Joint FAO/WHO Expert Committee on Food Additives

89th Meeting
Virtual meeting, 1 – 12 June 2020
SPECIAL NOTE

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Virtual meeting, 1 – 12 June 2020

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 89th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held virtually, 1 – 12 June 2020. 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:

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SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

New (N) or revised (R) specifications monographs were prepared for 7 food additives and these are presented in this publication.

Adenosine 5'-mono phosphate deaminase from \textit{Streptomyces murinus} (N)
D-Allulose 3-epimerase from \textit{Arthrobacter globiformis} expressed in \textit{Escherichia coli} (N)
Jagua (genepin-glycine) Blue (R)
Lipase from \textit{Mucor javanicus} (N)
Phosphatidylinositiol-specific phospholipase C expressed in \textit{Pseudomonas fluroescens} (N)
Magnesium stearate (R)
Polyvinyl alcohol(R)
ADENOSINE 5´-MONOPHOSPHATE DEAMINASE from STREPTOMYCES MURINUS

New specifications prepared at the 89th JECFA (2020) and published in FAO JECFA Monographs 25 (2020). The Committee concluded that the enzyme preparation would not pose a health concern when used in the applications specified, at the levels specified and in accordance with good manufacturing practice (89th JECFA, 2020).

SYNONYMS

Adenyllic acid deaminase; AMP deaminase; AMP aminase; adenylic deaminase; adenylate deaminase; 5-5´ deaminase; 5´-deaminase; adenosine 5-monophosphate deaminase; 5-adenylate deaminase; adenyl deaminase; 5-adenylic acid deaminase; adenosine monophosphate deaminase; adenylate aminohydrolase; adenylate desaminase; adenosine 5-phosphate aminohydrolase; 5-adenylate deaminase

SOURCES

Produced by controlled aerobic batch fermentation of a non-pathogenic, non-toxigenic strain of Streptomyces murinus. The AMP deaminase is secreted into the fermentation broth. The cell material is separated from the broth containing the enzyme, followed by filtration and centrifugation. The liquid enzyme is concentrated and further filtered. The liquid AMP-deaminase concentrate is spray-dried and standardized with food-grade dextrin to the desired activity.

Active principles

AMP deaminase

Systematic names and numbers

AMP aminohydrolase; EC 3.5.4.6; CAS No. 1618683-38-7

Reaction catalysed

Deamination of adenosine 5´-monophosphate (AMP) via hydrolysis to produce inosine 5´-monophosphate (IMP)

Secondary enzyme activities

No significant levels of secondary enzyme activities

DESCRIPTION

White to dark brown powder; soluble in water

FUNCTIONAL USES

Enzyme preparation

Used as a processing aid in the manufacture of yeast products, for cereals and production of flavourings of vegetable, animal, or microbial origin.

GENERAL SPECIFICATIONS

Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.
CHARACTERISTICS
IDENTIFICATION

AMP deaminase activity

The sample shows AMP deaminase activity.

See description under TESTS

TESTS

METHOD OF ASSAY

AMP deaminase activity

Principle

AMP deaminase activity is determined by measuring decrease of absorbance at 265 nm that results from the hydrolysis of the amine group in a AMP substrate to produce IMP.

1,000 U of AMP deaminase activity is defined as the quantity of enzyme required to decrease the absorbance at 265 nm by 0.1 units per hour at specified conditions.

Apparatus

UV-VIS Spectrophotometer

Water bath with circulation

Reagents and solutions

- AMP disodium salt (Sigma-Aldrich catalogue number 01930 Disodium adenosine 5’-phosphate ≥ 99.0% purity, or equivalent)
- 0.1 N Hydrochloric acid
- 0.06 M phosphate buffer, pH 5.6
- Trichloroacetic acid (10%)
- Sodium hydroxide
- Deionized water

10% Trichloroacetic acid Solution: Dilute 10 ml of trichloroacetic acid (100%) to 100 ml with water.

0.06 M phosphate buffer, pH 5.6:

- Potassium dihydrogen phosphate solution: Dissolve 4.54 g potassium dihydrogen phosphate in 250 ml deionized water. Dilute to 500 ml with deionized water.
- Disodium hydrogen phosphate solution: Dissolve 9.46 g disodium hydrogen phosphate in 500 ml deionized water. Dilute to 1 litre with deionized water.
- 0.06 M phosphate buffer, pH 5.6: Add disodium hydrogen phosphate solution to potassium dihydrogen phosphate solution until the pH stabilizes at 5.60.

Substrate

Weigh 330 mg AMP disodium salt and dissolve in 25 ml deionized water. Adjust to pH 5.6 with 0.1 N hydrochloric acid or sodium hydroxide. Dilute to 50 ml with deionized water. Label as Stock Substrate. This solution is stable for 1 week when refrigerated.
Prior to use, mix the Stock Substrate with 0.06 M phosphate buffer (pH 5.6) at a ratio of 1:2. Label as Working Substrate.

**AMP Deaminase sample preparation**

Dissolve an accurately weighed amount of AMP deaminase in deionized water in a volumetric flask of appropriate size to prepare a solution of approximately 55,000 U/ml. Label as Sample Preparation.

**Procedure**

Transfer 3 ml Working Substrate into a test tube (18x180 mm). Prepare in duplicate. Place the test tubes in a water bath maintained at 37 ± 0.5° for 15 min. At t = 0 min, add 1 ml Sample Preparation to each test tube, mix, and return the test tubes to the water bath. At t = 15 min, add 4 ml trichloroacetic acid solution to inactivate the enzyme. Shake the test tubes vigorously. Transfer 2 ml of this solution to a 100-ml volumetric flask and make up to volume with water. Measure the absorbance (A₂) at 265 nm in a 10-mm cuvette.

Prepare blank as follows. Transfer 3 ml Working Substrate into a test tube (18x180 mm). Prepare in duplicate. Add 4 ml trichloroacetic acid solution to each test tube and shake vigorously. Add 1 ml Sample Preparation to each test tube and mix. Measure the absorbance (A₁) at 265 nm in a 10-mm cuvette.

**Calculation of AMP deaminase activity**

Calculate the AMP deaminase activity, expressed as U/mg:

\[
U/\text{mg} = (A₂ - A₁) \times \frac{10}{0.001} \times \frac{8}{2} \times \frac{60}{15} \times \frac{n}{1000}
\]

where

- A₁ is the Absorbance at t = 15 min of the Sample Preparation (average of duplicates)
- A₂ is the Absorbance of the blank (average of duplicates)
- 10/0.001 is the conversion factor of AMP deaminase activity based on the definition of unit activity
- 8 is the total volume of reaction solution (ml)
- 2 is the volume of transferred reaction solution (ml)
- 60/15 is the conversion factor based on the AMP deaminase reaction time (15 min) and the definition of activity (change in absorbance per hour; 60 min)
- n is the dilution factor of the AMP deaminase solution (ml of Sample Preparation/g of enzyme)
D-ALLULOSE 3-EPIMERASE from ARTHROBACTER GLOBIFORMIS EXPRESSED in ESCHERICHIA COLI

New specifications prepared at the 89th JECFA (2020) and published in FAO JECFA Monographs 25 (2020). An ADI of “not specified” was established at the 89th JECFA (2020).

SYNONYM
Psicose epimerase, allulose epimerase

SOURCES
Produced by controlled aerobic batch fermentation of a genetically modified strain of Escherichia coli (E. coli) K-12 which contains a gene coding for D-allulose 3-epimerase from Arthrobacter globiformis. The secreted enzyme is separated from the biomass solids via filtration, followed by concentration and additional filtration steps. The liquid enzyme concentrate is then either freeze dried into a powdered enzyme preparation or formulated into a liquid enzyme preparation after stabilization with food grade D-sorbitol.

Active principles
Epimerase

Systematic names and numbers
D-Allulose 3-epimerase, D-Psicose 3-epimerase; EC 5.1.3.30; CAS No. 1618683-38-7

Reaction catalysed
Epimerization of D-fructose and D-allulose

Secondary enzyme activities
Epimerization of D- and L- forms of keto-hexoses, keto-pentoses, and keto-tetroses

DESCRIPTION
Yellow to brown liquid or grayish tan powder

FUNCTIONAL USES
Enzyme preparation
Used as a processing aid in the manufacture of D-allulose and other keto sugars.

GENERAL SPECIFICATIONS
Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.

CHARACTERISTICS

IDENTIFICATION
Epimerase activity
The sample shows D-allulose 3-epimerase activity.
See description under TESTS.
TESTS

METHOD OF ASSAY

Epimerase activity

Principle

D-Allulose 3-epimerase activity is determined by measuring the production of D-fructose that results from the epimerization of D-allulose, used as a substrate. After a 10-min reaction, a high-performance liquid chromatography (HPLC) technique enables determination of the epimerase activity based on the amount of D-fructose produced from the substrate.

One unit of epimerase activity is defined as the quantity of enzyme required to produce 1 μmol D-fructose per minute at the specified conditions.

Apparatus

HPLC system and operating conditions:
Column: MCI GEL CK08EC (8x 300 mm) (Mitsubishi Chemical) or equivalent
Column temperature: 80°
Detector: Refractive index (RI)
Mobile phase: Water, LC grade
Flow rate: 0.4 ml/min
Injection volume: 10 μl

Reagents and solutions

- Amberlite-IRA67 resin, weak basic anion exchange resin (free base form, dried).
- Amberlite-200CT resin, strong acidic cation exchange resin (H+ form, dried).
- Phosphate buffer (0.05 mol/l): Dissolve 7.8 g of sodium dihydrogen phosphate (NaH2PO4.2H2O) in deionized water in a 1litre volumetric flask and dilute to volume with deionized water (Solution A). Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate (Na2HPO4.12H2O) in deionized water in a 1-litre volumetric flask and dilute to volume with deionized water (Solution B). Mix Solution A and Solution B to achieve a pH of 8.0.
- 1.0 M Magnesium chloride solution (MgCl2.6H2O): Dissolve 203.3 g of magnesium chloride hexahydrate in deionized water in a 1-litre volumetric flask. Dilute to volume with deionized water.
- Fructose Standard Stock Solution (2.7 mg/ml D-fructose): Accurately weigh 270 mg of D-fructose into a 100-ml volumetric flask; dissolve in and dilute to volume with deionized water.
- Fructose Standard Solutions: Prepare a series of dilutions of the D-Fructose Standard Stock Solution in deionized water to contain 0.18, 0.54, 0.9 and 1.8 mg/ml of D-fructose. These correspond to 1, 3, 5 and 10 mmol/litre. Label as Standard Solutions 1 to 4.
Substrate
Transfer 3.6 g of D-allulose (>99.0% C₆H₁₂O₆) to a 100-ml volumetric flask; dissolve in and dilute to volume with deionized water. Label as Substrate Solution.

Sample preparation
Transfer 1 g of D-allulose 3-epimerase enzyme, accurately weighed, to a 200-ml volumetric flask and add sufficient phosphate buffer to dissolve the enzyme. Add 1 ml of 1.0 M magnesium chloride solution, mix, then dilute to volume with the phosphate buffer. Further dilute with phosphate buffer as needed to achieve a concentration of 4-10 U/ml using volumetric flasks of appropriate size. Label as Sample Preparation.

Procedure
Pipette 100 μl Sample Preparation and 400 μl phosphate buffer into a microcentrifuge tube. Cap the tube and heat in a water bath maintained at 50° ± 0.5° for 5 min. At time equals zero, add 500 μl Substrate Solution to the tube and mix well. Immediately return the reaction mixture to the water bath at 50°. At time equals 10 min, place the tube in a boiling water bath for 2 minutes to end the reaction.

Allow the reaction mixture to cool to room temperature, add about 100 mg strongly acidic cation-exchange resin and about 100 mg weakly basic anion-exchange resin for desalting, shake for 15 minutes, and filter through a membrane filter (pore size: 0.2 μm). Analyse the reaction mixture and Standard Solutions 1 - 4 by HPLC using the instrument conditions described under Apparatus.

Calculation
From the chromatograms obtained, construct a standard curve by plotting the peak areas versus the concentration of D-fructose in Standard Solutions 1 - 4, in µmol/litre. Use the standard curve to determine the concentration of D-fructose in the reaction mixture (F).

Calculate the epimerase activity, expressed as U/g enzyme:

\[ \text{U/g} = \frac{F}{T} \times \frac{V_r}{C \times V_s} \]

where
- \( F \) is the concentration of D-fructose in the reaction mixture (µmol/litre)
- \( T \) is the reaction time (10 min)
- \( V_s \) is the volume of Sample Preparation in the reaction mixture (0.1 ml)
- \( V_r \) is the volume of the reaction mixture (0.001 litre)
- \( C \) is the concentration of epimerase enzyme in the Sample Preparation (g/ml)
JAGUA (GENIPIN-GLYCINE) BLUE

Prepared at the 89th JECFA (2020) and published in FAO JECFA Monographs 25 (2020), superseding specifications prepared at the 84th JECFA (2017), and published in FAO JECFA Monographs 20 (2017). An ADI of 0-11 mg/kg bw on the polymer basis was established at the 89th JECFA (2020)

SYNONYMS

Jagua blue

DEFINITION

Jagua (Genipin-Glycine) Blue has a colour attributed mainly to a blue polymer that is formed by the reaction between genipin (methyl (1R,4aS,7aS)-1-hydroxy-7-(hydroxymethyl)-1,4a,5,7a-tetrahydro-cyclopenta[c]pyran-4-carboxylate) and glycine resulting in the combination of alternating dimeric moieties linked by a methylene bridge.

