,2-dioxiido-1H-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one</td>
<td>2236</td>
<td>C₁₈H₂₆N₄O₄S</td>
<td>Soluble</td>
<td>NA</td>
<td>&gt;95 %</td>
<td>394.49</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>236-238°C</td>
<td>&lt;5%</td>
<td>236-238°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>Molecular Formula</td>
<td>Solubility</td>
<td>Analytical Methods</td>
<td>mp (°C)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-(4-Methylphenoxy)-N-(1H-pyrazol-3-yl)-N-(thiophen-2-ylmethyl)acetamide</td>
<td><img src="image" alt="Structure" /></td>
<td>C_{17}H_{17}N_{3}O_{2}S</td>
<td>Slightly soluble at pH 2.8</td>
<td>MS, 1H-NMR, 13C-NMR, IR</td>
<td>NA 115-116.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Draft**

Soluble 99% NA

White to off-white solid

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Molecular Formula</th>
<th>Solubility</th>
<th>Analytical Methods</th>
<th>mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-(1H-pyrazol-3-yl)-N-(thiophen-2-ylmethyl)-2-(4-tolyloxy)acetamide</td>
<td><img src="image" alt="Structure" /></td>
<td>C_{17}H_{17}N_{3}O_{2}S</td>
<td>Slightly soluble at pH 2.8</td>
<td>MS, 1H-NMR, 13C-NMR, IR</td>
<td>NA 115-116.5</td>
</tr>
</tbody>
</table>

**Draft**

Soluble 99% NA

White to off-white solid
<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS</th>
<th>M.W</th>
<th>Physical Form</th>
<th>Odor</th>
<th>Assay min %</th>
<th>Solubility</th>
<th>Solubility in</th>
<th>B.P.</th>
<th>Acetyl value</th>
<th>Info. Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyldecanal</td>
<td></td>
<td>170.29</td>
<td>colorless liquid/ citrus/green odor</td>
<td>95%</td>
<td>Soluble</td>
<td>MS, 1H-NMR, 13C-NMR</td>
<td>1.4224 - 1.4421</td>
<td>4803 C</td>
<td>1.4256 - 1.4260</td>
<td></td>
</tr>
<tr>
<td>Methylnonanal</td>
<td></td>
<td>156.27</td>
<td>colorless, transparent liquid/Sweet aroma of fruit with green notes</td>
<td>&gt;95%</td>
<td>Soluble</td>
<td>MS, 1H-NMR, 13C-NMR</td>
<td>1.4224 - 1.4421</td>
<td>4803 C</td>
<td>1.4256 - 1.4260</td>
<td></td>
</tr>
<tr>
<td>Isodecanal, Isodecaldehyde</td>
<td></td>
<td>196.19</td>
<td>Colorless, transparent liquid/ Sweet aroma of fruit with green notes</td>
<td>95%</td>
<td>Soluble</td>
<td>MS, 1H-NMR, 13C-NMR</td>
<td>1.4224 - 1.4421</td>
<td>4803 C</td>
<td>1.4256 - 1.4260</td>
<td></td>
</tr>
</tbody>
</table>
GROUP 3: LINEAR AND BRANCHED-CHAIN ALIPHATIC, UNSATURATED, UNCONJUGATED ALCOHOLS, ALDEHYDES, ACIDS, AND RELATED ESTERS

<table>
<thead>
<tr>
<th>JECFA No.</th>
<th>Name</th>
<th>FEMA</th>
<th>Chemical Formula</th>
<th>Solubility in ethanol</th>
<th>ID test</th>
<th>R.I. (20°)</th>
<th>Other requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>2240</td>
<td>trans-6-Octenal (E)-Oct-6-enal</td>
<td>4787</td>
<td>C₉H₁₈O₂</td>
<td>Slightly soluble</td>
<td>1.4377</td>
<td>0.8536 (20°)</td>
<td>Information required</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>126.20</td>
<td>Soluble</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(E)-Oct-6-enal</td>
<td></td>
<td>Slightly soluble</td>
<td>95%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1H-NMR, 13C-NMR</td>
<td>1.428-1.459</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MS, 1H-NMR,</td>
<td>1.428-1.459</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13C-NMR, &gt;90%</td>
<td>0.794-0.904</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2241</td>
<td>2,6-Dimethyl-5-heptenol 2,6-Dimethylhept-5-en-1-ol</td>
<td>4789</td>
<td>C₉H₁₈O₂</td>
<td>Slightly soluble</td>
<td>142.24</td>
<td>0.794-0.904</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,6-dimethyl-5-hepten-1-ol, Melonol</td>
<td></td>
<td>142.24</td>
<td>Soluble</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colorless to light yellow, transparent liquid/Floral fruity aroma reminiscent of melon</td>
<td>204-206°</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Secondary component: 1-6% 2,6-dimethyl-5-heptenal (No. 349) The sum of 2,6-dimethyl-5-heptenol and 2,6-dimethyl-5-heptenal is ≥ 95%*
<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS</th>
<th>Chemical Formula</th>
<th>Solubility</th>
<th>Other Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLAVIS</td>
<td>2242</td>
<td>C₁₄H₂₂O₂</td>
<td>Practically insoluble to insoluble</td>
<td>Prerequisite: 30% to 50% of 2-M-chromene-2-carboxylic acid</td>
</tr>
<tr>
<td>Pinocarvyl isobutyrate</td>
<td>2243</td>
<td>C₁₄H₂₂O₂</td>
<td>Practically insoluble</td>
<td>ms 1H-NMR: NA; mp: 144-145°C</td>
</tr>
<tr>
<td>Carvyl palmitate</td>
<td>2244</td>
<td>C₂₆H₄₆O₂</td>
<td>Practically insoluble to insoluble</td>
<td>Mixture of (2R,4S)-carvyl palmitate and (2S,4S)-carvyl palmitate</td>
</tr>
<tr>
<td>Carvyl hexadecanoate</td>
<td>2245</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FLAVIS**

- Soluble
- Practically insoluble to insoluble
- Prerequisite: 30% to 50% of 2-M-chromene-2-carboxylic acid
- Prerequisite: 30% to 50% of 2-M-chromene-2-carboxylic acid
- Mixture of (2R,4S)-carvyl palmitate and (2S,4S)-carvyl palmitate
<table>
<thead>
<tr>
<th>2244</th>
<th><strong>6-Hydroxycarvone</strong></th>
<th>C_{10}H_{14}O_{2}</th>
<th>Slightly soluble</th>
<th>MS, 1H-NMR, IR</th>
<th>NA</th>
<th>mp: 185 °</th>
</tr>
</thead>
<tbody>
<tr>
<td>Draft</td>
<td>3-hydroxy-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one</td>
<td>166.22</td>
<td>Soluble</td>
<td>&gt;95%</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Solid/cool mint-like aroma
### Group 5: Menthol and Structurally Related Substances

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>FEMA No.</th>
<th>Solubility</th>
<th>Assay min %</th>
<th>B.P. °</th>
<th>Acid Value</th>
<th>Odor</th>
<th>Physical Form</th>
<th>Chemical Name</th>
<th>CAS</th>
<th>Status</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menthyl formate</td>
<td>4509</td>
<td>Practically insoluble to insoluble</td>
<td>&gt;95%</td>
<td>212-34</td>
<td>1.441-1.449</td>
<td>Clear, liquid</td>
<td>MS, IR</td>
<td>2-isopropyl-5-methylcyclohexyl formate</td>
<td>2246</td>
<td>Draft</td>
<td>2-isopropyl-5-methylcyclohexyl formate</td>
</tr>
<tr>
<td>Menthyl propionate</td>
<td>4510</td>
<td>Practically insoluble to insoluble</td>
<td>MS, IR</td>
<td>90-2</td>
<td>1.444-1.449</td>
<td>Clear, liquid</td>
<td>MS</td>
<td>2-isopropyl-5-methylcyclohexyl propionate</td>
<td>2247</td>
<td>Draft</td>
<td>2-isopropyl-5-methylcyclohexyl propionate</td>
</tr>
<tr>
<td>Compound</td>
<td>CAS Number</td>
<td>Molecular Formula</td>
<td>Physical Properties</td>
<td>Identification Methods</td>
<td>Melting Point (°C)</td>
<td>Notes</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>l-Menthyl butyrate</td>
<td>4524</td>
<td>C₁₃H₂₀O₂</td>
<td>Practically insoluble to insoluble</td>
<td>MS</td>
<td>1.445-1.450</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-isopropyl-5-methylcyclohexyl butyrate</td>
<td>226.36</td>
<td></td>
<td>Sparingly soluble</td>
<td>&gt;95%</td>
<td>0.912-0.915</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isomenthol</td>
<td>4729</td>
<td>C₁₀H₁₉O</td>
<td>Practically insoluble to insoluble</td>
<td>MS, 1H-NMR, IR</td>
<td>NA</td>
<td>m.p. 82 °; racemic mixture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cyclohexanol, 5-methyl-2-(propan-2-yl)cyclohexan-1-ol, (1S, 2R, 5R) (+)-Isomenthol, (1R, 2S, 5S) (-)-Isomenthol