Jagua (Genipin-Glycine) Blue is produced by a two-step process. In the first step the unripe fruit of Genipa americana is peeled, ground to pulp, pressed for the juice, and extracted with water. The extracted juice is filtered and checked for genipin content. In the next step the Jagua extract is treated with a stoichiometric amount of glycine and heated at 70° until all genipin is reacted. The resulting liquid is centrifuged and concentrated. The powder product in commerce is obtained after concentrating the Jagua (Genipin-Glycine) Blue to 20° Brix and mixing with a food-grade carrier such as maltodextrin or modified food starches, spray drying, or using other drying technologies and sieving

C.A.S. number

1314879-21-4
(Blue Polymer)

Chemical formula

(C\(_{27}\)H\(_{25}\)O\(_{8}\)N\(_{2}\))\(_{n}\)
(n is typically 10-12)
Structural formula

Molecular weight
Approximately 6000 Da (number average molecular weight, approximately a lognormal distribution between 4500 and 9500 Da).

Assay
Not less than 30% and not more than 40% of polymer by HPLC. See Method of Assay

DESCRIPTION
Blue to black powder; odourless

FUNCTIONAL USES
Colour
CHARACTERISTICS
IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water. Practically insoluble to insoluble in hexane and ethanol

Spectrophotometry (Vol. 4) The UV-Visible absorption spectrum of a sample dissolved in water shows absorption maximum between 590-594 nm.

HPLC The retention time of main peak in a chromatogram of Jagua (Genipin-Glycine) Blue matches that of the in-house reference standard (blue polymer); See Method of Assay.

Infared Spectrum (Vol. 4) Infared spectrum of the sample obtained by using potassium bromide disk corresponds to the reference spectrum

PURITY

Loss on drying (Vol 4) Not more than 6% (at 105°, to constant weight).

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

Ether-extractable Matter (Vol. 4) Not more than 0.2%

Total colouring matters 35 to 48% See description under Tests
(Vol. 4)

Genipin Passes test (< 0.01%)
See description under TESTS

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 method described in Volume 4

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 method described in Volume 4

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

Microbiological criteria (Vol. 4) Aerobic Plate Count: Not more than 1000 CFU/g
Total Coliforms: Not more than 10 CFU/g
E. coli: Absent in 25 g sample
Coagulase positive S. aureus: Absent in 1 g sample
Yeasts and moulds: Not more than 10 CFU/g

TESTS

PURITY TESTS

Genipin
Genipin in Jagua (Genipin-Glycine) Blue is extracted and determined by high performance liquid chromatography (HPLC)

Standards and reagents
- Genipin (>98% pure, HPLC grade), Sigma Aldrich G4796 or equivalent
- Ethyl acetate: Analytical grade or equiv.
- Methanol: LC grade
- Deionized water: LC grade
- Acetic acid: LC grade

Equipment
Use an HPLC system consisting of a high precision binary pump and an auto sampler
HPLC column: Phenomenex Luna C18 (2) (150 x 4.6 mm, 5µm), with guard column or equivalent.
Flow Rate: 1ml/min
Injection volume: 10 µl
Mobile Phase: A): 0.05% acetic acid in water; B): Methanol
Detector: UV/Diode Array at 240 nm
Temperatures:
  - Column: 30°
  - Detector: 30°

Gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Solvent A</th>
<th>% Solvent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>5</td>
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<tr>
<td>18</td>
<td>95</td>
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</tbody>
</table>

Preparation of standard solutions
Standard Stock Solution (100 mg/l): accurately weigh 10 mg genipin, dissolve in deionized water and make up to 100 ml in a volumetric flask.

Reference Solution (10 mg/l): dilute 1 ml Standard Stock Solution to 10 ml with 95:5 deionized water:methanol.
**Preparation of sample solutions**

Sample Solution A: Accurately weigh 300 mg sample in a 50 ml beaker, add 5 ml deionized water and sonicate until a homogeneous solution is obtained. Quantitatively transfer the solution to a 50 ml separatory funnel and extract with 3 x 10 ml portions of ethyl acetate. Combine the ethyl acetate fractions in a 50 ml round bottom flask and dry in a rotary evaporator (40° and pressure, 220 mbar). Dissolve the residue in 2 ml 95:5 deionized water:methanol and vortex to obtain a homogeneous solution. Filter using a 0.45 μm filter.

Sample Solution B: Accurately weigh 300 mg sample in a 50 ml beaker, spike with 300 μl genipin Standard Solution (100 mg/l), add 4.7 ml of deionized water and proceed as directed in sample solution A starting from the sonication step.

**Procedure**

Inject Reference Solution and Sample Solutions following the conditions described under HPLC. The retention time of genipin is approximately 12-13 min. Compare the chromatograms obtained for the genipin Reference Solution and Sample Solutions (A and B). The genipin peak should be present at approximately 12-13 min in the Reference Solution and in Sample B. The peak area of the genipin peak, if present in the chromatogram of Sample A, should not be more than half of that for Sample B.

**Total colouring matters**

Determine total colouring matters by spectrophotometry using water as the solvent, as described in the Combined Compendium of Food Additive Specifications, Vol. 4 (under Food Colours, total colouring matters content by spectrophotometry, procedure 1).

\[ \% \text{ Total Colouring Matter} = 100 \times \left( \frac{\text{Abs} \times 1L}{a \times 1 \text{ cm} \times F} \right) \times \frac{1}{W} \]

Where
- Abs is the absorbance of the sample solution at 592nm
- a is the absorptivity of the blue polymer: 6.08 (l/g.cm)
- F is the dilution factor
- W is weight of sample, g

**METHOD OF ASSAY**

Determine the percentage of blue polymer in Jagua (Genipin-Glycine) Blue by HPLC.

**Preparation of in-house reference standard for the polymer**

- Wash 5 g of Jagua (Genipin-Glycine) Blue with two separate 200-ml portions of ethyl acetate. Discard the supernatant.
- Extract the remaining residue with five separate 500-ml portions of methanol. Set the residue from this step (A) aside for later use.
- Reduce the volume of the methanol supernatant under vacuum, then load onto column (Sephadex LH-20).
- Elute with a solution of 50% (v/v) methanol in water to obtain two separate blue fractions; proceed using the first fraction. Reduce the volume of the fraction under vacuum (B).
- Load residue A onto column (Sephadex LH-20) and elute with a solution of 90% (v/v) methanol in water. Combine the first blue band to elute from the column with residue B.
- Load the combined material onto a reversed phase column (RP-C18) and elute with a solution of 67% (v/v) methanol in water.
- Combine the three blue bands obtained and lyophilize to obtain the In-House Reference Standard.

Preparation of In-House Reference Standard solutions
Accurately weigh 10 mg In-House Reference Standard and quantitatively transfer to a 20 ml volumetric flask. Dissolve in mobile phase.
Prepare by dilution in mobile phase a minimum of five In-House Reference Standards in the concentration range of 50-150 mg/l.

Sample preparation for the polymer determination
Prepare three independent samples by accurately weighing and dissolving 20 mg each of the sample in mobile phase. Dilute to volume in separate 10 ml volumetric flasks. Further dilute 1 ml of each Sample Solution to 10 ml with mobile phase. Filter before injection.

HPLC
Column: Bio Sec-2000 (300 mm x 4.6 mm, 5 µm), or equivalent
Flow rate: 1 ml/min
Injection volume: 20 µl
Mobile phase: 100 mM NaH₂PO₄ + 400 mM NaCl – Isocratic mode
Detector: PDA/UV-Vis (590 nm for peak identification and quantitation)
Temperatures:
  Column: 30°
  Detector: 30°

Procedure
Individually inject the five In-House Reference Standard solutions into the HPLC. Retention time of the polymer is around 5.3 min. Construct a standard curve. Individually inject the Sample Solutions and determine the concentration of the polymer from the standard curve.

Calculate the amount of polymer in the sample taken and report the average result of three samples.
IR Spectrum of the blue polymer in Jagua (Genipin-Glycine) Blue shows characteristic bands at: 3393, 2949, 1726, 1630 and 1540 cm\(^{-1}\).
LIPASE from *MUCOR JAVANICUS*

New specifications prepared at the 89th JECFA (2020) and published in FAO JECFA Monographs 25 (2020). An ADI of “not specified” was established at the 89th JECFA (2020).

**SYNONYM**
Triglyceride lipase; glycerol ester hydrolase; triglyceride hydrolase; triolein hydrolase; triglyceridase; triacylglycerol ester hydrolase; glycerol-ester hydrolase

**SOURCES**
Produced by controlled aerobic batch fermentation of a non-pathogenic, non-toxigenic strain of *Mucor javanicus* (*Mucor circinelloides*). The secreted lipase is separated from the biomass via a series of filtration steps, followed by concentration and purification. The lipase concentrate is spray dried, formulated and standardized with food grade dextrin to produce the lipase enzyme preparation.

Active principles
Triacylglycerol lipase

Systematic names and numbers
Triacylglycerol acylhydrolase; EC 3.1.1.3; CAS No. 9001-62-1

Reaction catalysed
Hydrolysis of ester bonds in triglycerides (yielding diglycerides, monoglycerides, glycerol and free fatty acids)

Secondary enzyme activities
No significant levels of secondary activities

**DESCRIPTION**
White to dark brown powder; soluble in water

**FUNCTIONAL USES**
Enzyme preparation
Used as a processing aid in the manufacture of baked goods, enzyme modified cheeses, flavourings and in the processing of egg whites.

**GENERAL SPECIFICATIONS**
Must conform to the latest edition of the JECFAP General Specifications and Considerations for Enzyme Preparations Used in Food Processing.

**CHARACTERISTICS**

**IDENTIFICATION**
Lipase activity
The sample shows lipase activity.
See description under TESTS.
TESTS

METHOD OF ASSAY

Lipase activity

**Principle**
Lipase activity is determined by measuring the release of free fatty acids that result from the hydrolysis of olive oil, used as a substrate. After a 30-minute reaction, the pH of the reaction mixture is increased by adding sodium hydroxide; back-titration with hydrochloric acid enables determination of the lipase activity based on the amount of fatty acid liberated from the substrate.

One unit of lipase activity is defined as the quantity of enzyme required to liberate 1 μmol free fatty acid per minute at the specified conditions.

**Apparatus**
Water bath with circulation
pH meter
Homogeniser (Ultra Turrax or equivalent)

**Reagents and solutions**
- Olive oil (Taiyo Pharmaceutical Co. Tokyo, Japan; product code 1041, or equivalent)
- 0.05 N Hydrochloric acid: Dilute 500 ml of 0.1 N hydrochloric acid to 1 litre with deionized water.
- Ethanol-acetone solution (1:1): Add 500 ml of ethanol (95%) to 500 ml of acetone and mix.
- 0.05 N Sodium hydroxide: Dilute 50 ml of 1 N sodium hydroxide to 1 litre with deionized water.
- Polyvinyl alcohol solution: Dissolve 18.00 g of polyvinyl alcohol (use fully hydrolysed polyvinyl alcohol with a saponification degree of 98.8 ± 0.2 mol%; available from Japan VAM & Poval Co., Ltd, Osaka, Japan as product number JF-17, or equivalent) and 2.00 g of polyvinyl alcohol (use partially hydrolysed polyvinyl alcohol with a saponification degree of 88.8 ± 1.0 mol%; available from Japan VAM & Poval Co., Ltd, Osaka, Japan as product number JP-05, or equivalent) in about 800 ml of deionized water preheated to 80° and cool. Dilute to 1 litre with deionized water. Filter if necessary.
- Phenolphthalein solution: Dissolve 1.0 g of phenolphthalein in 100 ml of ethanol (95%).
- 0.1 M Citric acid: Dissolve 21.0 g of citric acid monohydrate in 800 ml of deionized water and dilute to 1 litre with deionized water.
- 0.2 M Disodium hydrogen phosphate: Dissolve 28.4 g of disodium hydrogen phosphate in 500 ml of deionized water and dilute to 1 litre with deionized water.
- McIlvaine buffer (pH 7.0): Add 0.1 M citric acid to 0.2 M disodium hydrogen phosphate until the pH stabilizes at 7.0.

**Substrate**
Mix 150 ml polyvinyl alcohol solution with 50 ml olive oil and place on an ice water bath. Homogenize at 14,500 ± 300 rpm for a total of 10 min; alternate between mixing and cooling the solution for equal lengths of time until the mixture has been homogenized for a
full 10 min. Cool the mixture at 2-8° for at least 1 h before use. Label as Substrate Solution.

Sample preparation
Prepare the sample lipase solution at a dilution expected to require a volume of titrant (T₀-T₃₀) of 0.5 - 1.5 ml. Dissolve an accurately weighed amount of lipase in McIlvaine buffer and dilute to volume in a volumetric flask of appropriate size. Prepare serial dilutions as necessary. Label as Sample Preparation.

Procedure
Transfer 4 ml McIlvaine buffer and 5 ml Substrate Solution into a test tube and mix. Prepare in duplicate for each sample. Place the test tubes in a water bath maintained at 37 ± 0.5° for 10 min. At time equals zero, add 1 ml Sample Preparation to each test tube, mix and replace the tubes in the water bath. At time equals 30 min, add 10 ml ethanol-acetone solution to each test tube and mix. Next, add 10 ml 0.05 N sodium hydroxide followed by a second 10-ml aliquot ethanol-acetone solution and 2 drops of phenolphthalein solution to each test tube. Titrate the contents of each tube with 0.05 N hydrochloric acid until the pH reaches 10.0 while stirring and gently blowing nitrogen gas onto the solution.

Prepare a blank as follows. Transfer 4 ml McIlvaine buffer and 5 ml Substrate Solution into a test tube and mix. Prepare in duplicate. Add 10 ml of ethanol-acetone solution to each test tube and mix. Add 1 ml Sample Preparation to the tubes and mix. Next, add 10 ml 0.05 N sodium hydroxide followed by a second 10-ml aliquot ethanol-acetone solution and 2 drops of phenolphthalein solution to each test tube. Titrate the contents of each tube with 0.05 N hydrochloric acid until the pH reaches 10.0 while stirring and gently blowing nitrogen gas onto the solution.

Calculation
Calculate the lipase activity, expressed as U/g enzyme:

\[
U/g = (T₀ - T₃₀) \times f \times 50 \times n \times \frac{1}{30}
\]

where

- \(T₀\) is the titration volume of 0.05 N hydrochloric acid required for the blank solution (average of 2 values, ml)
- \(T₃₀\) is the titration volume of 0.05 N hydrochloric acid required for the reaction solution (average of 2 values, ml)
- \(f\) is a factor representing the exact concentration of the 0.05 N hydrochloric acid solution used divided by the target concentration of 0.0500 mol/litre
- 50 is the amount of fatty acid equivalent to 1 ml of 0.05 N hydrochloric acid (µmol)
- 30 is the reaction time (min)
- \(n\) is the dilution factor of the Sample Preparation (ml of Sample Preparation/g of enzyme)
New specifications prepared at the 89th JECFA (2020) and published in FAO JECFA Monographs 25 (2020). An ADI of “not specified” was established at the 89th JECFA (2020).

SYNONYM
Triphosphoinositide phosphodiesterase; phosphotinositidase C; 1-phosphatidylinositol-4,5-biphosphate phosphodiesterase; monophosphatidylinositol phosphodiesterase; phosphatidylinositol phospholipase C; PI-PLC

SOURCES
Phosphatidylinositol-Specific Phospholipase C (PI-PLC) is produced by controlled submerged aerobic fed-batch fermentation of a genetically modified, non-pathogenic, non-toxigenic strain of Pseudomonas fluorescens. The enzyme is released from the cells by a heat lysis step, then recovered from the fermentation broth. The recovery process includes separation of the enzyme from the cellular biomass using multiple filtration steps. PI-PLC is concentrated by ultrafiltration, and the concentrate is formulated and standardized with food grade glycerol to produce the liquid PI-PLC enzyme preparation.

Active principles
Phosphatidylinositol-specific phospholipase C

Systematic names and numbers
1-Phosphatidyl-1D-myoinositol-4,5-bisphosphate inositoltriphosphohydrolase; EC 3.1.4.11; CAS No. 6351-76-8

Reaction catalysed
Hydrolysis of phosphodiester bonds of phosphatidylinositol at the sn-3 position to produce inositol monophosphate and 1,2-diacylglycerol

Secondary enzyme activities
No significant levels of secondary activities

DESCRIPTION
Yellow to brown liquid

FUNCTIONAL USES
Enzyme preparation
Used in refining vegetable oils

GENERAL SPECIFICATIONS
Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.
CHARACTERISTICS

IDENTIFICATION

PI-PLC activity  The sample shows PI-PLC activity. See description under TESTS.

TESTS

METHOD OF ASSAY

PI-PLC activity  Principle
PI-PLC activity is measured in Inositol Phosphate Releasing Units (IPRU); it is determined by measuring the release of 4-methylumbelliferone that results from the hydrolysis of 4-methylumbelliferyl myo-inositol-1-phosphate substrate. The release of 4-methylumbelliferone is measured spectrophotometrically. The PI-PLC activity is calculated using a 4-methylumbelliferone standard curve.

One IPRU is defined as the quantity of enzyme required to liberate 1 μmol of 4-methylumbelliferone per minute at the specified conditions.

Equipment and reagents
Water bath with circulation
pH meter
UV-Vis spectrophotometer, thermostated
Disposable semi-micro cuvettes

Solutions
Phosphate buffer (0.2 M; 0.1% Triton X-100): Dissolve 27.6 g sodium phosphate monobasic, monohydrate (NaH₂PO₄·H₂O) and 1 g Triton X-100 in about 800 ml deionized water. Adjust the pH to 7.5 with 2 N sodium hydroxide solution and dilute to 1 litre with deionized water. This solution is stable for 1 month when kept refrigerated.

Methylumbelliferone Stock Solution (8.8 mg/ml 4-methylumbelliferone): Accurately weigh 88 mg of 4-methylumbelliferone (≥ 98% purity; Sigma-Aldrich M-1381 or equivalent) dissolve in dimethyl sulfoxide (≥ 99.7% purity) and dilute to 10 ml in a volumetric flask with dimethyl sulfoxide.

Methylumbelliferone Standard Solutions: Prepare a series of dilutions containing 0.176, 0.352, 0.528, 0.704 and 0.880 mg/ml of 4-Methylumbelliferone Stock in phosphate buffer. These correspond to 1.0, 2.0, 3.0, 4.0 and 5.0 mmol/litre. Label as Standard Solutions 1 through 5. CAUTION: Do NOT store the Standard Solutions on ice; methylumbelliferone may precipitate out of solution.

Substrate
Prepare the substrate just prior to use as it will undergo spontaneous degradation. The substrate must be prepared
accurately because the activity is strongly dependent on the substrate concentration. Prior to preparation of the substrate, rinse a 50-ml graduated cylinder as follows: rinse twice with 2 N hydrochloric acid solution; rinse three times with deionized water; rinse once with ethanol (≥ 95%). Allow the cylinder to dry at room temperature. Weigh an amount of 4-methylumbelliferyl myo-inositol-1-phosphate, N-methyl-morpholine salt (Biosynth Carbosynth M-5717, or equivalent; CAS No. 244145-23-1) into the cylinder and calculate the amount, in g, of Phosphate buffer to be added to obtain a solution of exactly 10.4 mg/ml 4-methylumbelliferyl myo-inositol-1-phosphate, N-methyl-morpholine salt. Add the calculated amount of Phosphate buffer to the cylinder, homogenize, and use immediately. Label as Substrate Solution.

Sample preparation
Prepare the PI-PLC sample at a dilution of approximately 0.6 lPRU/ml in Phosphate buffer. Transfer an accurately weighed amount of PI-PLC to a 100 ml volumetric flask and dissolve in 60 ml Phosphate buffer, then dilute to volume with Phosphate buffer. Prepare serial dilutions in Phosphate buffer, as needed, to achieve the desired sample dilution. Keep in an ice water bath until use; analyse on the day of preparation. Label as Sample Preparation.

Procedure
Equilibrate the Substrate Solution in a circulating water bath set at 37° ± 0.5°. Set the temperature of the spectrophotometer to 37°. Zero the instrument with an empty disposable semi-micro cuvette. Label five disposable semi-micro cuvettes as Standard Solutions 1 through 5. Transfer 2.8 ml of the equilibrated Substrate Solution into each of the cuvettes. Add 0.2 ml of the appropriate Methylumbelliferone Standard Solutions into the corresponding cuvette; place a cover on each cuvette and mix the contents by inverting twice. Measure the absorbance of each of the Standard Solutions at 380 nm. Construct a standard curve by plotting the absorbances at 380 nm versus the concentration of 4-methylumbelliferone in the Standard Solutions, in mmol/litre. Use the slope and y-intercept of the linear regression standard curve to determine the activity of the PI-PLC in the Sample preparation. If the intercept of the standard curve is OD₃₈₀ > 0.4, repeat the preparation of the Substrate Solution, the Standard Solutions and the analysis.

Transfer 2.8 ml of the equilibrated Substrate Solution into a disposable semi-micro cuvette. Place the cuvette in the spectrophotometer set to 37°. Allow the Substrate Solution to equilibrate for 5 min. At time equals zero, add 0.2 ml Sample Preparation to the cuvette containing the equilibrated Substrate Solution; place a cover on the cuvette, mix by inverting twice and place the cuvette back into the spectrophotometer. After 1 min, begin measuring the absorbance at 380 nm. Continue recording the absorbance for 5 min, taking a reading every 30 s. For each Sample Preparation being assayed, repeat the preceding steps (from “Transfer 2.8 ml...” to “...every 30 s; measurement in duplicate). Prepare a buffer blank (in triplicate) in the same manner using 0.2 ml of phosphate buffer in place of the Sample Preparation.
Calculate the average change in absorbance per min (ΔA/min) for each reaction mixture prepared with the Sample Preparation and for each buffer blanks. Average the results to obtain ΔA/min_{Sample} and ΔA/min_{Blank}. The average value should be between 0.05 – 0.20 OD/min for the Sample preparation; if the value does not fall in this range, prepare a new Sample Preparation diluted to achieve the desired ΔA/min and repeat the assay. The average ΔA/min at 380 nm for the buffer blank must be < 0.015 OD/min and the RSD for the three values shall not exceed 10%.

Calculation
Calculate the PI-PLC activity, expressed as IPRU/g enzyme:

\[
\text{IPRU/g} = (\text{ΔA/min}_{\text{Sample}} - \text{ΔA/min}_{\text{Blank}} - b) \times \left( \frac{1}{W} \right) \times \left( \frac{1}{S} \right)
\]

where
- ΔA/min_{Sample} is the average absorbance change per min for the Sample preparation (average of two)
- ΔA/min_{Blank} is the average absorbance change per min for the Buffer blank (average of three)
- b is the y-intercept of the standard curve
- f is the final dilution factor for the Sample Preparation (ml)
- S is the slope of the standard curve (Abs·litre/mmole 4-methylumbelliferone)
- W is the weight of PI-PLC in the Sample Preparation (g)
MAGNESIUM STEARATE


SYNONYMS
Magnesium distearate, dibasic magnesium stearate, INS No. 470(iii)

DEFINITION
Magnesium stearate is a mixture of magnesium salts of fatty acids obtained from edible fats and oils. The product consists mainly of magnesium stearate and palmitate in varying proportions. It is manufactured by one of the following processes: a) the direct process: fatty acids are directly reacted with a magnesium source, such as magnesium oxide to form magnesium salts of the fatty acids; b) indirect process: a sodium soap is produced by the reaction of fatty acids and sodium hydroxide in water, followed by the precipitation of magnesium salts of fatty acids by the addition of inorganic magnesium salts to the sodium soap.

Chemical names
Magnesium stearate, magnesium octadecanoate, fatty acids C16-C18 magnesium salts

C.A.S number
557-04-0 (magnesium stearate), 91031-63-9 (fatty acids C16-18 magnesium salts)

Chemical formula
\text{Mg(C_{18}H_{35}O_2)_2} (magnesium distearate)

Formula weight
591.27 (magnesium distearate)

Assay
Magnesium: Not less than 4.0% and not more than 5.0%, on the dried basis.
Fatty acids: Not less than 40.0% stearic acid in the fatty acid fraction; and not less than 90.0% as the sum of stearic and palmitic acids in the fatty acid fraction.

DESCRIPTION
Off-white to white, very fine and light powder; greasy to the touch

FUNCTIONAL USES
Anticaking agent, emulsifier, binder

CHARACTERISTICS
IDENTIFICATION
Solubility (Vol.4)
Practically insoluble in water
**Magnesium** *(Vol. 4)*

Present

Identify in the ashed sample following the procedure described in Volume 4 “(under identification tests, inorganic ions, magnesium)” or using the Method of Assay.

**Fatty acid composition**

Using the Method of Assay, identify the individual fatty acids

**PURITY**

**Loss on drying** *(Vol. 4)*

Not more than 6% (105°, constant weight, use 1 g of sample)

**Acidity or alkalinity**

Passes test

See description under TESTS

**Unsaponifiable matter**

Not more than 2%

See description under TESTS

**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 method described in Volume 4 (under “General Methods, Metallic Impurities”).

**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”).

**Nickel** *(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 method described in Volume 4 (under “General Methods, Metallic Impurities”). Use analytical line (emission wavelength): 231.60 nm, curve type: linear, and calibration range: 0.10 – 10.0 µg/ml

**TESTS**

**PURITY TESTS**

**Acidity or alkalinity**

To 1.0 g sample add 20 ml freshly prepared deionized water (carbon dioxide free) and boil for 1 min with continuous shaking. Cool and filter. To 10 ml filtrate add 0.05 ml bromothymol blue solution (prepared by dissolving 100 mg bromothymol blue in a mixture of equal volumes ethanol (96%) and water and dilute to 100 ml with the same mixture, filter if necessary). Not more than 0.05 ml 0.1 M hydrochloric acid or 0.1 M sodium hydroxide is required to change the colour of the indicator.
**Unsaponifiable matter**

Weigh about 5 g, to the nearest 0.01 g well-mixed sample into a 250 ml round-bottom flask. Add approximately 50 ml 0.5 N potassium hydroxide solution and some pumice, attach a reflux condenser, and boil gently for 1 h. Stop heating. Add 100 ml distilled water through the top of the condenser and swirl.

After cooling, transfer the solution to a separatory funnel. Rinse the flask and the pumice several times with diethyl ether (total 100 ml) and add the solvent to the separatory funnel. Stopper and shake vigorously for 1 min, periodically releasing pressure by inverting the separating funnel and opening the stopcock.

Allow to stand until the two phases are completely separated. Then draw off the soap solution as completely as possible into a second separating funnel. Extract this solution twice more with 100 ml diethyl ether each time. Combine the three ether extracts into one separating funnel containing 40 ml water.

Gently rotate the separating funnel containing the combined ether extracts and the 40 ml water. Violent agitation at this stage may result in emulsion formation. Allow the layers to separate completely and draw off the lower aqueous layer. Wash the ether layer twice more with 40 ml portions of water, shaking vigorously each time and discarding the lower aqueous layers after separation. Draw off each washing solution leaving approx. 2 ml, then rotate the separating funnel around its axis, wait some min to give the last remainders opportunity for collection and draw off the collected remainders, close stopcock when ether starts to pass into the bore of the stopcock.