Solid/cool minty aroma
Dimenthyl glutarate

C25H44O4

Practically insoluble to Insoluble

1H-NMR

mp: Approx. 40°

Dimenthyl bis(2-isopropyl-5-methylcyclohexyl) glutarate

09.935

408.61

Very slightly soluble

Dimenthyl Pentanedioic acid, bis-[5-methyl-2-(1-methylethyl)cyclohexyl] ester

(9CI); Glutaric acid, di-(p-menth-3-yl) Ester

Amber amorphous or crystalline solid/Fresh minty aroma

IR

Melting Range: 41-44° (-menthol);
Nonvolatile Residue: =< 0.05%;
Angular Rotation: -52° to -40° (-menthol):
-2° to +2° (dl-menthol)

Dimenthyl 3-p-Menthanol

156.27

95%; sum of (+/-) isomers

0.901 (20°);
0.891 (30°)

colourless, hexagonal crystals, usually needle-like;
and very soluble in alcohol

Dimenthyl Menthol

400.79-72.73

95% solution in alcohol

C10H20O

very soluble in alcohol

and volatile oils;
slightly soluble in water

IR

measurable at d=0.984 (+)
0.904 (-)

NA

mp: Approx. 40°

Dimenthyl glutarate

4H-NMR

Full

Dimenthyl 3-p-Menthanol

4H-NMR

Full

Dimenthyl Menthol

4H-NMR

Full

Dimenthyl 3-p-Menthanol
Draft

2-(2-((2-isopropyl-5-methylcyclohexyl)oxy)ethoxy)ethanol

Practically insoluble to insoluble MS, 1H-NMR, IR 1.444 - 1.484 (1R, 2S, 5R) (1S, 2R, 5S)

>95% (as a sum of the + and - isomers; racemic mixture) 0.947 - 0.987 (20°); 0.945 - 0.985 (25°)

Colorless viscous liquid/Minty herbal aroma with fruity notes 97° (0.2 mmHg) NA
<table>
<thead>
<tr>
<th>Name</th>
<th>Chemical Name</th>
<th>Chemical Formula</th>
<th>Solubility in Ethanol</th>
<th>Solubility in Water</th>
<th>Solubility in Alcohol</th>
<th>Solubility in Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLAVIS</td>
<td>Ethyl maltol isobutyrate</td>
<td>C₁₁H₁₄O₄</td>
<td>Soluble</td>
<td>Soluble to insoluble</td>
<td>Insoluble</td>
<td>Insoluble</td>
</tr>
<tr>
<td></td>
<td></td>
<td>210-23</td>
<td>93-94%</td>
<td>58-65° (2 mm Hg)</td>
<td>1.132-1.138</td>
<td>1.480-1.496</td>
</tr>
</tbody>
</table>

Yellow light yellow liquid; sweet fruity aroma.
# Group 7: Alicyclic Primary Alcohols, Aldehydes, Acids and Related Esters

<table>
<thead>
<tr>
<th>JECFA No.</th>
<th>Name</th>
<th>FEMA</th>
<th>Chemical Formula</th>
<th>Solubility</th>
<th>ID test</th>
<th>R.I. (20°)</th>
<th>Other requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>2253</td>
<td>Mixture of 1-Vinyl-3-cyclohexenecarbaldehyde and 4-Vinyl-1-cyclohexenecarbaldehyde</td>
<td>FLAVIS</td>
<td>C₉H₁₂O</td>
<td>Very slightly soluble</td>
<td>MS, 1H-NMR, 13C-NMR</td>
<td>1.4870 - 1.4930</td>
<td>60%-70% 1-vinyl-3-cyclohexenecarbaldehyde and 25-35% 4-vinyl-1-cyclohexenecarbaldehyde</td>
</tr>
<tr>
<td>86</td>
<td>1-Vinylcyclohex-3-ene-1-carbaldehyde and 4-vinylcyclohex-1-ene-1-carbaldehyde</td>
<td>CE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>973</td>
<td>p-Mentha-1,8-dien-7-al</td>
<td>CAS</td>
<td>C₁₀H₁₄O</td>
<td>Insoluble in water; soluble in alcohols and oils</td>
<td>NMR</td>
<td>1.504-1.513</td>
<td>Safety evaluation not completed</td>
</tr>
<tr>
<td>86</td>
<td>1-Vinylcyclohex-3-ene-1-carbaldehyde</td>
<td></td>
<td></td>
<td>Miscible at room temperature</td>
<td></td>
<td>0.948-0.956</td>
<td></td>
</tr>
<tr>
<td>2111-75-3</td>
<td>2111-75-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Methyl 2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol

C_{15}H_{26}O

Soluble

Very slightly soluble

MS, 1H-NMR, 13C-NMR

1.4820 - 1.4880

Racemic mixture of R- and S-

166.09

Racemic mixture of R- and S-

4.790 C_{10}H_{20}O

1.047 (20°)

Soluble

Clear liquid; Floral aroma with earthy fermented undertones

1% 71.9% 21.2% 0.007 - 1.117

1% 71.9% 21.2% 0.007 - 1.117

1% 71.9% 21.2% 0.007 - 1.117

Very slightly soluble

Racemic mixture of R- and S-

Soluble

Clear liquid; Floral aroma with earthy fermented undertones

1% 71.9% 21.2% 0.007 - 1.117

1% 71.9% 21.2% 0.007 - 1.117

1% 71.9% 21.2% 0.007 - 1.117

Very slightly soluble

Racemic mixture of R- and S-

Soluble

Clear liquid; Floral aroma with earthy fermented undertones

1% 71.9% 21.2% 0.007 - 1.117

1% 71.9% 21.2% 0.007 - 1.117

1% 71.9% 21.2% 0.007 - 1.117

Very slightly soluble

Racemic mixture of R- and S-

Soluble

Clear liquid; Floral aroma with earthy fermented undertones

1% 71.9% 21.2% 0.007 - 1.117

1% 71.9% 21.2% 0.007 - 1.117

1% 71.9% 21.2% 0.007 - 1.117

Very slightly soluble

Racemic mixture of R- and S-
### GROUP 8: FURAN SUBSTITUTED ALIPHATIC HYDROCARBONS, ALCOHOLS, ALDEHYDES, KETONES, CARBOXYLIC ACIDS AND RELATED ESTERS, SULFIDES, DISULFIDES AND ETHERS

<table>
<thead>
<tr>
<th>JECFA No.</th>
<th>Name</th>
<th>FEMA</th>
<th>Chemical Formula</th>
<th>Solubility</th>
<th>ID test</th>
<th>R.I. (20°)</th>
<th>Other requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1491</td>
<td>2-Pentylfuran</td>
<td>3317</td>
<td>C₆H₁₀O</td>
<td>Slightly soluble in water</td>
<td>NMR</td>
<td>1.443-1.449</td>
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<td>1492</td>
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<td>Colourless to yellowish liquid; Nutty, coffee-like aroma</td>
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Decylfuran

- Molecular formula: C_{14}H_{24}O
- Insoluble in water
- NMR
- mp: 30°

Methyl-(3-methylbut-2-enyl)-furan

- Molecular formula: C_{10}H_{14}O
- Slightly soluble in water
- MS
- 1.473 - 1.479

Rosefuran; 2-(3-methylbut-2-enyl)-3-methylfuran

- Molecular formula: C_{10}H_{16}O
- Colourless liquid; Caramel aroma
- mp: 70° (11 mm Hg)