Wash the ether layer successively with 40 ml 0.5 N potassium hydroxide solution, 40 ml water, and again with 40 ml potassium hydroxide solution, then at least twice more with 40 ml water. Continue to wash with water until the wash-water no longer gives a pink colour on addition of a drop of phenolphthalein solution.

Transfer the ether layer quantitatively a little at a time through the top of the separating funnel into a flask previously dried and weighed to the nearest 0.0001 g.

Evaporate the solvent by distillation on a boiling-water bath. Add 5 ml acetone and remove the solvent completely in a gentle air current, holding the flask obliquely while turning it in a boiling-water bath.

Dry the residue at 103 ± 2° for 30 min, placing the flask in an almost horizontal position. Cool in a desiccator and weigh to the nearest 0.0001 g (m1). Repeat the drying for successive 15 min periods until the loss of weight between two successive weighings is less than 0.002 g.

After weighing the residue dissolve in 4 ml diethyl ether and add 20 ml ethanol, previously neutralized to a faint pink colour using phenolphthalein TS as indicator. Titrate with standard 0.1 N ethanolic potassium hydroxide solution (prepared by dissolving 6 g potassium hydroxide in about 5 ml water and making up to 1 liter with ethanol) to the same final colour.
Correct the weight of the residue for the free acidity content of the blank.
Calculate the percent unsaponifiable matter using the formula:

\[
\text{Unsaponifiable matter (\%) } = \frac{100 \times (m_1 - 0.281 \times T \times V)}{m}
\]

where
- \(m\) is the mass, in g, of the test portion
- \(m_1\) is the mass, in g, of the residue
- \(V\) is the number of ml of the standardized potassium hydroxide solution used
- \(T\) is the exact normality of the potassium hydroxide solution used

**METHOD OF ASSAY**

**Magnesium**
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”).

**Fatty acid composition**
Fatty acids in the sample are methylated and the resultant fatty acid methyl esters are determined by gas chromatography (Vol.4).

**Procedure**

**Sample solution**
In a conical flask fitted with a reflux condenser, dissolve 0.10 g sample in 5 ml boron trifluoride-methanol solution (commercially available or prepared by dissolving 14 g boron trifluoride in 100 ml anhydrous methanol). Boil under a reflux for 10 min. Add 4 ml heptane through the condenser and boil again under reflux for 10 min. Allow to cool. Add 20 ml saturated sodium chloride solution, shake and allow layers to separate. Remove the organic layer and dry over 0.1 g anhydrous sodium sulfate (previously rinsed with heptane).

**Reference solution**
Prepare in the same manner as the test solution using 50.0 mg palmitic acid (>96% pure) and 50.0 mg stearic acid (>96% pure).

**Gas chromatography**
Use a suitable capillary GC-FID system under isothermal conditions.
- Column: Polyethylene glycol 20000, 30 m x 0.32 mm id x 0.5 \(\mu\)m film thickness (Macrogol 20 000 R or equiv.)
- Carrier gas: helium (>99.995 % pure); flow rate: 1 ml/min
- Column temperature: 180°
- Injector temperature: 250°
- Detector temperature: 260°

**System suitability**
Resolution between the peaks of methyl palmitate and methyl stearate in the reference solution should be >5.0. Relative standard deviation, determined on areas of 6 injections using reference solution, should be <3.0% for methyl palmitate and <1.0% for methyl stearate.
Analysis
condition the GC using the conditions above; inject 1 µl reference
solution and record retention times for the constituent fatty acid
methyl esters.

Calculation
Using area normalization (area %) determine the relative
percentages of palmitic and stearic acid esters in the reference
solution. Inject 1 µl of test solution and determine the relative
percentages of fatty acids in the sample solution.
POLYVINYL ALCOHOL


SYNONYMS
Vinyl alcohol polymer, PVOH, INS No. 1203

DEFINITION
Polyvinyl alcohol is a synthetic resin prepared by the polymerization of vinyl acetate, followed by partial hydrolysis of the ester in the presence of an alkaline catalyst. The physical characteristics of the product depend on the degree of polymerization and the degree of hydrolysis.

Chemical names
Ethenol homopolymer

C.A.S. number
9002-89-5

Chemical formula
(C\(_2\)H\(_3\)OR)\(_n\) where R=H or COCH\(_3\) (randomly distributed)

Structural formula
\[
\begin{array}{c}
\text{CH}_2 \\
\frac{\text{CH}}{\text{OR}} \\
\end{array}
\] 
\(n\)

where R=H or COCH\(_3\) (randomly distributed)

DESCRIPTION
Odourless, translucent, white or cream-coloured granular powder.

FUNCTIONAL USES
Coating, binder, sealing agent and surface-finishing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4)
Soluble in water, practically insoluble or insoluble in ethanol

pH (Vol.4)
5.0 – 6.5 (1 in 25 solution)

Infrared spectrum (Vol.4)
The infrared absorption spectrum of a potassium bromide dispersion of the sample corresponds to that of a poly vinyl alcohol standard (see infrared spectrum below).
**Colour reaction A**  
Dissolve 0.01 g of the sample in 100 ml of water with warming and let the solution cool to room temperature. To 5 ml of the solution, add one drop of iodine TS and a few drops of boric acid solution (1 in 25). A blue colour is produced.

**Colour reaction B**  
Dissolve 0.5 g of the sample in 10 ml of water with warming and let the solution cool to room temperature. Add 1 drop of iodine TS to 5 ml of solution and allow to stand. A dark red to blue colour is produced.

**Precipitation reaction**  
Add 10 ml of ethanol to the remaining 5 ml of solution prepared for Colour reaction B. A white, turbid or flocculent precipitate is formed.

**PURITY**

**Loss on drying (Vol.4)**  
Not more than 5.0% (105°, 3 h)

**Residue on ignition (Vol.4)**  
Not more than 1.0%

**Water insoluble substances (Vol.4)**  
Not more than 0.1%  
Substitute a 100-mesh screen for the sintered-glass filter specified in Volume 4

**Particle size**  
Not less than 99.0% material to pass through a 100 mesh sieve  
Determine by sieving for 30 min 100g of sample through a 100 mesh sieve and weigh the material passing through the sieve.

**Methanol and methyl acetate**  
Not more than 1.0 % of each  
See description under TESTS

**Acid value**  
Not more than 3.0  
See description under TESTS

**Ester value**  
Between 125 and 153 mg KOH/g  
See description under TESTS

**Degree of hydrolysis**  
Between 86.5 and 89.0%  
See description under TESTS

**Viscosity**  
4.8 - 5.8 mPa•s (4% solution at 20°)  
See description under TESTS

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

PURITY TESTS

**Methanol and methyl acetate**

Determine by GC (Vol.4)

**Sample preparation**
Place 2.0 g of the sample into a 100 ml screw-cap bottle, and add a magnetic stirrer. Add 98 ml of water and 30 µl of acetone. Cap the bottle tightly and heat in a water-bath, stirring continuously. Once the solution becomes clear, remove the bottle from the water bath and allow it to cool to room temperature.

**Standard preparation**
Prepare a standard by taking 2 ml of a mixed solution of methanol and methyl acetate (1.2 % v/v solution), 98 ml of water and 30 µl acetone; proceed as above starting from “Cap the bottle… room temperature”.

**GC conditions**
- Column: Sunpak A (3.2 mm i.d. x 3 m) or equivalent
- Column temperature: 160°
- Injector temperature: 160°
- Detector temperature: 160°

**Procedure**
Inject 0.4 ± 0.1 µl of the standard solution into the gas chromatograph and record the peak areas (PA) for methanol, methyl acetate and acetone. Inject 0.4 ± 0.1 µl of the sample solution and record the peak areas (PA) for methanol, methyl acetate, and acetone.

**Calculation**

Calculate the methanol and methyl acetate content using the formulae:

\[
\text{Methanol (wt%)} = \frac{\text{PA(methanol)}}{\text{PA(acetone)}} \times \frac{\text{PR}_1 \times 0.024}{100/2}
\]

\[
\text{Methyl acetate (wt %)} = \frac{\text{PA(methyl acetate)}}{\text{PA(acetone)}} \times \frac{\text{PR}_2 \times 0.024}{100/2}
\]

where

- 0.024 is the conversion factor to obtain the individual mass of methanol and methyl acetate added to 30 µl acetone (density = 0.8) in the methanol/methyl acetate standard
- \(\text{PR}_1\) is the PA(acetone)/PA(methanol) peak area ratio
- \(\text{PR}_2\) is the PA(acetone)/PA(methyl acetate) peak area ratio of the standard 1.2% methanol and methyl acetate aqueous solution
**Acid value**

Determine the acid value by titration.

Add 200 ml water and a stir bar into a 500-ml round-bottom flask, attach a reflux condenser and begin heating in a boiling water bath. Add 10.0 g of the sample and continue heating for 30 min while stirring continuously. Remove the flask from the water bath and continue stirring until the solution reaches room temperature. Quantitatively transfer this solution to a 250-ml volumetric flask and dilute to volume with water. Take 50 ml of the solution, add 1 ml of phenolphthalein TS and titrate with 0.05 M potassium hydroxide until the pink colour persists for 15 sec; record the titre in ml (V). Calculate the acid value, A:

\[
A = 5.0(56.1\times V\times M)/W
\]

where
- 56.1 is the formula weight of KOH
- M is the molarity of the KOH solution
- W is the weight of sample (g)

**Ester value**

Determine the ester value as follows:

Accurately weigh about 1.0 g of sample into a 250-ml round-bottom flask, add 25 ml 0.5 M alcoholic potassium hydroxide, 25.0 ml of water and a few glass beads. Attach a condenser and allow the contents to reflux for 30 minutes in a boiling water-bath. Let cool to room temperature, remove the condenser, add 1 ml of phenolphthalein TS and titrate immediately with 0.5 M hydrochloric acid; record the titre in ml (V1).

Carry out a blank test under the same conditions. Titrate with 0.5 M hydrochloric acid and record the titre in ml (V2). Calculate the saponification value, S:

\[
S = 56.1(V_2 - V_1) \times M/W
\]

where
- 56.1 is the formula weight of KOH
- M is the molarity of the hydrochloric acid solution
- W is the weight of the sample in (g)

Calculate the ester value, E:

\[
E = S - A
\]

where
- S is the saponification value
- A is acid value

**Degree of hydrolysis**

Convert the saponification value obtained during the determination of the ester value to the “dried basis” (S\textsubscript{db}):

\[
S_{\text{db}} = (S \times 100)/(100 - \text{LOD})
\]

where
- LOD is Loss on Drying
The degree of hydrolysis is:

$$100 - \left[7.84 \frac{S_{db}}{(100 - 0.075 S_{db})}\right]$$

### Viscosity Calibration of capillary-type viscometers
An oil of known viscosity is used to determine the viscometer constant (k).

**Ostwald-Type Viscometer:** Fill the tube with the exact amount of oil (adjusted to 20.0 ± 0.1º), as specified by the manufacturer. Use either pressure or suction to adjust the meniscus of the column of liquid in the capillary tube to the level of the top graduation line. Allow the liquid to flow into the reservoir against atmospheric pressure by opening both the filling and capillary tubes. If either tube is not open, false values might be obtained. Record the time (seconds), for the liquid to flow from the upper mark to the lower mark of the capillary tube (efflux time).

**Ubbelohde-Type Viscometer:** Place a quantity of the oil (adjusted to 20.0 ± 0.1º) in the filling tube, and transfer to the capillary tube by gentle suction. Keep the air vent tube closed in order to prevent bubble formation in the liquid. Adjust the meniscus of the column of liquid in the capillary tube to the level of the top graduation line. Allow the liquid to flow into the reservoir against atmospheric pressure by opening both the filling and capillary tubes. If either tube is not open, false values might be obtained. Record the efflux time (sec).

The viscosity constant (k) for capillary-type viscometers is given by:

$$k = \frac{v}{d} \times t$$

where
- v is the known viscosity (mPa·s) of the oil used for the viscometer calibration
- d is the density (g/ml) of the liquid tested at 20º/20º
- t is the efflux time in sec.

### Procedure
Weigh a quantity of undried sample equivalent to 6.00 g on the dried basis. Into a tared 250-ml flask containing a magnetic stir bar and approximately 140 ml of water, quickly (seconds) transfer the sample, while simultaneously stirring slowly and continuously. Once the sample appears thoroughly saturated, slowly increase the stirring rate to minimize the entrainment of air in the mixture. Heat the mixture to 90º and maintain it at this temperature for approximately 5 minutes; discontinue heating and continue stirring for 1 hour. Add water in small amounts to attain a total mixture weight of 150 g, and resume stirring until the mixture appears homogenous. Filter the mixture through a tared 100-mesh screen into a 250 ml conical flask, cool the filtrate to about 15º, mix, and determine its viscosity at 20º using an appropriate viscometer (follow the manufacturer’s instructions).

**NOTE:** The temperature at which the viscosity measurement is made must be strictly controlled.
For measurements using capillary-type viscometers, the viscosity is given by:

\[ v = k \times d_s \times t \]

where
- \( k \) is the viscosity constant
- \( t \) is the efflux time for the sample solution
- \( d_s \) is the density of the sample solution at 20º

**Infrared spectrum of polyvinyl alcohol**

![Infrared spectrum of polyvinyl alcohol](image)
RIBOFLAVIN FROM ASHYBYA GOSSYPII

Riboflavin from *Ashbya gossypii* was on the agenda of the current meeting at the request of the Codex Committee on Food Additives at its fifty-first session (1) for assessment of its safety and dietary exposure and for preparation of new specifications. Because of time constraints, the assessments of safety and dietary exposure were not completed.

Riboflavin or 7,8-dimethyl-10-(1’-D-ribityl)isoalloxazine (IUPAC name: 7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl]benzo[g]pteridine-2,4-dione; CAS No. 83-88-5), commonly known as vitamin B2, was last evaluated for specifications by JECFA as a synthetic product in 1987 and as a product of fermentation from a genetically modified strain of *Bacillus subtilis* in 1999 (Annex 1, reference 185).