(2-Furyl)acrolein

- Molecular formula: C_{7}H_{6}O_2
- Insoluble in water
- NMR
- mp: 49-52°
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<th>CAS Registry Number</th>
<th>Molecular Formula</th>
<th>Solubility in Water</th>
<th>NMR (13C)</th>
<th>IR</th>
<th>Solubility (%</th>
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<td>3-(5-Methyl-2-furyl)prop-2-enal</td>
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<td>Acetyl-3,5-dimethylfuran</td>
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<td>1-(2-Furyl)-1-butanone</td>
<td>C₇H₁₄O₂</td>
<td>Soluble 97%</td>
<td>1.074-1.080</td>
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<td>colourless liquid, aroma suggestive of radish</td>
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<td>(2-Furyl)-2-propanone</td>
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<td>(2-Furanyl)-1-pentanone</td>
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<td>2,5-Dimethyl-3-thioacetoxyfuran</td>
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<td>1.527-1.533</td>
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<td>Furfuryl ether</td>
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<td>1.113-1.114</td>
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<td>2,5-Dimethyl-3-thiophenylacetate</td>
<td>C₁₀H₁₀O₂S</td>
<td>Soluble</td>
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<td>colourless liquid, fruity floral</td>
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Notes:
- MS: Mass Spectrum
- mp: Melting Point
- NMR: Nuclear Magnetic Resonance
- IR: Infrared Spectroscopy
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<th>4120</th>
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<th>Slightly soluble in water</th>
<th>MS</th>
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<td>1-(2-Furyl)butan-3-one</td>
<td>13.138</td>
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<td>4-(2-Furyl)-2-butanone;</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Colourless solid; Spicy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>caramel aroma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1511</th>
<th><strong>4-(2-Furyl)-3-buten-2-one</strong></th>
<th>2495</th>
<th>C₈H₁₀O₂</th>
<th>Insoluble in water</th>
<th>NMR</th>
<th>mp: 37-40°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>4-(2-Furyl)but-3-en-2-one</td>
<td>13.044</td>
<td>136.15</td>
<td>Soluble</td>
<td>98%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Furylidene acetone; Furfural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>acetone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colourless needle crystals;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spicy aroma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1513</th>
<th><strong>Ethyl 3-(2-furyl)propionate</strong></th>
<th>2435</th>
<th>C₉H₁₂O₃</th>
<th>Very slightly soluble in water</th>
<th>NMR</th>
<th>mp: 24-25°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>Ethyl 3(2-furyl)propionate</td>
<td>13.022</td>
<td>168.19</td>
<td>Soluble</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethyl furfurylacetate; Ethyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>furylpropionate; Ethyl 2-furan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>propionate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Low melting solid, turning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>yellow on exposure to air;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fruity aroma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Isobutyl 3-(2-furan)propionate

- Very slightly soluble in water
- NMR: 1.531 - 1.537

- Full

Isoamyl 3-(2-furan)propionate

- Insoluble in water
- NMR: 1.549 - 1.557

- Full

2-Isoamyl furfurylacetate; 3-Methylbutyl 3-(2-furyl)propionate; Isobutyl 2-furanpropionate

- Colourless to pale yellow liquid; Fruity, winey, brandy-like aroma
- Boiling point: 263-265° (3 mm Hg)

- Full

Isoamyl 4-(2-furan)butyrate

- Insoluble in water
- NMR: 1.551 - 1.555

- Full

3-Methylbutyl 4-(2-furan)butanoate

- Soluble 95%
- NMR: 1.975 - 0.981

- Full

Isopentyl 2-furanbutyrate; alpha-Isoamyl furfurylpropionate; 3-Methylbutyl 2-furanbutyrate

- Pale yellowish liquid; Sweet, buttery, fruity and caramel-like aroma
- Boiling point: 263-265° (3 mm Hg)

- Full

2-Methylbutyl 3-(2-furan)propionate
<table>
<thead>
<tr>
<th>Code</th>
<th>Compound</th>
<th>CAS Number</th>
<th>Molecular Formula</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1517</td>
<td>Phenethyl 2-furoate</td>
<td>2685</td>
<td>C_{13}H_{12}O_{3}</td>
<td>Insoluble in water; Soluble in oils; NMR 1.585-1.593</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.006</td>
<td>216.24</td>
<td>Soluble 96% 1.136-1.142</td>
</tr>
<tr>
<td></td>
<td>2-Phenylethyl 2-furoate; Phenylethyl 2-furoate</td>
<td></td>
<td></td>
<td>Colourless liquid; Warm, fruity-caramel, slightly earthy, oily aroma 275° 5</td>
</tr>
</tbody>
</table>

86

| 1520 | Furfuryl methyl ether           | 3159       | C_{6}H_{8}O_{2}   | Insoluble in water; Soluble in ether; NMR 1.454-1.460                    |
|      |                                  | 13.052     | 112.13            | Soluble 99% 1.013-1.019                                                   |
|      | Unlabeled Compound               | 7149-32-8  |                   |                                                                           |
|      | Methyl furfuryl ether            |            |                   |                                                                           |
|      |                                  | 13679-46-4 |                   |                                                                           |

86

| 1521 | Ethyl furfuryl ether            | 4114       | C_{7}H_{10}O_{2}  | Slightly soluble in water                                                 |
|      |                                  | 13.123     | 126.15            | Soluble 95% 0.982-0.988                                                   |
|      |                                  |            |                   |                                                                           |
|      | Furfuryl ethyl ether             |            |                   |                                                                           |

86
1524  **Furfuryl 2-methyl-3-furyl disulfide**

Full

Furfuryl 2-methyl-3-furyl disulfide
3-(2-Furanylmethyl)dithio]-2-methylfuran; (2-Methyl-3-furyl)furfuryl disulfide; 2-Methyl-3-[(2-furanylmethyl)dithio]furan; 3-(Furfuryldithio)-2-ethylfuran

Slightly soluble in water; Soluble in pentane, diethyl ether

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>HNMR</td>
<td>1.581-1.587</td>
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<tr>
<td>IR</td>
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<tr>
<td>SC</td>
<td>6-7%</td>
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</tbody>
</table>

109537-55-5

1525  **3-[(2-Methyl-3-furyl)thio]-2-butanone**

Full

3-[(2-Methyl-3-furyl)thio]-2-butanone
3-[(2-Methyl-3-furanyl)sulfanyl]-2-butanone; 3-[(2-Methyl-3-furyl)thio]-2-butanone; 3-[(2-Methyl-3-furyl)sulfanyl]-2-butanone

Soluble in ethyl acetate, triacetin, Practically insoluble in water

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>HNMR</td>
<td>1.510-1.516</td>
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<tr>
<td>MS</td>
<td></td>
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<tr>
<td>Soluble</td>
<td>99%</td>
</tr>
<tr>
<td>SC</td>
<td>1-1.104</td>
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</tbody>
</table>

61295-44-1
O-Ethyl S-(2-furylmethyl)thiocarbonate

**Chemical Structure:**

- Molecular Formula: C\textsubscript{8}H\textsubscript{10}O\textsubscript{3}S
- Pratically insoluble in water; soluble in diethyl ether, ethyl acetate.

**Physical Properties:**

- Colourless liquid; spicy, floral aroma
- Mp: 130-135°C
- Soluble in fats
- NMR: 1.56-1.63
- IR: 13.07
- MS: 333
- Spicy aroma; clear to yellow liquid; Nutty
- mp: 153°C
- Soluble in fats
- NMR: 1.03-1.07
- IR: 14.06
- MS: 336
- Colourless solid; Floral, fruity aroma
- mp: 153°C
- Insoluble in water
- HNMR IR MS