The Committee at its present meeting drafted a chemical and technical assessment and new specifications for riboflavin from *A. gossypii* from the data submitted by the sponsor, but they were not finalized for publication. The Committee recognized the benefits of simultaneous review and harmonization of new specifications with existing specifications for riboflavin as a synthetic product and as a product of *B. subtilis*. The Committee recommends that this work be undertaken at a future meeting.
SORBITAN ESTERS of FATTY ACIDS (INS 491, INS 492 and INS 495)

Sorbitan monostearate (INS 491), sorbitan tristearate (INS 492) and sorbitan monopalmitate (INS 495) were on the agenda of the current meeting at the request of the CCFA at its Fifty-first Session (1) to replace the specification for congealing range. The Committee noted that the method for identifying the congealing range reported in the JECFA monographs for INS 491, 492 and 495 is empirical and difficult to perform (Annex 1, reference 63), and the results are not repeatable. Determination of the congealing range was included as an identification method because it is correlated with the type and content of fatty acids (sorbitan monostearate vs sorbitan tristearate) and their length (sorbitan monopalmitate vs sorbitan monostearate). The congealing range also depends, however, on variations in the content of minor constituents, such as that of stearic acid in edible palmitic acid, a raw material for the manufacture of sorbitan monopalmitate. The Committee considered that a specification for the fatty acid components (a minimum–maximum range) and additional compositional parameters would be more appropriate.

The Committee noted that toxicological evaluations of the above additives were often performed with samples for which the congealing range was not reported. JECFA established a group ADI of 0–25 mg/kg bw for sorbitan monostearate (INS 491), sorbitan tristearate (INS 492), sorbitan monopalmitate (INS 495), sorbitan monolaurate (INS 493) and sorbitan monooleate (INS 494) at its 26th meeting in 1982 (Annex 1, reference 59). Sorbitan monolaurate and sorbitan monooleate were not on the agenda of the present meeting because their specifications do not include a congealing range. The Committee noted that the group ADI is not expressed based on sorbitan content, although the sorbitan content of the five esters varies widely.

JECFA established the specifications for sorbitan esters of fatty acids at its 17th, 33rd and 39th meetings of (Annex 1, references 32, 83 and 101, respectively). The specifications in those early evaluations, such as for solubility and impurities, should be revised, the manufacturing information updated and the ADI re-evaluated. The Committee recommends that a new call for data be issued in order to proceed with an updated safety evaluation and specifications for the five sorbitan esters of fatty acids at the same time.

The Committee also noted that five polyoxyethylene sorbitan esters (polysorbates) were evaluated by JECFA at its 17th meeting Annex 1, reference 32), and specifications were established. The Committee recommends that a new call for data be issued for their full evaluation.

In view of these recommendations and because of the limited time available at the current meeting, the agenda of the meeting was changed, and the specifications for sorbitan esters of fatty acids were not revised.
SPECIFICATIONS FOR CERTAIN FLAVOURING AGENTS

At the 89th meeting, the Committee prepared specifications of identity and purity for 15 new flavouring agents with the numbers 2256 - 2270. Full specifications were prepared for 12 of the agents, and tentative specifications were prepared for three, (2260, 2263, 2264) as the safety evaluations for these flavouring agents were not completed. The Committee also re-evaluated 14 flavourings (2002, 1575, 1604, 2077, 1125, 380.1, 1491, 1497, 1502, 1504, 1506, 1511, 1513 and 1517); full specifications were maintained for 13 of these flavouring agents and one was maintained as tentative (380.1).

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.

The flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents and a list of conclusions is given in Annex 1.
1. Phenol and phenol derivatives used as flavouring agents

<table>
<thead>
<tr>
<th>JECFA No.</th>
<th>Name</th>
<th>Chemical Name</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>2256</td>
<td>(±)-Homoeriodictyol, sodium salt</td>
<td>5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-2,3-dihydro-4H-1-benzopyran-4-one—sodium (1/1) HED sodium salt; 4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-, sodium salt;</td>
<td>C_{16}H_{14}O_6Na</td>
<td>Soluble Practically insoluble to insoluble</td>
<td>MS, IR, HNMR</td>
<td>NA</td>
<td>mp: Decomposition &gt;130° SC: 3-5% Eriodictyol-7-methyl ether (racemic mixture); 1-2% Homoeriodictyol-7-methyl ether (racemic mixture)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Status</th>
<th>Chemical Name</th>
<th>Physical form; Odour</th>
<th>B.P. °</th>
<th>Acid value</th>
<th>Information required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session</td>
<td>COE</td>
<td>CAS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 89 | 462631-45-4 |
**Naringenin**

**Chemical Structure:**

\[
\text{C}_{15}\text{H}_{12}\text{O}_{5}
\]

- Practically insoluble to insoluble in MS, HNMR, CNMR, IR.
- Melting point (mp): 251°C
- Isomeric composition: R isomer 50.6% (17654-19-2); S isomer 49.4% (480-41-1)

**Properties:**
- Pale yellow powder
- Practically insoluble in MS, HNMR, CNMR, IR.
- Optical rotation: [α]_D^28 93.6°
- Slight citrus aroma
- Pale yellow powder
- Melting point (mp): 134-135°C
- Optical rotation: [α]_D^28 8.1°

**Chemical Structure:**

\[
\text{C}_{16}\text{H}_{14}\text{O}_{5}
\]

- Practically insoluble to insoluble in MS, HNMR, CNMR, IR.
- Melting point (mp): 175-176°C
- Isomeric composition: R isomer 49.7% (17654-19-2); S isomer 50.3%
- Optical rotation: [α]_D^28 93.6°
- Slight citrus aroma
- Pale yellow powder
- Melting point (mp): 225°C
<table>
<thead>
<tr>
<th></th>
<th><strong>2259</strong> 7,8-Dihydroxyflavone</th>
<th>4830</th>
<th><strong>C₁₅H₁₀O₄</strong></th>
<th>Very slightly soluble</th>
<th>HNMR</th>
<th>NA</th>
<th>mp: 244-249°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>7,8-dihydroxy-2-phenylchromen-4-one</td>
<td>-</td>
<td>254.24</td>
<td>Very slightly soluble</td>
<td>95%</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7,8-Dihydroxy-2-phenyl-4H-1-benzopyran-4-one; 7,8-Dihydroxy-2-phenyl-4-benzopyrone; 7,8-Dihydroxy-2-phenyl-4H-chromen-4-one</td>
<td>-</td>
<td>Pale yellow powder;</td>
<td>Bland aroma</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|   | **2260** (2S)-3',7-Dihydroxy-8-methyl-4'-methoxyflavan | 4833 | **C₁₇H₁₈O₄** | Practically insoluble to insoluble | MS, HNMR | NA | mp: 130-131° |
|   | (S)-2-(3-hydroxy-4-methoxyphenyl)-8-methylchroman-7-ol | 286.32 | Very slightly soluble | 95% | NA | Optical rotation: -45.7° (c = 14.0 mg/mL, CHCl₃) |
| Tentative | (S)-2-(3-Hydroxy-4-methoxyphenyl)-8-methylchroman-7-ol; (2S)-7,3'-Dihydroxy-4'-methoxy-8-methylflavane | - | White powder; | Bland aroma | NA | - | Safety evaluation not completed |

<table>
<thead>
<tr>
<th></th>
<th>89</th>
<th>87733-81-1</th>
</tr>
</thead>
</table>

89   38183-03-8
<table>
<thead>
<tr>
<th>Compound Description</th>
<th>C</th>
<th>H</th>
<th>O</th>
<th>Molecular Formula</th>
<th>mp</th>
<th>Optical Rotation</th>
<th>pKa</th>
<th>Solubility</th>
<th>MS, IR, HNMR</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3-(3-hydroxy-4-methoxy-phenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one</strong></td>
<td>16</td>
<td>16</td>
<td>6</td>
<td>304.29</td>
<td>&gt;120°C</td>
<td>+25° (20 °C, c = 1.0 g/100 mL in absolute ethanol)</td>
<td>3.1</td>
<td>Practically insoluble</td>
<td>MS, IR, HNMR</td>
<td>Full</td>
</tr>
<tr>
<td><strong>Hesperetin dihydrochalcone</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Decomposition</td>
<td>-</td>
<td>-</td>
<td>Very slightly soluble; Slightly grey solid</td>
<td>MS, IR, HNMR</td>
<td>Full</td>
</tr>
</tbody>
</table>
## 2. Alicyclic ketones, secondary alcohols and related esters used as flavouring agents

<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>FLAVIS</td>
<td>M.W</td>
<td>Solubility in ethanol</td>
<td>Assay min %</td>
<td>S.G. (25°)</td>
<td>Synonyms</td>
</tr>
<tr>
<td>Synonyms</td>
<td>CAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 2263      | trans-4-tert-Butylcyclohexanol | 4724 | C₁₀H₂₀O | Practically insoluble to insoluble | MS, IR, HNMR | NA | mp: 80° |
| Tentative | (1r,4r)-4-tert-Butylcyclohexan-1-ol; trans-1-tert-Butylcyclohexan-4-ol; trans-p-tert-Butylcylohexanol; trans-4-(1,1-dimethyl)cyclohexanol | 156.27 | Soluble | 95% | NA |

| 89        | 21862-63-5 | | | | | | |

![Chemical structure of trans-4-tert-Butylcyclohexanol]
Practically insoluble to insoluble in water
Practically insoluble

HNMR, C\textsuperscript{13}H\textsuperscript{2}O

Isomeric composition:
25% (38224-26-3)
75% (34298-31-2)

Soluble

95% (sum of isomers)

0.968

Mixture of 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]-undecan-5-ol and 4,11,11-trimethyl-8-methylenebicyclo[7.2.0]-undecan-5-ol

Isomeric composition:
220.35

Spicy, woody

310°

Safety evaluation not completed

Mixtures of 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]-undecan-5-ol and 4,11,11-trimethyl-8-methylenebicyclo[7.2.0]-undecan-5-ol
### 3. Amino acids and related substances used as flavouring agents

<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>2265</td>
<td>Betaine</td>
<td>FLAVIS</td>
<td>C₅H₁₁NO₂</td>
<td>Very soluble</td>
<td>IR</td>
<td>NA</td>
<td>mp: 293°</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M.W</td>
<td>Physical form; Odour</td>
<td>Assay min %</td>
<td>S.G. (25°)</td>
<td>Information required</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>COE</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAS</td>
<td></td>
<td></td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full</td>
<td>2-(trimethylazaniumyl)acetate</td>
<td>4223</td>
<td>117.15</td>
<td>Soluble</td>
<td>95%</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-Carboxy-N,N,N-trimethylmethanaminium hydroxide inner salt; (Carboxymethyl)trimethylaminum hydroxide inner salt; Glycine betaine; Glycyldetaine; N,N,N-Trimethylglycine; Trimethylglycine hydroxide inner salt; Trimethylglycine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Free-flowing white crystals; Savoury</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>107-43-7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Glutamyl-2-aminobutyric acid

C₁₀H₁₆N₂O₅

Freely soluble in IR, HNMR,
CNMR

mp: 180-182°
Optical rotation: -3° to -2°

Glutamyl-2-norvalyl-glycine

C₁₂H₂₁N₃O₆

Soluble in IR, HNMR,
CNMR

mp: 191-192°
Optical rotation: -25° to -24° (c=1.0 g/100 mL, H₂O)

Glutamyl-L-glutamine; L-gamma-glutamyl-L-2-aminobutyric acid; N-(DL-gamma-glutamyl)-L-2-aminobutyric acid; N-[(2S)-3-amino-5-oxopentan-2-yl]oxypropyl]L-glutamate; L-gamma-Glu-Abu-OH; H-Glu(Abu-OH)
**Glutamyl-norvaline**

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th>Molecular Weight</th>
<th>Solubility</th>
<th>Spectroscopic</th>
<th>Optical Rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₃₀H₃₈N₂O₅</td>
<td>4740</td>
<td>Freely soluble</td>
<td>IR, HNMR, CNMR</td>
<td>-14° to -13°</td>
</tr>
</tbody>
</table>

Full name: L-gamma-Glutamyl-L-norvaline; N-(N-L-gamma-Glutamyl)-L-norvaline; gamma-Glu-Nva;

- Off-white powder;
- Kokumi

**N-Acetyl glutamate**

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th>Molecular Weight</th>
<th>Solubility</th>
<th>Spectroscopic</th>
<th>Optical Rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₇H₁₁NO₅</td>
<td>4752</td>
<td>Soluble</td>
<td>HNMR, CNMR</td>
<td>-25.6° (22 °C in water)</td>
</tr>
</tbody>
</table>

Full name: (2S)-2-(acetylamino)pentanedioic acid

- White powder or white crystals;
- Savoury, cooked, roasted

89 71133-09-0

89 1188-37-0
<table>
<thead>
<tr>
<th>CAS Number</th>
<th>Methyl (2R)-2-amino-3-sulfanylpropanoate hydrochloride; Mecysteine methyl ester hydrochloride; laevo-cysteine methyl ester hydrochloride</th>
<th>mp</th>
<th>Optical rotation</th>
<th>HNMR</th>
<th>Soluble</th>
<th>Fully soluble</th>
<th>95%</th>
<th>Soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>2270</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>171.65</td>
<td></td>
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<tr>
<td>18598-63-5</td>
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</tr>
</tbody>
</table>