- Full
- 2,4-Difuranylfuran
- 146.2
- 146.2
- 2,3-Dimethylbenzofuran
- 13.074
- 14.76
- 2,4-Difurfurylfuran
- 13.107
- 228.24
- 376-696-5
- O-Ethyl S-(2-furylmethyl)thiocarbonate
- 696.2
- 496.0
- O-Ethyl S-(2-furylmethyl)thiocarbonate
- 696.2
<table>
<thead>
<tr>
<th>CAS No.</th>
<th>Name</th>
<th>Molecular Formula</th>
<th>Properties</th>
<th>NMR</th>
<th>Boiling Point</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500</td>
<td>2-Furfurylidenebutyraldehyde</td>
<td>C₉H₁₀O₂</td>
<td>Insoluble in water; Soluble in oils</td>
<td>1.570-1.576</td>
<td>152.18</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2-Furfurylidene-2-butanal</td>
<td>C₉H₁₀O₂</td>
<td>Pale yellowish liquid; Mild, warm, vegetable-like aroma</td>
<td>1.575-1.581</td>
<td>150.18</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>3-(5-Methyl-2-furyl)-butanal</td>
<td>C₉H₁₂O₂</td>
<td>Colourless liquid; Vegetable, fruity aroma</td>
<td>1.006-1.012</td>
<td>88-91° (12 mm Hg)</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>3-(5-Methyl-2-furyl)-butanal</td>
<td>C₉H₁₀O₂</td>
<td>Pale yellowish liquid; Mild, warm, cinnamon-like aroma</td>
<td>1.057-1.063</td>
<td>150.18</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2-Methyl-3(2-furyl)acrolein</td>
<td>C₉H₁₀O₂</td>
<td>Pale yellowish liquid; Mild, warm, cinnamon-like aroma</td>
<td>1.567-1.573</td>
<td>136.15</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>3-(2-Furyl)-2-methylprop-2-enal</td>
<td>C₉H₁₀O₂</td>
<td>Pale yellowish liquid; Mild, warm, cinnamon-like aroma</td>
<td>1.097-1.103</td>
<td>136.15</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2-Methyl-3-(2-furyl)propenal;alpha-Methylfurylacroleine;Furfurylidene-2-propanal</td>
<td>C₉H₁₀O₂</td>
<td>Pale yellowish liquid; Mild, warm, cinnamon-like aroma</td>
<td>1.097-1.103</td>
<td>136.15</td>
<td>86</td>
</tr>
<tr>
<td>No.</td>
<td>Name</td>
<td>CAS No.</td>
<td>Molecular Formula</td>
<td>Solubility/Property</td>
<td>MP (°C)</td>
<td>NMR</td>
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<tr>
<td>-----</td>
<td>------------------------------------------------</td>
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<td>-------------------</td>
<td>------------------------------------------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>1502</td>
<td>2-Phenyl-3-(2-furyl)prop-2-enal</td>
<td>3586</td>
<td>C_{13}H_{10}O_{2}</td>
<td>Insoluble in water</td>
<td>NMR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Full</td>
<td>13.137</td>
<td>198.22</td>
<td>Soluble</td>
<td>99%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-(2-Furyl)-2-phenylprop-2-enal</td>
<td></td>
<td></td>
<td>White solid; Berry aroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-Furfurylidene phenylacetaldehyde</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td></td>
<td>65545-81-5</td>
<td></td>
<td>Slightly soluble in water; Soluble in propylene glycol, most fixed oils</td>
<td>NMR</td>
<td>1.488-1.490</td>
</tr>
<tr>
<td>1506</td>
<td>3-Acetyl-2,5-dimethylfuran</td>
<td>3391</td>
<td>C_{8}H_{10}O_{2}</td>
<td>Soluble</td>
<td>99%</td>
<td>1.037-1.039</td>
</tr>
<tr>
<td></td>
<td>Full</td>
<td>13.066</td>
<td>138.17</td>
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<tr>
<td></td>
<td>3-Acetyl-2,5-dimethylfuran</td>
<td></td>
<td></td>
<td>Clear to yellow liquid; Powerful, slightly roasted, nutty aroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,5-Dimethyl-3-acetylfuran</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td></td>
<td>10599-70-9</td>
<td></td>
<td>Slightly soluble in water</td>
<td>NMR</td>
<td>1.490-1.496</td>
</tr>
<tr>
<td>1512</td>
<td>Pentyl 2-furyl ketone</td>
<td>3418</td>
<td>C_{10}H_{14}O_{2}</td>
<td>Soluble</td>
<td>99%</td>
<td>0.992-0.998</td>
</tr>
<tr>
<td></td>
<td>Full</td>
<td>13.070</td>
<td>166.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-Hexanoylfuran</td>
<td></td>
<td></td>
<td>Colourless to yellow liquid; Apricot, peach-like aroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-Furyl pentyl ketone</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>14360-50-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1518</td>
<td><strong>Propyl 2-furanacrylate</strong></td>
<td>2945</td>
<td>C(<em>{10})H(</em>{12})O(_3)</td>
<td>Insoluble in water</td>
<td>NMR</td>
<td>1.071-1.077</td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Full</td>
<td>Propyl 3(2-furyl)prop-2-enoate</td>
<td>13.047</td>
<td>180.2</td>
<td>Soluble</td>
<td>97%</td>
<td>(20°)</td>
</tr>
<tr>
<td></td>
<td>2-Propenoic acid, 3-(2-furanyl)-, propyl ester, Propyl 3-(2-furyl)acrylate</td>
<td></td>
<td>Colourless to pale yellow liquid; Light strawberry, pear-like aroma</td>
<td></td>
<td>119° (7 mm Hg)</td>
<td>5</td>
</tr>
</tbody>
</table>

86

<table>
<thead>
<tr>
<th>1519</th>
<th><strong>2,5-Dimethyl-3-oxo-(2H)-furan-4-yl butyrate</strong></th>
<th>3970</th>
<th>C(<em>{10})H(</em>{14})O(_4)</th>
<th>Insoluble in water</th>
<th>NMR</th>
<th>1.467-1.473</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>4-Butyroxy-2,5-dimethyl-3(2H)-furanone</td>
<td></td>
<td>Soluble</td>
<td>93%</td>
<td>1.095-1.103</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colourless to pale yellow liquid; Spicy, sweet aroma</td>
<td></td>
<td>287°</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

86

<table>
<thead>
<tr>
<th>2103</th>
<th><strong>(E)-Ethyl 3-(2-furyl)acrylate</strong></th>
<th>4541</th>
<th>C(<em>{9})H(</em>{10})O(_3)</th>
<th>Practically insoluble to insoluble in water</th>
<th>MS</th>
<th>1.542-1.548</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>Ethyl (2E)-3-(furan-2-yl)prop-2-enoate</td>
<td>166.17</td>
<td>Soluble</td>
<td>95%</td>
<td>1.090-1.096</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethyl(E)-3-(2-furyl)-2-propenoate</td>
<td>Viscous liquid; Sweet aroma</td>
<td>230-233°</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2,2'-Methanediyldifuran

Colourless clear liquid; Rich roasted aroma

194 - 195°

95%

1.097 - 1.103 (20°)

C₉H₈O₂

4540

Practically insoluble to insoluble in water

MS 1.501 - 1.507

Full

2-Methylbenzofuran

Colourless liquid; Burnt phenolic aroma

197 - 198°

95%

1.052 - 1.057

C₉H₈O

4543

Practically insoluble to insoluble in water

MS 1.548 - 1.560

Full

2-Methyl-1-benzofuran

Colourless clear liquid; Rich roasted aroma

194 - 195°

95%

1.097 - 1.103 (20°)

C₉H₈O₂

4540

Practically insoluble to insoluble in water

MS 1.501 - 1.507

Full

2'-Methyl-1-benzofuran

Colourless clear liquid; Rich roasted aroma

194 - 195°

95%

1.097 - 1.103 (20°)

C₉H₈O₂

4540

Practically insoluble to insoluble in water

MS 1.501 - 1.507

Full

2,2'-Methanediyldifuran

Colourless clear liquid; Rich roasted aroma

194 - 195°

95%

1.097 - 1.103 (20°)

C₉H₈O₂

4540

Practically insoluble to insoluble in water

MS 1.501 - 1.507

Full
### REVISIONS TO EXISTING FLAVOUR SPECIFICATIONS

<table>
<thead>
<tr>
<th>JECFA No.</th>
<th>Name</th>
<th>FEMA</th>
<th>Chemical Formula</th>
<th>Solubility</th>
<th>ID test</th>
<th>R.I. (20°)</th>
<th>Other requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status</td>
<td>Chemical Name</td>
<td>Physical form; Odour</td>
<td>M.W</td>
<td>Solubility in ethanol</td>
<td>Assay min %</td>
<td>S.G. (25°)</td>
<td>Information required</td>
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<tr>
<td>Synonyms</td>
<td>CAS</td>
<td>COE</td>
<td>Solubility in ethanol</td>
<td>Assay min %</td>
<td>S.G. (25°)</td>
<td>Information required</td>
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<td>Session</td>
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<td>B.P. °</td>
<td>Acid value</td>
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<td>433</td>
<td>FLAVIS</td>
<td>COE</td>
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</tr>
<tr>
<td>Full</td>
<td>I-methyl L-lactate</td>
<td>COE</td>
<td>C13H24O3</td>
<td>IR</td>
<td>97%</td>
<td></td>
<td>Melting point c.25°</td>
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<td></td>
<td>(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl</td>
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<tr>
<td></td>
<td>(2S)-2-Hydroxypropanoate</td>
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<td></td>
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<tr>
<td>86</td>
<td>619</td>
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</tr>
<tr>
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<td>L-malic acid</td>
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<tr>
<td>Full</td>
<td>2-Hydroxybutanedioic acid</td>
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<td>86</td>
<td></td>
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<tr>
<td>Full</td>
<td>Hydroxybutanedioic acid</td>
<td></td>
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<tr>
<td>Full</td>
<td>Glutamyly-valyl-glycine</td>
<td></td>
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<td>2123</td>
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<td>Full</td>
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<td>86</td>
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<td></td>
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</tr>
<tr>
<td>Full</td>
<td>L-gamma-glutamyl-L-valyl-glycine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SPECTRA OF CERTAIN FLAVOURING AGENTS

433  Methyl lactate, (-)

619  Methyl ester

974  2,3,4,6-Tetra-O-acetyl-1-thio-

975  β-D-galactopyranose

980  2,3,4,6-Tetra-O-acetyl-1-thio-

981  β-D-galactopyranose
## CORRIGENDUM

The following requests for corrections, reported to the JECFA secretariats, were evaluated by the 86th JECFA meeting and found to be necessary. These corrections, however, will only be made in the electronic versions and in the on-line database.

<table>
<thead>
<tr>
<th>Food additive</th>
<th>Original text</th>
<th>New text</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium disodium ethylenediaminetetraacetate (INS 385) Monograph 1 (2006)</td>
<td>CAS No. 662-33-9</td>
<td>CAS No. 62-33-9</td>
<td>Transcription error</td>
</tr>
<tr>
<td>Chlorophyllins, copper complexes sodium and potassium salts (INS 141(ii)) Monograph 5 (2008) Test for &quot;Free ionisable copper&quot;</td>
<td>Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil….</td>
<td>Accurately weigh about 1 g of the sample and mix in 20 ml of arachid oil….</td>
<td>Correction</td>
</tr>
<tr>
<td>Curcumin (INS: 100(ii)) Monograph 1 (2006)</td>
<td>The criteria for several residual solvents are listed under the heading &quot;Residual solvents&quot; (see Fig. 1).</td>
<td>Acetone: Not more than 30 mg/kg Hexane: Not more than 25 mg/kg Methanol: Not more than 50 mg/kg Ethanol: Not more than 50 mg/kg Isopropanol: Not more than 50 mg/kg Ethyl acetate: Not more than 50 mg/kg</td>
<td>Improves readability It was unclear whether the criterion “Not more than 50 mg/kg” extended to methanol, ethanol, isopropanol and ethyl acetate.</td>
</tr>
<tr>
<td>Ethyl acetoacetate ethyleneglycol ketal JECFA No: 1969 JECFA 73 (2010)</td>
<td>CAS No. 1648615</td>
<td>CAS No. 6413-10-1</td>
<td>Transcription error</td>
</tr>
<tr>
<td>Ethyl 2-methyl pentanoate JECFA No: 214 JECFA 55 (2000)</td>
<td>CAS No. 28959-02-6</td>
<td>CAS No. 39255-32-8</td>
<td>Wrong CAS number</td>
</tr>
<tr>
<td>cis-3-Hexen-1-ol JECFA No.: 315 JECFA 51 (1998)</td>
<td>98.0% (sum of (Z) and (E) isomers, =&lt;92.0% (Z))</td>
<td>98.0% (sum of (Z) and (E) isomers, =&gt;92.0% (Z))</td>
<td>Transcription error</td>
</tr>
<tr>
<td>Myrcene JECFA No.: 1327 JECFA 63 (2004)</td>
<td>Specific gravity: 0.789–1.793</td>
<td>Specific gravity: 0.789–0.793</td>
<td>Transcription error</td>
</tr>
<tr>
<td>Food additive</td>
<td>Original text</td>
<td>New text</td>
<td>Additional information</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------</td>
<td>----------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Polyoxyethylene (20) sorbitan monostearat (Polysorbate 60) (INS 435) Monograph 16 (2014)</td>
<td>CAS No. 9005-07-6</td>
<td>CAS No. 9005-67-8</td>
<td>Wrong CAS number</td>
</tr>
<tr>
<td>Sodium aluminium silicate (INS 554) Monograph 20 (2017)</td>
<td>Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed “on dried basis”.</td>
<td>Within the assay, the limits for silicon dioxide, aluminium oxide and sodium oxide are expressed “on ignited basis”.</td>
<td>Transcription error</td>
</tr>
<tr>
<td>Silicon dioxide, amorphous (INS 551) Monograph 20 (2017)</td>
<td>CAS No. 112696-00-8 (hydrated silica)</td>
<td>CAS No. 112926-00-8 (hydrated silica)</td>
<td>Transcription error</td>
</tr>
<tr>
<td>Sodium thiosulfate (INS 539) Monograph 1 (2006)</td>
<td>CAS No. 7772-98-7</td>
<td>CAS No. 10102-17-7</td>
<td>CAS No. 7772-98-7 refers to the anhydrous form. The specifications in the monograph refer to the pentahydrate form.</td>
</tr>
<tr>
<td>Brown HT and its aluminium lake (FAO JECFA Monographs 19, 82nd meeting, 2016)</td>
<td>Text in the Table 1 “Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry”</td>
<td>See Table 1, below</td>
<td></td>
</tr>
<tr>
<td>Fast Green FCF (FAO JECFA Monographs 19, 82nd meeting, 2016)</td>
<td>Chemical structure in Table 1 “Values for synthetic colours for use in performing tests for colouring matters content by spectrophotometry”</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CAS: Chemical Abstracts Service; INS: International Numbering System for Food Additives; No.: number

Bolding and underlining for clarity only. This formatting will not be shown in the online database.
The criteria for several residual solvents are listed under the heading “Residual solvents” (see Fig. 1).

**Figure 1**: Residual solvent criteria for curcumin as displayed in Monograph 1, 2006

<table>
<thead>
<tr>
<th>Residual solvents (Vol. 4)</th>
<th>Acetone: Not more than 30 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hexane: Not more than 25 mg/kg</td>
</tr>
<tr>
<td>Methanol:</td>
<td></td>
</tr>
<tr>
<td>Ethanol:</td>
<td>Not more than 50 mg/kg</td>
</tr>
<tr>
<td>Isopropanol:</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate:</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1**

Replacement of the text for the spectrophotometric data for Brown HT and its aluminium lake originally published in “Table 1. Values for synthetic colours for use in performing tests for Colouring Matters Content by Spectrophotometry” (FAO JECFA Monographs 19, 82nd meeting, 2016)

<table>
<thead>
<tr>
<th>JECFA name</th>
<th>Sample weight</th>
<th>Structure</th>
<th>Spectral data</th>
<th>Visible absorption spectrum</th>
</tr>
</thead>
</table>
| Brown HT   | 245.6 mg      | ![Structure](image) | Water, pH 7  
\(\lambda_{\text{max}} = 464\)  
\(A = 0.9957\)  
Spec abs = 403  
a = 40.3  
  
Water  
\(\lambda_{\text{max}} = 464\)  
\(A = 0.9804\)  
Spec abs = 397  
a = 39.7  
  
0.04 N AmAc  
\(\lambda_{\text{max}} = 461\)  
\(A = 0.9206\)  
Spec abs = 373  
a = 37.3 | ![Spectral Data](image) |
| Brown HT Aluminiu m Lake | 53.3 mg | ![Structure](image) | Straight colour (blue)  
0.04 N AmAc  
\(\lambda_{\text{max}} = 461\)  
\(A = 0.9206\)  
  
Lake (red)  
0.04 N AmAc  
\(\lambda_{\text{max}} = 458\)  
\(A = 1.0451\) | ![Spectral Data](image) |
ANNEX I: SUMMARY OF RECOMMENDATIONS FROM THE 86th JECFA

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held in Geneva, Switzerland, from 12 to 21 June 2018. The purpose of the meeting was to evaluate certain food additives (including flavouring agents).

Dr A. Mattia, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, served as Chairperson, and Dr. Richard Cantrill, Canada, served as Vice-Chairperson.

Dr M. Lipp, Office for Food Safety, Food and Agriculture Organization of the United Nations, and Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, served as Joint Secretaries.

The present meeting was the eighty-sixth in a series of similar meetings. The tasks before the Committee were (a) to undertake safety evaluations of certain food additives (including flavouring agents); and (b) to review and prepare specifications for certain food additives (including flavouring agents).

The Committee evaluated the safety of eight food additives, revised the specifications for 19 other food additives (including 16 modified starches), evaluated 69 flavouring agents according to the revised Procedure for the Safety Evaluation of Flavouring Agents and revised the specifications for three flavouring agents.

The report of the meeting will be published in the WHO Technical Report Series. Its presentation will be similar to that of previous reports – namely, general considerations, comments on specific substances and recommendations for future work. An annex will include detailed tables (similar to the tables in this report) summarizing the main conclusions of the Committee in terms of acceptable daily intakes and other toxicological, dietary exposure and safety recommendations. Information on the specifications for the identity and purity of certain food additives (including flavouring agents) examined by the Committee will also be included.

The participants in the meeting are listed in Annex 1. Items of a general nature that the Committee would like to disseminate quickly are included in Annex 2. Future work and recommendations are listed in Annex 3.