**Notes:**
- White powder;
- Sulphur-like odour;
- mp: 140-141°C;
- HNMR, CNMR.
### 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>Synonyms</td>
<td>Physical form; Odour</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>Session</td>
<td>CAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid gamma-lactone</td>
<td>FLAVIS</td>
<td>C11H16O2</td>
<td>Soluble in non-polar solvents; insoluble in water</td>
<td>HNMR</td>
<td>1.560-1.575</td>
<td>SC: 2-3% 3,4-Dimethylfuran-2,5-dione</td>
</tr>
<tr>
<td>Full</td>
<td>3,4-Dimethyl-5-pentylidenefuran-2(5H)-one</td>
<td>COE</td>
<td>10.042</td>
<td>180.24</td>
<td>Clear and colourless liquid; Spicy-herbal to mint-like aroma</td>
<td>Soluble</td>
<td>93%</td>
</tr>
<tr>
<td>89</td>
<td>5-Pentylidene-3,4-dimethyl-2,5-dihydrofuran-2-one; Bovolide</td>
<td>CAS</td>
<td>774-64-1</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Status</th>
<th>Chemical Name</th>
<th>Synonyms</th>
<th>Physical form; Odour</th>
<th>Solubility in ethanol</th>
<th>Assay min %</th>
<th>S.G. (25°)</th>
<th>Information required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session</td>
<td>CAS</td>
<td></td>
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<tr>
<td>2002</td>
<td>4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid gamma-lactone</td>
<td>FLAVIS</td>
<td>C11H16O2</td>
<td>Soluble in non-polar solvents; insoluble in water</td>
<td>HNMR</td>
<td>1.560-1.575</td>
<td>SC: 2-3% 3,4-Dimethylfuran-2,5-dione</td>
</tr>
<tr>
<td>Full</td>
<td>3,4-Dimethyl-5-pentylidenefuran-2(5H)-one</td>
<td>COE</td>
<td>10.042</td>
<td>180.24</td>
<td>Clear and colourless liquid; Spicy-herbal to mint-like aroma</td>
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</tr>
<tr>
<td>89</td>
<td>5-Pentylidene-3,4-dimethyl-2,5-dihydrofuran-2-one; Bovolide</td>
<td>CAS</td>
<td>774-64-1</td>
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<tr>
<td>Molecule</td>
<td>Molecular Formula</td>
<td>CAS No.</td>
<td>mp (°C)</td>
<td>Relative Abundance (%)</td>
<td>Aroma Description</td>
<td>Solubility</td>
<td>MS SC %</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-------------------</td>
<td>---------</td>
<td>---------</td>
<td>------------------------</td>
<td>----------------------------------</td>
<td>------------</td>
<td>---------</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>C_{15}H_{24}O_{2}</td>
<td>1139-30-6</td>
<td>-</td>
<td>-</td>
<td>Colourless solid; Fishy aroma</td>
<td>Insoluble in water; Soluble in fat</td>
<td>-</td>
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<tr>
<td>Humulene-1,2-epoxide</td>
<td>C_{15}H_{22}NO</td>
<td>85213-22-4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Acetyl-1-pyrroline</td>
<td>C_{4}H_{9}NO</td>
<td>103475-43-0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4,5-Epoxy-4,12,12-trimethyl-8-bicyclo(8.2.0)dodecane</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Full</td>
<td></td>
<td>85-63.0</td>
<td>1139-30-6</td>
<td>-</td>
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<tr>
<td>Full</td>
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<td>1139-30-6</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
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*Note: HNMR = proton nuclear magnetic resonance; MS = mass spectrometry; SC = specific content.*
<table>
<thead>
<tr>
<th><strong>Compound</strong></th>
<th><strong>CAS Number</strong></th>
<th><strong>Formula</strong></th>
<th><strong>Solubility</strong></th>
<th><strong>Densities</strong></th>
<th><strong>Isomers</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>(2E,6E/Z,8E)-N-(2-Methylpropyl) 2,6,8-decatrienamide</td>
<td>4668</td>
<td>C_{14}H_{23}NO</td>
<td>Insoluble in water</td>
<td>MS IR 95% (mixture of isomers)</td>
<td>1.491-1.541</td>
</tr>
<tr>
<td>(1Z,2E/6Z,8E)-N-(2-Methylpropyl)deca-2,6,8-trienimide</td>
<td>Full</td>
<td>221.34</td>
<td>Soluble</td>
<td>0.945-0.949</td>
<td></td>
</tr>
<tr>
<td>2E,6Z,8E-Decatrienoic acid N-isobutylamide; N-Isobutyldeca-trans-2,cis-6,trans-8-trienamide; (2E,6Z,8E)-N-(2-Methylpropyl)-2,6,8-decatrienamide; Spilanthol</td>
<td>504-48-3; 25394-57-4</td>
<td>140-160° (13.3 Pa)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Hexen-3-one</td>
<td>3352</td>
<td>C_{6}H_{10}O</td>
<td>Slightly soluble in water; soluble in oil</td>
<td>NMR 95% (sum of isomers)</td>
<td>1.437-1.443</td>
</tr>
<tr>
<td>4-Hexen-3-one</td>
<td>Full</td>
<td>98.14</td>
<td>Miscible at room temperature</td>
<td>0.855-0.861</td>
<td></td>
</tr>
<tr>
<td>2-Hexen-4-one</td>
<td>718</td>
<td></td>
<td>93° (150 mm Hg)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Isomers: 73-80% (2E,6Z,8E); 15-18% (2E,6E,8E); 3-7% (2E,6Z,8Z); 1-2% (2Z,6Z,8E); 1-2% (2Z,6E,8E)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Molecular Formula</td>
<td>Solubility and Physical Properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------</td>
<td>------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carvone</td>
<td>C₁₀H₁₄O</td>
<td>Colourless liquid; fruity aroma.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>Soluble in water.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMR IR</td>
<td></td>
<td>1.45, 1.41</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IR</td>
<td></td>
<td>1.49, 1.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optical rotation</td>
<td></td>
<td>+50° to +60°</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 2-Amylthran               |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylthran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylfuran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Amylthran               |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylthran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylfuran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Amylthran               |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylthran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylfuran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Amylthran               |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylthran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylfuran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Amylthran               |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylthran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylfuran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Amylthran               |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylthran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylfuran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Amylthran               |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

| 2-Pentylthran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |

<p>| 2-Pentylfuran             |                   | Soluble in propylene glycol and most fixed oils. Insoluble in glycerol.    |
|                          |                   | Miscible with alcohol.           |
|                          |                   | IR 1.45, 1.41                     |
|                          |                   | Optical rotation: +50° to +60°   |</p>
<table>
<thead>
<tr>
<th>1497</th>
<th>3-(2-Furyl)acrolein</th>
<th>2494</th>
<th>C\textsubscript{7}H\textsubscript{6}O\textsubscript{2}</th>
<th>Insoluble in water</th>
<th>NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Full</strong></td>
<td>3-(2-Furyl)prop-2-enal</td>
<td>13.034</td>
<td>122.12</td>
<td>Soluble</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td>Furylacrolein;3-(2-Furyl)acrylaldehyde;3-(2-Furyl)-2-propenal;2-Furanacrolein</td>
<td>-</td>
<td>White or yellow needles; Cooked spicy-herb aroma</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>623-30-3</td>
<td>42-54°</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1502</th>
<th>2-Phenyl-3-(2-furyl)prop-2-enal</th>
<th>3586</th>
<th>C\textsubscript{13}H\textsubscript{10}O\textsubscript{2}</th>
<th>Insoluble in water</th>
<th>NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Full</strong></td>
<td>3-(2-Furyl)-2-phenylprop-2-enal</td>
<td>13.137</td>
<td>198.22</td>
<td>Soluble</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>2-Furfurylidene phenylacetaldehyde</td>
<td>-</td>
<td>Orange crystalline solid; warm spicy aroma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>65545-81-5</td>
<td>56-57°</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1504</th>
<th>2-Acetyl-5-methylfuran</th>
<th>3609</th>
<th>C\textsubscript{7}H\textsubscript{6}O\textsubscript{2}</th>
<th>Slightly soluble in water; Soluble in corn oil</th>
<th>HNMR IR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Full</strong></td>
<td>2-Acetyl-5-methylfuran</td>
<td>13.083</td>
<td>124.14</td>
<td>Soluble</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>Methyl 5-methyl-2-furyl ketone;1-(5-Methyl-2-furyl) ethanone</td>
<td>-</td>
<td>Clear to almost yellow orange liquid; strong, nutty, hay-coumarin odor</td>
<td>1.511-1.517</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>1193-79-9</td>
<td>71-72° (8 mm Hg)</td>
<td></td>
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</tbody>
</table>

**Notes:**
- Full: Full name of the compound.
- IR: Infrared spectroscopy.
- HNMR: NMR spectroscopy.
<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS Number</th>
<th>Physical Properties</th>
<th>NMR Data</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-2,5-dimethylfuran</td>
<td>83-70-4</td>
<td>Colorless to yellow liquid; sweet nutty hazelnut aroma with earthy undertones</td>
<td>Full</td>
<td>Insoluble in water; slightly soluble in mixed oils, propylene glycol, water</td>
</tr>
<tr>
<td>Furfurylidene acetone</td>
<td>4-(2-Furyl)-3-buten-2-one</td>
<td>Amber to brown crystals; spicy warm cinnamon aroma</td>
<td>I 1.49</td>
<td>Soluble</td>
</tr>
<tr>
<td>4-(2-Furyl)-3-buten-2-one</td>
<td>1511</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,5-Dimethyl-3-acetylfuran</td>
<td>89-62-3</td>
<td>Amber to brown crystals; spicy warm cinnamon aroma</td>
<td></td>
<td>Insoluble in water; slightly soluble in mixed oils, propylene glycol, water</td>
</tr>
<tr>
<td>3-Acetyl-2,5-dimethylfuran</td>
<td>1506</td>
<td>Amber to brown crystals; spicy warm cinnamon aroma</td>
<td></td>
<td>Slightly soluble in most mixed oils, propylene glycol, water; soluble in mixed oils</td>
</tr>
<tr>
<td>1513</td>
<td><strong>Ethyl 3-(2-furyl)propanoate</strong></td>
<td>2435</td>
<td>C_{9}H_{12}O_{3}</td>
<td>Very slightly soluble in water</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Full</td>
<td>Ethyl 3-(2-furyl)propionate</td>
<td>13.022</td>
<td>168.19</td>
<td>Soluble</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low melting solid, turning yellow on exposure to air; Fruity aroma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethyl furfurylacetae;Ethyl furylpropionate;Ethyl 2-furanpropionate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yellow liquid; fruity pineapple aroma</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1517</th>
<th><strong>Phenethyl 2-furoate</strong></th>
<th>2865</th>
<th>C_{13}H_{12}O_{3}</th>
<th>Insoluble in water</th>
<th>NMR 1.540-1.550</th>
<th>1.138-1.150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>Phenethyl 2-furoate</td>
<td>13.006</td>
<td>216.23</td>
<td>Soluble</td>
<td>96%</td>
<td>1.138-1.150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Colourless to yellow orange liquid; honey rose aroma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-Phenylethyl 2-furoate; Phenylethyl 2-furoate</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>275°</td>
<td>5</td>
</tr>
<tr>
<td>89</td>
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<td></td>
<td></td>
<td></td>
<td>7149-32-8</td>
<td></td>
</tr>
</tbody>
</table>
## CORRIGENDA

The following requests for corrections, reported to the Joint JECFA Secretariat, were evaluated by the 89th 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>Revised text</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium disodium ethylenediaminetetraacetate (INS 385)</td>
<td>CAS No. 662-33-9</td>
<td>CAS No. 62-33-9 (anhydrous) 6766-87-6 (dihydrate) 23411-34-9 (hydrated)</td>
<td>Correction to CAS number (for the anhydrous form)</td>
</tr>
<tr>
<td></td>
<td>Chemical formula ( \text{C}<em>{10}\text{H}</em>{12}\text{CaN}<em>{2}\text{Na}</em>{2}\text{O}<em>{8}\cdot 2\text{H}</em>{2}\text{O} )</td>
<td>Chemical formula ( \text{C}<em>{10}\text{H}</em>{12}\text{CaN}<em>{2}\text{Na}</em>{2}\text{O}<em>{8} ) (anhydrous) ( \text{C}</em>{10}\text{H}<em>{12}\text{CaN}</em>{2}\text{Na}<em>{2}\text{O}</em>{8} \cdot \text{H}<em>{2}\text{O} ) (monohydrate) ( \text{C}</em>{10}\text{H}<em>{12}\text{CaN}</em>{2}\text{Na}<em>{2}\text{O}</em>{8} \cdot 2\text{H}_{2}\text{O} ) (dihydrate)</td>
<td>CAS No. for hydrated forms; chemical formula and formula weight for anhydrous and monohydrate also included</td>
</tr>
<tr>
<td></td>
<td>Formula weight 410.31</td>
<td>Formula weight 374.37 (anhydrous) 392.31 (monohydrate) 410.31 (dihydrate)</td>
<td></td>
</tr>
<tr>
<td>Pentasodium triphosphate (INS 451(i))</td>
<td>Dowex F x 8</td>
<td>Dowex 1 x 8</td>
<td>Correction to the resin in the procedure of method of assay</td>
</tr>
<tr>
<td>Talc (INS 553(iii))</td>
<td>A range of length:width ratios of 20:1 to 100:1 or higher for fibres longer than 5 m</td>
<td>A range of length:width ratios of 20:1 to 100:1 or higher for fibres longer than 5 µm</td>
<td>Length of fibre corrected</td>
</tr>
<tr>
<td>Annatto extracts (norbixin-based) (INS 160b(ii))</td>
<td>CAS numbers ( \text{cis-Norbixin: 542-40-5} ) ( \text{cis-Norbixin dipotassium salt: 33261-80-2} ) ( \text{cis-Norbixin disodium salt: 33261-81-3} )</td>
<td>CAS numbers ( \text{cis-Norbixin: 626-76-6} ) ( \text{cis-Norbixin dipotassium salt} ) ( \text{cis-Norbixin disodium salt:} )</td>
<td>Correction to the CAS number of ( \text{cis-norbixin} ) and deletion of the incorrect CAS numbers for the dipotassium and disodium salts</td>
</tr>
<tr>
<td></td>
<td>For: 1. Alkali processed norbixin, acid precipitated 2. Alkali processed norbixin, not acid precipitated 3. Solvent extracted norbixin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ANNEX 1: SUMMARY OF RECOMMENDATIONS FROM THE 89th JECFA

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held on a virtual online platform, on 1–12 June 2020. The purpose of the meeting was to evaluate the safety of certain food additives and flavourings. The present meeting was the 89th in a series of similar meetings. The tasks before the Committee were (a) to further elaborate principles governing the evaluation of food additives, (b) to undertake safety evaluations of certain food additives, (c) to review and prepare specifications for certain food additives and (d) to establish specifications for certain flavouring agents.

The 89th meeting of JECFA was originally scheduled for 2–11 June 2020 at WHO headquarters in Geneva, Switzerland. Because of the travel restrictions and lock-downs due to the COVID-19 pandemic in many countries, the joint FAO/WHO JECFA secretariat was unable to convene the meeting as scheduled. Therefore, the meeting was held as a video-conference.

In view of the countries of origin of the invited experts, the only possible time for a video-conference was restricted to a 4-h time slot (12:00–16:00 CEST) a day. This allowed approximately 40% of the usual daily time (8–10 h) of a JECFA 8-day face-to-face meeting.

As under the circumstances less meeting time had been available, compared to an normal JECFA meeting, the food additives natamycin (INS 234), natamycin (INS 235), β-glucanase from *Streptomyces violaceoruber* expressed in *S. violaceoruber*, collagenase from *S. violaceoruber* expressed in *S. violaceoruber*, phosphodiesterase from *Penicillium citrinum* and phospholipase A2 from *S. violaceoruber* expressed in *S. violaceoruber*, which were originally scheduled for discussion, had therefore not been considered. Furthermore, it became quickly apparent early in the meeting that the experts of the 89th JECFA would not have been able to complete the evaluations for alicyclic ketones, secondary alcohols and related esters and a toxicological evaluation of riboflavin from *Ashbya gossypii*. Therefore, these two evaluations have also been deleted from the meeting agenda. All compounds that had been deleted from the agenda of the 89th JECFA will be re-scheduled for evaluation at future JECFA meetings. More details can be found in Annex 4.

Dr Antonia Mattia served as Chairperson and Professor Cantrill as Vice-Chairperson.

Mr Kim Petersen, World Health Organization (WHO), and Dr Markus Lipp, Food and Agriculture Organization of the United Nations (FAO), served as joint secretaries.

The Committee evaluated the safety of six food additives, conducted an exposure assessment for one group of food additives and revised the specifications for three other food additives (including one group). The Committee also evaluated the safety of two groups of flavouring agents and revised the specifications for 12 flavouring agents. Tentative specifications were prepared for three, as the safety evaluations were not completed.

The report of the meeting will be published in the WHO Technical Report Series. The report will summarize the main conclusions of the Committee in terms of acceptable daily intakes and other toxicological, dietary exposure and safety recommendations. Information on deliberations and conclusion with regard to the
specifications for the identity and purity of certain food additives examined by the Committee and on the specifications for the flavouring agents will also be included.

The participants are listed in Annex 1. Information of a general nature that the Committee wishes to disseminate quickly is provided in Annex 2. Future work and recommendations arising from the meeting are summarized in Annex 3. Annex 4 details the selection of compounds and observations by experts with regard to the feasibility of holding these expert meetings online rather than in-person.

Toxicological monographs summarizing the data that were considered by the Committee in establishing ADIs will be published in WHO Food Additives Series No. 80. Monographs summarizing the data that were considered by the Committee in recommending MRLs will be published in FAO JECFA Monographs No. 25.

More information on the work of JECFA is available at:


and

https://www.who.int/foodsafety/en/

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Please note that the annexes referred to in this document are to be found in the original summary of the 89th meeting and are not those in this volume of the FAO JECFA Monographs series.
Toxicological and dietary exposure information and information on specifications

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 conclusions on toxicology and dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine 5´-monophosphate deaminase from <em>Streptomyces murinus</em></td>
<td>N</td>
<td>Negative results were observed in genotoxicity tests, and a NOAEL of 500 mg/kg bw per day (equal to 69 mg TOS/kg bw per day) was identified in a 13-week oral toxicity study. Comparison of the dietary exposure estimate of 0.075 mg TOS/kg bw per day with the NOAEL of 69 mg TOS/kg bw per day gives a margin of exposure (MOE) of 920. The Committee concluded that the AMP deaminase enzyme preparation from <em>S. murinus</em> would not pose a health concern when used in the applications specified, at the levels specified and in accordance with good manufacturing practice.</td>
</tr>
<tr>
<td>D-Allulose 3-epimerase from <em>Arthrobacter globiformis</em> expressed in <em>Escherichia coli</em></td>
<td>N</td>
<td>Negative results were observed with D-allulose in genotoxicity tests. A NOAEL of 1100 mg TOS/kg bw per day was identified, the highest dose tested in a short-term (90-day) oral toxicity study in rats. When the dietary exposure estimate for the highest consumers (90th percentile for infants and children) of 0.38 mg TOS/kg bw per day was compared with the NOAEL of 1100 mg TOS/kg bw per day, an MOE of nearly 3000 was calculated. The Committee established an ADI “not specified” for D-allulose 3-epimerase from <em>A. globiformis</em> M30 expressed in <em>E. coli</em> K-12 W3110 when the enzyme is used in the applications specified, at the levels specified and in accordance with good manufacturing practice.</td>
</tr>
<tr>
<td>Carbohydrate-derived fulvic acid (CHD-FA)</td>
<td>No&lt;sup&gt;a&lt;/sup&gt;</td>
<td>The Committee concluded that the available data are inadequate for evaluating the safety of CHD-FA. The Committee assessed the chemical and technical information received and concluded that there was insufficient information to prepare specifications for CHD-FA.</td>
</tr>
<tr>
<td>Jagua (genipin-glycine) blue (Jagua blue)</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;</td>
<td>The Committee considered that the new toxicological data and additional characterization of the test compound provided adequate information to complete the safety evaluation of Jagua blue. The new 12-month study of rats exposed in utero was conducted for a longer exposure time and at higher doses of Jagua blue, as recommended by the Committee at its 84th meeting. Although no new toxicokinetics study was available, newly developed analytical methods for the dimers provided acceptable characterization of the test article, thus reducing the uncertainty of the safety assessment due to limited biochemical information. An ADI of 0–11 mg/kg bw was established by the Committee for Jagua blue, on a blue-polymer basis. This ADI was based on the absence of treatment-related...</td>
</tr>
</tbody>
</table>
long-term toxicity and of reproductive and developmental toxicity in the 12-month rat dietary study with in-utero exposure, in which the NOAEL was identified as 1127 mg/kg bw per day of the blue polymer, the highest dose tested. The ADI was established by applying an uncertainty factor of 100 to the NOAEL.

The Committee noted that the upper end of the high-level dietary exposure estimate for Jagua blue, on a blue-polymer basis, for infants and toddlers of 11.5 mg/kg bw per day is in the region of the upper bound of the ADI. In view of the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained Jagua blue on a blue-polymer basis at the maximum use level, and because the ADI was based on a NOAEL that was the highest dose tested, the Committee concluded that the estimated dietary exposure to Jagua blue, on a blue-polymer basis, does not represent a health concern.

Lipase from *Mucor javanicus*  N

Negative results were obtained in genotoxicity tests, and no treatment-related adverse effects were seen at the highest dose tested (800 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the estimated dietary exposure of 0.84 mg TOS/kg bw per day with the highest dose tested of 800 mg TOS/kg bw per day gives an MOE of at least 900.

The Committee established an ADI “not specified” for the lipase enzyme preparation from *M. javanicus*, used in the applications specified and in accordance with good manufacturing practice.

Phosphatidylinositol-specific phospholipase C expressed in *Pseudomonas fluorescens* (PI-PLC)  N

Negative results were obtained in genotoxicity tests, and no treatment-related adverse effects were seen with PI-PLC enzyme concentrate at the highest dose tested (1871 mg TOS/kg bw per day) in the 13-week study of oral toxicity in rats. A comparison of the highest estimated dietary exposure of 0.01 mg TOS/kg bw per day with the highest dose tested of 1871 mg TOS/kg bw per day gives an MOE of at least 187 100.

The Committee established an ADI “not specified” for the PI-PLC enzyme preparation expressed in *P. fluorescens*, used in the applications specified and in accordance with good manufacturing practice.

Riboflavin from *Ashbya gossypii*  No<sup>c</sup>

Because of time constraints, the assessments of safety and dietary exposure were not completed.

N: new specifications; R: existing specifications revised

<sup>a</sup> No specifications were prepared. Information is required to prepare specifications (see Annex 3).

<sup>b</sup> The specifications were revised and the tentative status removed.

<sup>c</sup> As the evaluation was postponed, specifications will be published later (see Annex 3).

**Food additives assessed only for dietary exposure**

<table>
<thead>
<tr>
<th>Food additives</th>
<th>Conclusions on dietary exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose esters of fatty acids (INS 473) (SEFs) and sucrose oligoesters type I and type II (INS 473a) (SOEs)</td>
<td>At its 49th meeting, the Committee established a group ADI of 0–30 mg/kg bw for SEFs and sucroglycerides on the basis of their potential to induce laxative effects in adult volunteers at doses &gt; 30 mg/kg bw per day, without</td>
</tr>
</tbody>
</table>
applying an uncertainty factor. At its 71st meeting, the Committee noted that some of the components of SEFs may be present in significant amounts in SOEs and established a group ADI of 0–30 mg/kg bw for SEFs, SOEs and sucroglycerides.

The high dietary exposure estimate of the sum of SEFs and SOEs of 113 mg/kg bw per day for children aged 3–9 years exceeds the group ADI of 0–30 mg/kg bw per day by a factor of about 4. The Committee also noted that the dietary exposure estimates for some other age groups also exceeded the ADI.

The Committee noted that the high dietary exposure estimates are conservative, predominantly due to the assumptions that

- all foods that could contain SOEs and SEFs do in fact contain these food additives, whereas other food additives with the same functions in foods are available; and
- when SEFs or SOEs are used, they are always present at the reported use levels.

Therefore, the Committee considered that more refined dietary exposure estimates should be provided.

### Food additives considered for specifications only

<table>
<thead>
<tr>
<th>Food additive</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium stearate (INS 470(iii))</td>
<td>R&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Polyvinyl alcohol (INS 1203)</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sorbitan esters of fatty acids (INS 491, INS 492, INS 495)</td>
<td>No&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> For the assay of magnesium, the reference to the ICP-AES method was replaced by a general term, to read “Use a method appropriate to the specified level”.

<sup>b</sup> The solubility criterion was changed to “practically insoluble or insoluble in ethanol”. For additional remarks, see Annex 3.

<sup>c</sup> No specifications were prepared. Information is required to prepare specifications (see Annex 3).
Flavouring agents evaluated by the revised Procedure for the Safety Evaluation of Flavouring Agents

A. Amino acids and 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>Structural class I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betaine</td>
<td>2265</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>N-Acetyl-glutamate</td>
<td>2269</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>L-Cysteine methyl ester hydrochloride</td>
<td>2270</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Glutamyl-2-aminobutyric acid</td>
<td>2266</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Glutamyl-norvaline</td>
<td>2268</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>Glutamyl-norvalyl-glycine</td>
<td>2267</td>
<td>N</td>
<td>No safety concern</td>
</tr>
</tbody>
</table>

B. Phenol and phenol derivatives

<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>(±)-Homoeriodictyol sodium salt</td>
<td>2256</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>(±)-Naringenin</td>
<td>2257</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>(2R)-3’,5-Dihydroxy-4´-methoxyflavanone</td>
<td>2258</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>7,8-Dihydroxyflavone</td>
<td>2259</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>(2S)-3’,7-Dihydroxy-8-methyl-4´-methoxyflavan</td>
<td>2260</td>
<td>N</td>
<td>Genotoxicity data for this agent raise concern about potential genotoxicity</td>
</tr>
<tr>
<td>(R)-5-Hydroxy-4-(4´-hydroxy-3´-methoxyphenyl)-7-methylchroman-2-one</td>
<td>2261</td>
<td>N</td>
<td>No safety concern</td>
</tr>
<tr>
<td>3-(3-Hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one</td>
<td>2262</td>
<td>N</td>
<td>No safety concern</td>
</tr>
</tbody>
</table>