Toxicological and dietary exposure monographs on most of the substances that were considered will be published in WHO Food Additives Series No. 77. New and revised specifications for the identity and purity of the compounds will be published in FAO JECFA Monographs 22.
More information on the work of JECFA is available at:


and

http://www.who.int/foodsafety/areas_work/chemical-risks/jecfa/en/

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### Food additives evaluated toxicologically and assessed for dietary exposure

<table>
<thead>
<tr>
<th>Food additive</th>
<th>Specifications</th>
<th>Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anionic methacrylate copolymer (AMC)</td>
<td>N, Ta</td>
<td>The Committee was unable to complete the evaluation of AMC. While the copolymer itself is not of health concern, genotoxicity concerns remains for the residual monomer methacrylic acid. The specifications were made tentative pending the completion of the safety evaluation of AMC.</td>
</tr>
<tr>
<td>Basic methacrylate copolymer (BMC)</td>
<td>N</td>
<td>The Committee established an ADI “not specified” for basic methacrylate copolymer. The Committee concluded that the use of BMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes and micronutrient encapsulation for food fortification. The NOAELs for BMC ranged from 750-2000 mg/kg bw per day which were the highest doses tested. The Committee evaluated exposure to BMC for the copolymer and its monomers (n-butyl methacrylate, 2-(dimethylamino)ethyl methacrylate and methyl methacrylate). Estimated exposures to BMC range from 3.0 to 135 mg/kg bw per day. The total monomeric content of BMC is less than 0.3%. The Committee concluded that the toxicological data on the residual monomers do not give rise to concerns when taking into account the low dietary exposures.</td>
</tr>
<tr>
<td>Erythrosine</td>
<td>Rb</td>
<td>The Committee concluded that the new data that have become available since the previous evaluation of erythrosine do not give reason to revise the ADI and confirmed the previous ADI of 0–0.1 mg/kg bw. The Committee noted that the dietary exposure estimate for erythrosine of 0.09 mg/kg bw per day (95th percentile for children) was close to the upper bound of the ADI. Given that this estimate of exposure is for children and it is a high percentile for consumers only, such a level is unlikely to occur every day over a lifetime. Therefore, the Committee concluded that dietary exposures to erythrosine for all age groups do not present a health concern.</td>
</tr>
<tr>
<td>Food additive</td>
<td>Specifications</td>
<td>Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Indigotine</td>
<td>$R^b$</td>
<td>The Committee considered the new data that had become available since the previous evaluation as well as previously evaluated studies and <strong>concluded that there are no reasons to revise the ADI and confirmed the previous ADI of 0–5 mg/kg bw.</strong> The Committee noted that the conservative dietary exposure estimate of 0.8 mg/kg bw per day (95th percentile for children and toddlers) is less than the upper bound of the ADI of 0–5 mg/kg bw. The Committee concluded that dietary exposure to indigotine for all age groups does not present a health concern.</td>
</tr>
<tr>
<td>Lutein</td>
<td>$R^{c,d}$</td>
<td>Free lutein, lutein esters and free zeaxanthin including meso-zeaxanthin are biochemically and toxicologically equivalent. At the present meeting the Committee concluded that there were sufficient toxicological data to complete a safety assessment of lutein and lutein esters from <em>Tagetes erecta</em>, synthetic zeaxanthin and meso-zeaxanthin. Free lutein, lutein esters and free zeaxanthin and meso-zeaxanthin are substances of low toxicity for which no adverse effects have been observed in a broad range of toxicological studies in laboratory animals and clinical studies in humans. Based on the absence of toxicity in a wide range of studies, the <strong>Committee established a group ADI “not specified” for lutein from Tagetes erecta, lutein esters from Tagetes erecta and zeaxanthin (synthetic).</strong> Meso-zeaxanthin was not included in this group ADI, as specifications are not currently available. The <strong>group ADI of 0–2 mg/kg bw for lutein from Tagetes erecta and zeaxanthin (synthetic) was withdrawn.</strong></td>
</tr>
<tr>
<td>Neutral methacrylate copolymer (NMC)</td>
<td>$N, T$</td>
<td>The <strong>Committee established an ADI “not specified” for NMC. The ADI “not specified” was made temporary because the specifications are tentative.</strong> The Committee concluded that the use of NMC that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a coating or glazing agent for solid food supplements and for foods for special medical purposes. The NOAELs for NMC ranged from 454–2000 mg/kg bw per day, and these were the highest doses tested. The Committee evaluated exposure to NMC for the copolymer and its monomers (methyl methacrylate and ethyl acrylate). Estimated exposures to NMC range from 5.8 to 86 mg/kg bw per day. The total monomeric content of NMC is less than 0.01%. Toxicological data on the residual monomers do not</td>
</tr>
<tr>
<td>Food additive</td>
<td>Specifications</td>
<td>Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Sorbitol syrup</td>
<td>-</td>
<td>Sorbitol syrup (INS 420(ii)) is currently included in the Codex General Standard for Food Additives (GSFA) although it has not been assigned an ADI or determined, on the basis of other criteria, to be safe. The Committee was therefore requested to consider the previous evaluations of sorbitol, hydrogenated glucose syrups and other relevant substances, and advise on the need for a separate evaluation of sorbitol syrup or if the ADI “not specified” for sorbitol is also applicable for sorbitol syrup. Based on the similarity of the chemical constituents of sorbitol syrup to the previously evaluated sorbitol, maltitol syrup and polyglycitol syrup, the Committee concluded that there is no need for a separate evaluation of sorbitol syrup and established an ADI “not specified” for sorbitol syrup.</td>
</tr>
<tr>
<td>Spirulina extract</td>
<td>N, T</td>
<td>The Committee established a temporary ADI “not specified” for spirulina extract. The ADI was based on the absence of toxicity in repeated-dose animal studies with spirulina extract and dried spirulina. The ADI “not specified” was made temporary due to the tentative nature of the specifications. Expressed as phycocyanins, estimated dietary exposure from the use of spirulina extract as a food colour based on the Budget method and exposure to spirulina extract and dried spirulina from other dietary sources, including food ingredients, dietary supplements, and coatings of food supplements was 190 mg/kg bw for adults (60 kg/person) and 650 mg/kg bw for a child (15 kg/person). The Committee concluded that this dietary exposure does not present a health concern.</td>
</tr>
</tbody>
</table>

- : no specifications prepared; N: new specifications; R: existing specifications revised; T: tentative specifications

a The specifications were made tentative pending the completion of the safety evaluation of AMC.
b At the current meeting, high-performance liquid chromatographic (HPLC) methods were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.
c The specifications for lutein esters from *Tagetes erecta* and zeaxanthin (synthetic) were maintained.
d At the current meeting, the identity test for melting range was deleted, the identity tests for carotenoids and spectrophotometry were updated, the test for propylene glycol was incorporated verbatim and the previous reference removed, and the method of assay was updated.
Food additives considered for specifications only

<table>
<thead>
<tr>
<th>Food additive</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassia gum</td>
<td>Ra</td>
</tr>
<tr>
<td>Citric and fatty acid esters of glycerol</td>
<td>R, T</td>
</tr>
<tr>
<td>Glycerol ester of wood rosin</td>
<td>Rc</td>
</tr>
<tr>
<td>Modified starches</td>
<td>Rd, T</td>
</tr>
</tbody>
</table>

R: existing specifications revised; T: tentative specifications

a The Committee, at its current meeting, received analytical methods and included the most suitable validated method in the specifications monograph. However, this method uses chloroform for the extraction of anthraquinones. Extraction with n-hexane and diethyl ether resulted in poor recovery of anthraquinones. The Committee recommends that the JECFA Secretariat be notified if an alternative extraction solvent is identified. The specifications were revised and the tentative status was removed.

b The Committee did not receive a replacement method for the obsolete packed column gas chromatographic method for the determination of total citric acid, in its specifications monograph. The Committee noted further that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform. Specifications were revised and made tentative pending the availability of data. Specifications will be withdrawn if suitable information is not provided by December 2019.

c The Committee received information on the manufacture of GEWR from the rosin obtained from the stumps of two additional species namely Pinus halepensis and Pinus brutia as source materials. Recognizing the natural variability of the composition of wood rosin, the Committee removed the restriction to certain pine species within the specifications. Since the specifications monograph for GEWR does not contain an assay, the Committee recommended that the JECFA Secretariat be notified upon the development and validation of an appropriate assay. The existing specifications were revised.

d The Committee reviewed data on the method of manufacture, identity, and purity of all 16 modified starches. Based on the information received, and available information the Committee noted that:

- All processes are performed under similar manufacturing conditions and result in minor chemical modifications. Given the chemical and physical similarities of modified starches, the Committee at previous meetings considered the application of a read-across approach to be appropriate for the toxicological evaluation of these substances.
- All 16 modified starches had been assigned an ADI of “not specified”.
- All modified starches can be additionally bleached or fragmented; therefore revision in the specifications of bleached or fragmented starches would imply the revision of all 16 monographs;
- Microbiological specifications were not present in the existing specifications for all modified starches.
- Several specifications were common to all modified starches (such as for heavy metals impurities content and microbiological considerations). Revision of those common specifications would affect all 16 monographs;
- As a result of the wide range of products manufactured, the identification tests required to unambiguously chemically characterize each modified starch in individual specifications may be cumbersome, potentially unavailable, and unlikely to reflect market requirements.
- It may not be possible to publish identification tests based on market requirements without unduly revealing proprietary information.
- Based on the points noted above, individual specifications for several modified starches may remain tentative for an indefinite period or may need to be withdrawn.

The Committee therefore recommended that a new approach to the specifications monographs should be introduced to account for the chemical similarity between all modified starches, their functional diversity, the variety of chemicals used in their manufacture, and the corresponding diversity of impurities. The Committee recommended that all modified starches be included in a
modular monograph titled ‘Modified Starches’ that contains common requirements [General specifications for modified starches] consisting of specifications that apply to all 16 modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451), and annexes with specifications applicable to each individual modified starch based on the treatment(s) received. The Committee drafted a new modular specifications monograph titled “Modified starches” consisting of an explanatory introduction, “General specifications for modified starches,” and eight annexes. The new modular specifications monograph for modified starches is printed in FAO Monograph 22, and will replace the 16 existing individual specifications for modified starches (INS 1400, 1401, 1402, 1403, 1404, 1405, 1410, 1412, 1413, 1414, 1420, 1422, 1440, 1442, 1450, 1451).

The specification for lead included in the General specifications be decreased from 2 mg/kg to 0.2 mg/kg. The limit of lead for starch sodium octenylsuccinate for use in infant formula and formula for special medical purposes intended for infants was set to 0.1 mg/kg in the General specifications.

The methods for the determination of free adipic acid and adipate groups, residual vinyl acetate, free octenyl succinic acid and octenyl succinate esters were revised and a method for the determination of propylene chlorohydrins was added.

**Flavouring agents evaluated by the revised Procedure for the Safety Evaluation of Flavouring Agents**

### A. Alicyclic primary alcohols, aldehydes, acids and related esters

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
<th>Conclusion based on current estimated dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural class I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture of 1-Vinyl-3-cyclohexene-carbaldehyde and 4-Vinyl-1-cyclohexene-carbaldehyde</td>
<td>2253</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>p-Mentha-1,8-dien-7-ol</td>
<td>974</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>p-Mentha-1,8-dien-7-yl acetate</td>
<td>975</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene</td>
<td>980</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Myrtenol</td>
<td>981</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Myrtenyl acetate</td>
<td>982</td>
<td>M</td>
<td>No safety concern</td>
</tr>
<tr>
<td><strong>Structural class II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1-Methyl-2-(1,2,2-trimethylbicyclo[3.1.0]hex-3-ylmethyl)cyclopropyl)methanol</td>
<td>2254</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td><strong>Structural class III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(±)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid, ethyl ester</td>
<td>2255</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td><strong>Flavouring agent excluded at Step 1 of the Procedure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Mentha-1,8-dien-7-al (Perillaldehyde)</td>
<td>973</td>
<td>M</td>
<td>Genotoxicity data for p-mentha-1,8-dien-7-al raise concerns for potential genotoxicity</td>
</tr>
</tbody>
</table>

N: new specifications  
M: existing specifications maintained;
### B. Carvone and structurally related substances

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
<th>Conclusion based on current estimated dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural class I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pinocarvyl isobutyrate</td>
<td>2242</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Carvyl palmitate</td>
<td>2243</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td><strong>Structural class III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Hydroxycarvone</td>
<td>2244</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td><strong>Flavouring agents not evaluated according to the revised Procedure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+)-Carvone</td>
<td>380.1</td>
<td>M</td>
<td>The Committee did not re-evaluate (+)-carvone (No. 380.1) according to the revised Procedure given the lack of information on the oral exposure from all sources and the need to review the ADI. A review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for oral exposure to (+)-carvone from all sources to complete the evaluation for (+)-carvone.</td>
</tr>
<tr>
<td>(-)-Carvone</td>
<td>380.2</td>
<td>M</td>
<td>The Committee did not re-evaluate (-)-carvone (No. 380.2) according to the revised Procedure given the lack of information on the oral exposure from all sources and the lack of toxicological data.</td>
</tr>
</tbody>
</table>

M: existing specifications maintained; N: new specifications

### C. Furan-substituted aliphatic hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids and related esters, sulfides, disulfides and ethers

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
<th>Conclusion based on current estimated dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural class III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Pentylfuran</td>
<td>1491</td>
<td>M&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Heptylfuran</td>
<td>1492</td>
<td>M&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Decylfuran</td>
<td>1493</td>
<td>M&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No safety concern</td>
</tr>
<tr>
<td>3-Methyl-2-(3-methylbut-2-enyl)-furan</td>
<td>1494</td>
<td>M&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Flavouring agent</td>
<td>No.</td>
<td>Specifications</td>
<td>Conclusion based on current estimated dietary exposure</td>
</tr>
<tr>
<td>------------------------------------------------------</td>
<td>-------</td>
<td>----------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>2,3-Dimethylbenzofuran</td>
<td>1495</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2,4-Difurfurylfuran</td>
<td>1496</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>3-(2-Furyl)acrolein</td>
<td>1497</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Methyl-3(2-furyl)acrolein</td>
<td>1498</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>3-(5-Methyl-2-furyl)prop-2-enal</td>
<td>1499</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>3-(5-Methyl-2-furyl)butanal</td>
<td>1500</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Furfurylidene-butyreraldehyde</td>
<td>1501</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Phenyl-3-(2-furyl)prop-2-enal</td>
<td>1502</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Furyl methyl ketone</td>
<td>1503</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Acetyl-5-methylfuran</td>
<td>1504</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Acetyl-3,5-dimethylfuran</td>
<td>1505</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>3-Acetyl-2,5-dimethylfuran</td>
<td>1506</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Butyrylfuran</td>
<td>1507</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>(2-Furyl)-2-propanone</td>
<td>1508</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Pentanoylfuran</td>
<td>1509</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>1-(2-Furyl)butan-3-one</td>
<td>1510</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>4-(2-Furyl)-3-buten-2-one</td>
<td>1511</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Pentyl 2-furyl ketone</td>
<td>1512</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Ethyl 3-(2-furyl)propanoate</td>
<td>1513</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Isobutyl 3-(2-furan)propionate</td>
<td>1514</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Isoamyl 3-(2-furan)propionate</td>
<td>1515</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Isoamyl 3-(2-furan)butyrate</td>
<td>1516</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Phenethyl 2-furoate</td>
<td>1517</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Propyl 2-furanacrylate</td>
<td>1518</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2,5-Dimethyl-3-oxo-(2H)-fur-4-yl butyrate</td>
<td>1519</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Furfuryl methyl ether</td>
<td>1520</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Ethyl furfuryl ether</td>
<td>1521</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Difurfuryl ether</td>
<td>1522</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2,5-Dimethyl-3-furanthiol acetate</td>
<td>1523</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Furfuryl 2-methyl-3-furyl disulfide</td>
<td>1524</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>3-[(2-Methyl-3-furyl)thio]-2-butanone</td>
<td>1525</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>O-Ethyl S-{2-furylmethyl}thiocarbonate</td>
<td>1526</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>(E)-Ethyl 3-(2-furyl)acrylate</td>
<td>2103</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>di-2-Furylmethane</td>
<td>2104</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-Methylbenzofuran</td>
<td>2105</td>
<td>M*</td>
<td>No safety concern</td>
</tr>
</tbody>
</table>

M: existing specifications maintained
a The text indicating that the safety evaluation for these flavouring agents had not been completed was removed from the specifications and the specifications were maintained as full.

**D. Linear and branched-chain aliphatic, unsaturated, unconjugated alcohols, aldehydes, acids and related esters**

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
<th>Conclusion based on current estimated dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural class I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trans-6-Octenal</td>
<td>2240</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2,6-Dimethyl-5-heptenol</td>
<td>2241</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td><strong>E. Maltol and related substances</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Structural class II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltol</td>
<td>1480</td>
<td>M</td>
<td>No safety concern&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Structural class III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl maltol isobutyrate</td>
<td>2252</td>
<td>N</td>
<td>No safety concern</td>
</tr>
</tbody>
</table>

M: existing specifications maintained
N: new specifications

<sup>a</sup> The previously established ADI for maltol was withdrawn by the Committee.