Flavouring agents considered for specifications only

<table>
<thead>
<tr>
<th>Flavouring agent</th>
<th>No.</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid</td>
<td>2002</td>
<td>R^a</td>
</tr>
<tr>
<td>ß-Caryophyllene oxide</td>
<td>1575</td>
<td>R^b</td>
</tr>
<tr>
<td>2-Acetyl-1-pyrroline</td>
<td>1604</td>
<td>R^c</td>
</tr>
<tr>
<td>(2E,6/E/8E)-N-(2-Methylpropyl)-2,6,8-decatrienamide</td>
<td>2077</td>
<td>R^d</td>
</tr>
<tr>
<td>4-Hexen-3-one</td>
<td>1125</td>
<td>R^e</td>
</tr>
<tr>
<td>d-Carvone</td>
<td>380.1</td>
<td>R^f</td>
</tr>
</tbody>
</table>
Flavouring agent | No. | Specifications |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Pentylfuran</td>
<td>1491</td>
<td>R^g</td>
</tr>
<tr>
<td>3-((2-Furyl)acrolein</td>
<td>1497</td>
<td>R^h</td>
</tr>
<tr>
<td>2-Phenyl-3-(2-furyl)prop-2-enal</td>
<td>1502</td>
<td>R^i</td>
</tr>
<tr>
<td>2-Acetyl-5-methylfuran</td>
<td>1504</td>
<td>R^j</td>
</tr>
<tr>
<td>3-Acetyl-2,5-dimethylfuran</td>
<td>1506</td>
<td>R^k</td>
</tr>
<tr>
<td>4-(2-Furyl)-3-buten-2-one</td>
<td>1511</td>
<td>R^l</td>
</tr>
<tr>
<td>Ethyl 3-(2-furyl) propanoate</td>
<td>1513</td>
<td>R^m</td>
</tr>
<tr>
<td>Phenethyl 2-furoate</td>
<td>1517</td>
<td>R^n</td>
</tr>
</tbody>
</table>

R: revised

a The specific gravity was revised to 0.950–1.000 at 20 °C, and the assay minimum was maintained at 93%, with a change of the secondary component from 1–2% 3,4-dimethyl 5-ketobutanoic acid \(\gamma\)-lactone to 2–3% 3,4-dimethylfuran-2,5-dione.

b The melting-point was revised to 55–63 °C and the assay minimum to 95% (sum of isomers). Specifications for the isomeric composition were also established: 84–89% (1R,4R,6R,10S) (CAS No. 1139-30-6), 7–9% (1R,4S,6S,10S) (CAS No. 60594-22-1), 0.3–2% (1R,4S,6S,10S) (CAS No. 103475-43-0) and 1–2% humulene-1,2-epoxide.

c The assay minimum was revised to 90%, with a secondary component of ≤ 5–6% 5,6-dihydro-2-methyl-3-(4H)-pyridinone.

d The isomeric composition was updated to be 73–80% (2E,6Z,8E), 15–18% (2E,6E,8E), 3–7% (2E,6Z,8Z), 1–2% (2Z,6Z,8E) and 1–2% (2Z,6E,8E).

e The assay minimum was set to 95% (sum of isomers), and the specifications for the isomeric composition were established as: 90–95% \(trans\)-4-hexen-3-one and 1–5% cis-4-hexene-3-one.

f The refractive index was revised to 1.496–1.502 and the specific gravity to 0.956–0.961.

g The refractive index was revised to 1.445–1.451 and the assay minimum to 95%.

h The refractive index was revised to 1.445–1.451 and the assay minimum to 95%.

i The melting point was revised to 42–54 °C.

j The specific gravity was revised to 1.065–1.074 and the assay minimum to 95%; the physical form and odour were also revised.

k The specific gravity was revised to 1.034–1.048, and the physical form and odour were also revised.

l The melting-point was revised to 28–40 °C, and the physical form and odour were also revised.

m The physical form and odour were revised, and specifications for the refractive index and the specific gravity were established as 1.455–1.462 and 1.051–1.058, respectively.

n The refractive index was revised to 1.540–1.550 and the specific gravity to 1.138–1.150; the physical form and odour were updated.

Secondary components of flavouring agents with revised specifications with minimum assay values of less than 95%

<table>
<thead>
<tr>
<th>JECFA No.</th>
<th>Flavouring agent</th>
<th>Minimum assay value</th>
<th>Secondary components</th>
<th>Comments on secondary components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic lactones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>4-Hydroxy-2,3-dimethyl-2,4-nonadienoic acid (\gamma)-lactone</td>
<td>93%</td>
<td>3,4-Dimethylfuran-2,5-dione (2–3%)</td>
<td>The SPET value for No. 2002 is 62.5 µg/day, and 3% of this value is 2 µg/day, which is below the class III threshold of toxicological concern.</td>
</tr>
<tr>
<td>Aliphatic and aromatic amines and amides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1604</td>
<td>2-Acetyl-1-pyrroline</td>
<td>&gt; 90%</td>
<td>5,6-Dihydro-2-methyl-3-(4H)-pyridinone (5–6%)</td>
<td>The SPET value for No. 1604 is 160 µg/day, and 6% of this value is 10 µg/day, which is below the class III threshold.</td>
</tr>
</tbody>
</table>
of toxicological concern.

<table>
<thead>
<tr>
<th>JECFA No.</th>
<th>Flavouring agent</th>
<th>Minimum assay value</th>
<th>Secondary components</th>
<th>Comments on secondary components</th>
</tr>
</thead>
<tbody>
<tr>
<td>2256</td>
<td>(±)-Homoeriodictyol, sodium salt</td>
<td>&gt; 90%</td>
<td>Eriodictyol-7-methyl ether (3–5%); Homoeriodictyol-7-methyl ether (1–2%)</td>
<td>Structurally related (±)-eriodictyol (No. 2172) has been evaluated by the Committee and found to be of no safety concern at estimated dietary exposure when used as a flavouring agent.</td>
</tr>
</tbody>
</table>
ANNEX 2. GENERAL CONSIDERATIONS


Update of guidance on dose–response assessment and derivation of health-based guidance values (revision of Environmental Health Criteria (EHC) 240, Chapter 5)

Since the last update to the Committee in June 2019 (WHO Technical Report Series (TRS) No. 1020, 2019), revision of Chapter 5 of EHC 240, on dose–response assessment and derivation of health-based guidance values, has continued, and a draft of the chapter was sent for public consultation in December 2019. In response, the Secretariat received about 300 comments from 14 organizations or individuals, indicating a high level of interest. The comments included many helpful suggestions for further revision and clarification of the text. Most of the comments have now been considered and addressed, and the work will be completed soon. After editing, the text will be published online as an updated chapter of EHC 240.

Update of guidance on evaluation of enzyme preparations (revision of EHC 240, Chapter 9.1.4.2)

The Committee was given an update on progress made in revising guidance on the evaluation of enzymes for use in food. An expert working group was established in 2018 to discuss the available information on the safety of enzymes used in food and current practices of the food enzyme industry. Several documents and definitions were amended and submitted for public comment late in 2019. The comments received were evaluated, and the text of a revised version of Chapter 9.1.4.2 of EHC 240 was edited further as necessary.

The working group made a series of recommendations to this Committee, which came to the following consensus.

1a. The Committee adopted the proposed definitions of “safe food enzyme production strain” and “presumed safe progeny strain” (Annex 2) with minor editorial changes.

1b. The Committee adopted the proposed revisions to Chapter 9.1.4.2 of EHC 240 pertaining to enzymes, including a revision of the classification of enzymes and their definitions. The text for Class I Type iii and Class II enzymes was modified to state that “an ADI may be established.”

1c. The Committee approved the proposed checklist of data requirements for the risk assessment of enzyme preparations in submissions for review by JECFA, with a change to one of the test requirements. The Committee debated the value of including on the checklist a request for information on “Bioinformatics analysis of the amino acid sequence for potential matches with known toxins” (checklist item #29). The Committee decided that it should remain on the checklist, and the usefulness of such information should be evaluated once sufficient experience has been gained.

1d. The Committee adopted the proposed list of terms and definitions related to submissions on enzyme preparations for use in food and added a definition of “total organic solids”.

2. The Committee recommended that allergenicity should be assessed only for enzyme preparations proposed for inclusion in Class I Type iii or Class II.

3. The Committee debated whether it would be appropriate to combine consideration of immobilized enzyme preparations that are in contact with foods only during processing with consideration of enzyme preparations added to foods but removed from the final products. Differing points of view were expressed, and the Committee was reminded that such
consideration did not apply to other situations in which food-grade carriers and formulation ingredients are used. Furthermore, the Committee considered that the levels of residues of immobilizing agents in the final product would be extremely low; the levels of these substances or their contaminants permitted in the final product should be at the lowest levels that are technologically feasible. The Committee decided that the wider issue of food contact materials was not one of their current terms of reference, and their consideration would have to be initiated by the Codex Alimentarius Commission or others before it could be taken up.

4. The Committee supported establishment of a separate online database for toxicological data and specifications for enzyme preparations for use in food evaluated by JECFA in order to simplify presentation of the data to users (similar to that currently used for flavourings).

5. The Committee supported establishment of a separate JECFA numbering system for identifying enzyme preparations for which JECFA had completed safety evaluations (similar to that used for flavourings).

6. The Committee supported development of an enzyme-specific template for the submission of information on analytical methods, including method performance characteristics (method validation data) and quality control data.

**Update of guidance on evaluation of the genotoxicity of chemical substances in food**

Since the last update provided to the Committee, in June 2019 (TRS 1020), on revision of Chapter 4.5 of EHC 240, guidance on evaluating the genotoxicity of chemical substances in food, a draft of the chapter was sent for public consultation in December 2019. In response, the Secretariat received about 300 comments from 14 organizations or individuals, indicating a high level of interest. The comments included many helpful suggestions for further revision and clarification of the text. Most of the comments have now been considered and addressed, and the work will be completed soon. After editing, the text will be published online as an updated chapter of EHC 240.

**Withdrawal of the ADI for lipase from Aspergillus oryzae, var.**

In evaluating lipase from Mucor javanicus, the Committee noted that the specifications for lipase from Aspergillus oryzae, var. had been withdrawn by the Committee at its 55th meeting, but that it had not addressed the consequences of the withdrawal of specifications on its acceptable daily intake (ADI). The Committee at its current meeting decided to withdraw the ADI "not specified" for lipase from Aspergillus oryzae, var.

The Committee also noted that specifications for other food additives had been withdrawn at the 55th meeting without addressing the consequences for the respective ADIs. The Committee recommends reconsideration of the ADIs concerned at a future meeting.
ANNEX 3. FUTURE WORK AND RECOMMENDATIONS

Carbohydrate-derived fulvic acid
The Committee requires data to characterize the products of commerce in order to evaluate the product for use as a preservative. The required information includes a detailed description of the manufacturing processes and thorough chemical characterization of the commercial products.

The following information is required:

- the full composition of the products;
- a detailed description of the manufacturing process;
- analytical methods and data on method validation; and
- analytical data for five non-consecutive batches of commercial products, including information on impurities.

The sponsor is encouraged to offer a rationale for whether a single monograph covering all products or individual monographs should be prepared.

Given the deficiencies of the toxicological database, the Committee recommends that the following studies be conducted. The test protocols should be in accordance with the relevant current guidelines, and the test materials should be well characterized in relation to the article(s) of commerce:

- absorption, distribution, metabolism and excretion;
- repeated-dose 90-day oral toxicity in rodents;
- two-generation reproductive toxicity or extended one-generation reproductive toxicity;
- prenatal developmental toxicity;
- additional studies, including an in vitro micronucleus test in mammalian cells, might be required, depending on elucidation of the article(s) of commerce and the provision of full information on their composition; and
- information on the potential of the material to induce antimicrobial resistance.

In addition, use levels should be provided for estimating dietary exposure.

Withdrawal of the ADI for lipase from Aspergillus oryzae, var.
The Committee also noted that specifications for other food additives had been withdrawn at the 55th meeting without addressing the consequences for the respective ADIs. The Committee recommends reconsideration of the ADIs concerned at a future meeting.

Riboflavin from A. gossypii
The Committee drafted a chemical and technical assessment and new specifications for riboflavin from A. gossypii from the data submitted by the sponsor, but did not finalize them for publication. The Committee recognized the benefits of simultaneous review and harmonization of new specifications with existing specifications for riboflavin as a synthetic product and as a product of B. subtilis and recommended that this work be undertaken at a future meeting.

Sucrose esters of fatty acids (INS 473) and sucrose oligoesters types I and II (INS 473a)
To refine the dietary exposure estimates of SEFs and SOEs, either alone or summed, the Committee recommends that sponsors submit information on:

- typical or mean and high use levels for foods in which the food additives are used; and
- foods (or food categories) in which the use of SEFs and/or SOEs is permitted but in which they are never used.
In both cases, the information should be as specific as possible, and the foods should be classified according to the FoodEx2 classification system, which is that used for the CIFOCOss and GIFT food consumption databases, or another appropriate system.

The Committee did not use the CIFOCOss and GIFT databases to assess dietary exposure to SEFs and SOEs, partly because calculations of exposure would have been laborious in view of the number of broad food categories for which use levels were provided. In order to use these data for dietary exposure assessment of food additives that are present in large numbers of food categories, a table should be developed to map the foods recorded in both databases according to the FoodEx2 classification to the food categories of the GSFA. That will also ensure that mapping is consistent for all meetings.

The Committee recommends that more detailed information on the use of SEFs and SOEs in foods and a mapping table be made available within 2 years.

**Polyvinyl alcohol**

The Committee recommended that the CCFA determine whether the food-grade PVOH products currently available in commerce comply with the narrow range of viscosity (4.8–5.8 mPa × s) and degree of hydrolysis (86.5–89%) in the specifications. Any deviations would necessitate a review of its safety evaluation.

The Committee also noted that the gas chromatographic method for determining methanol and methyl acetate in PVOH is a packed-column method and recommended that it be replaced by a suitable capillary or wide-bore column gas chromatographic method.

**Sorbitan esters of fatty acids (INS 491, INS 492 and INS 45)**

The Committee recommends that a new call for data be issued in order to proceed with an updated safety evaluation and specifications for the five sorbitan esters of fatty acids at the same time.

The Committee also noted that five polyoxyethylene sorbitan esters (polysorbates) were evaluated by JECFA at its 17th meeting Annex 1, reference 32), and specifications were established. The Committee recommends that a new call for data be issued for their full evaluation.
This document contains food additive specification monographs, analytical methods, and other information prepared at the eighty-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held virtually from 1 to 12 June 2020. 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.