**F. Menthol and structurally related substances**

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
<th>Conclusion based on current estimated dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural class I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menthyl formate</td>
<td>2246</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Menthyl propionate</td>
<td>2247</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>l-Menthyl butyrate</td>
<td>2248</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>dl-Isomenthol</td>
<td>2249</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Dimethyl glutarate</td>
<td>2250</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Menthol</td>
<td>427</td>
<td>M</td>
<td>No safety concern&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Structural class III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(±)-2-[(2-p-Menthoxy)ethoxy]ethanol</td>
<td>2251</td>
<td>N</td>
<td>No safety concern</td>
</tr>
</tbody>
</table>

M: existing specifications maintained
N: new specifications

<sup>a</sup> The ADI of menthol of 0–4 mg/kg bw established at the fifty-first meeting was maintained.
### G. Miscellaneous nitrogen-containing substances

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
<th>Conclusion based on current estimated dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural class III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-(((3-(2,3-Dimethoxyphenyl)-1H-1,2,4-triazol-5-yl)thio)methyl)pyridine</td>
<td>2235</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>S)-1-(((4-Amino-2,2-dioxido-1H-benzo[c][1,2,6]thiadiazin-5-yl)oxy)methyl)piperidin-1-yl)-3-methylbutan-1-one</td>
<td>2236</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>2-(4-Methylphenoxy)-N-(1H-pyrazol-3-yl)-N-(thiophen-2-ylmethyl)acetamide</td>
<td>2237</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N: new specifications</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### H. Saturated aliphatic acyclic branched-chain primary alcohols, aldehydes, and acids

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
<th>Conclusion based on current estimated dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural class I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-Methyldecanal</td>
<td>2238</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>8-Methylnonanal</td>
<td>2239</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>N: new specifications</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Flavouring agents considered for specifications only**

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>L-menthyl lactate</td>
<td>433</td>
<td>R&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>L-malic acid</td>
<td>619</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Glutamyl-valyl-glycine</td>
<td>2123</td>
<td>R&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The CAS number was changed from 59259-38-0 to 61597-98-6 and the name to L-menthyl L-lactate.

<sup>b</sup> The specification for specific rotation were removed

<sup>c</sup> The melting point range was revised
ANNEX 2. GENERAL INFORMATION

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ANNEX 3. FUTURE WORK AND RECOMMENDATIONS

SPECIFIC FOOD ADDITIVES (OTHER THAN FLAVOURING AGENTS)

Anionic Methacrylate Copolymer

The Committee noted that there were insufficient data to reach a conclusion on the genotoxic potential of methacrylic acid. Further studies to clarify the in vivo carcinogenic potential are required.

Citric and fatty acid esters of glycerol

The specifications of CITREM were made tentative, requiring a suitable validated method for the determination of total citric acid content, along with performance characteristics of the method and data on the total citric acid content in at least five batches of products currently available in commerce, determined using that method.

The Committee noted that the method for total glycerol still uses chloroform. The Committee encouraged the submission of a method for total glycerol that eliminates the use of chloroform. Specifications were revised and made tentative. Specifications will be withdrawn if suitable information is not provided by December 2019.

Neutral Methacrylate Copolymer

The Committee noted that there was no data submitted for a suitable method of assay. Tentative specifications for NMC were prepared and made tentative requiring a suitable validated method of assay.

Spirulina extract

The Committee received limited analytical data on spirulina extract. In order to remove the tentative designation from the specifications, the following information on the products of commerce is requested by December 2019:

- Full compositional characterization of commercial products in both liquid and powder forms.
- Full compositional characterization of the aqueous extract before formulation/standardization.
- Validated analytical methods for identification of the substance with a suitable specificity (including validation data and representative batch data).
- Validated analytical methods for the determination of the purity of the substance with a suitable specificity (including validation data and representative batch data).

Modified starches

The Committee requested additional data and a suitable method for the determination of propylene chlorohydrins in Hydroxypropyl starch (INS 1440) and Hydroxypropyl distarch phosphate (INS 1442) in order to consider lowering this limit.

The Committee requests suitable microbiological acceptance criteria and supporting data for all modified starches.
Table 1. The annexes and the modified starches to which they apply along with required information:

<table>
<thead>
<tr>
<th>ANNEX</th>
<th>Modification</th>
<th>Starches to which it applies</th>
<th>Information required</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Minor fragmentation</td>
<td>INS 1400: Dextrin roasted starch; INS 1401: Acid treated starch; INS 1402: Alkaline treated starch; INS 1405: Enzyme-treated starch All modified starches that are additionally fragmented.</td>
<td>A suitable method for dispersion and a method for reducing sugars and data on at least 5 representative batches using the method(s) from each of the fragmentation processes.</td>
</tr>
<tr>
<td>2</td>
<td>Bleaching</td>
<td>INS 1403: Bleached starch All modified starches if additionally bleached.</td>
<td>Suitable method(s) for the determination of residual reagents and data on at least 5 representative batches using the method(s).</td>
</tr>
<tr>
<td>3</td>
<td>Esterification and/or crosslinking with phosphorus containing compounds</td>
<td>INS 1410: Monostarch phosphate; INS 1412: Distarch phosphate; INS 1413: Phosphated distarch phosphate; INS 1414: Acetylated distarch phosphate; INS 1442: Hydroxypropyl distarch phosphate</td>
<td>A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches.</td>
</tr>
<tr>
<td>4</td>
<td>Acetylation</td>
<td>INS 1420: Starch acetate; INS 1414: Acetylated distarch phosphate; INS 1422: Acetylated distarch adipate; INS 1451: Acetylated oxidized starch</td>
<td>Currently no additional information required.</td>
</tr>
<tr>
<td>5</td>
<td>Oxidation</td>
<td>INS 1404: Oxidized starch; INS 1451: Acetylated oxidized starch</td>
<td>A suitable method for determination of residual hypochlorite and data on at least 5 representative batches using the method.</td>
</tr>
<tr>
<td>6</td>
<td>Esterification with octenyl succinic anhydride</td>
<td>INS 1450: Starch sodium octenyl succinate</td>
<td>Currently no additional information required.</td>
</tr>
<tr>
<td>7</td>
<td>Etherification with propylene epoxide</td>
<td>INS 1440: Hydroxypropyl starch; INS 1442: Hydroxypropyl distarch phosphate</td>
<td>A suitable method for the determination of propylene chlorohydrin with detection limit lower than 0.1 mg/kg and data on at least 5 representative batches of Hydroxypropyl starch using the method</td>
</tr>
<tr>
<td>8</td>
<td>Crosslinking with adipic anhydride</td>
<td>INS 1422: Acetylated distarch adipate</td>
<td>A suitable method for identification of crosslinking and data on at least 5 representative batches of crosslinked and non-crosslinked starches. Levels of free adipic acid in at least 5 representative batches</td>
</tr>
</tbody>
</table>
FLAVOURING AGENTS

Carvone and structurally related substances

For (+)-carvone (No. 380.1), the Committee concluded that a review of the ADI is recommended based on the evaluation of all biochemical and toxicological data. Also, data are needed for an exposure assessment for the oral exposure to (+)-carvone from all sources.

The ADI for (+)-carvone is maintained pending review of the ADI at a future meeting. The Committee recommends that the re-evaluation is completed within three years.

For (-)-carvone (No. 380.2), the Committee concluded that toxicological data on (-)-carvone are necessary. Also, data are needed for an exposure assessment for the oral exposure to (-)-carvone from all sources.

Maltol and related substances

The Committee could not verify the NOEL of 100 mg/kg bw in rats that was used to derive the ADI of 0–1 mg/kg bw for maltol (No. 1480) during its twenty-fifth meeting because of uncertainties in the administered dose levels and the effects observed in several studies described in the monograph of that meeting.

The Committee withdrew the ADI for maltol. The Committee concluded that access to either the original studies or submission of new data would be needed to reaffirm or amend the ADI.

The ADI for ethyl maltol was maintained.
Joint FAO/WHO Expert Committee on Food Additives
84th Meeting 2018

This document contains food additive specification monographs, analytical methods, and other information prepared at the eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, 12–21 June 2018. The specification monographs provide information on the identity and purity of food additives used directly in foods or in food production. The main three objectives of these specifications are to identify the food additive that has been subjected to testing for safety, to ensure that the additives are of the quality required for use in food or in processing and to reflect and encourage good manufacturing practice. This publication and other documents produced by JECFA contain information that is useful to all those who work with or are interested in food additives and their safe use in food.