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19

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

82nd Meeting 2016

Food and Agriculture Organization of the United Nations

World Health Organization

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
WORLD HEALTH ORGANIZATION
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Geneva, 7 – 16 June, 2016

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 82nd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome on 7 - 16 June 2016. The specifications monographs are one of the outputs of JECFA's risk assessment of food additives, and should be read in conjunction with the safety evaluation, reference to which is made in the section at the head of each specifications monograph. Further information on the meeting discussions can be found in the summary report of the meeting (see Annex 1), and in the full report which will be published in the WHO Technical Report series. Toxicological monographs of the substances considered at the meeting will be published in the WHO Food Additive Series.

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

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

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

Contact and Feedback

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

jecfa@fao.org

SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

New (N) or revised (R) specifications monographs were prepared for 30 food additives (including 16 modified starches) and these are presented in this publication.

Allura Red AC (R)
Aspartame (R)
Cassia gum (R,T)
Carob bean gum (R)
Citric and fatty acid esters of glycerol (R)
Lutein esters from Tagetes erecta (R)
Modified Starches:
- Acetylated distarch adipate (R,T)
- Acetylated distarch phosphate (R,T)
- Acetylated oxidized starch (R)
- Acid treated starch (R,T)
- Alkaline treated starch (R,T)
- Bleached starch (R,T)
- Dextrin roasted starch (R,T)
- Distarch phosphate (R,T)
- Enzyme-treated starch (R,T)
- Hydroxypropyl distarch phosphate (R,T)
- Hydroxypropyl starch (R,T)
- Monostarch phosphate (R,T)
- Oxidized starch (R)
- Phosphated distarch phosphate (R,T)
- Starch acetate (R)
- Starch sodium octenyl succinate (R,T)

Octanoic acid (R)
Octenyl succinic acid (OSA)–modified gum arabic (R)
Pectin (R)
Quinoline Yellow (R)
Rosemary extract (T)
Steviol glycosides (N, T)
Tartrazine (R)
Xanthan gum (R)

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

Modified Starches

The Committee at the seventy-ninth meeting recommended the separation of the combined specification for the modified starches into 16 separate specifications. Based on the limited information received, the Committee prepared full specifications for 3 modified starches and tentative specifications for other 13 which requires information for the removal of the tentative status.
The Committee also noted that all the modified starches may additionally be subjected to bleaching and therefore included the appropriate purity tests in the revised specifications

ALLURA RED AC

Prepared at the 82nd JECFA and published in JECFA Monograph 19 (2016) superseding specifications prepared at the 28th JECFA (1984) and published in FNP 31/1 (1984) and FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-7 mg/kg bw was established at the 25th JECFA (1981).

SYNONYMS INS No. 129, CI Food Red 17, CI (1975) No.16035, FD&C Red No. 40

DEFINITION Consists of disodium 6-hydroxy-5-(2-methoxy-5-methyl-4- sulfonato-phenylazo)-2-naphthalenesulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by coupling diazotized 4-amino-5-methoxy-2-methylbenzenesulfonic acid with 6-hydroxy-2-naphthalene sulphonic acid. The resulting dye is purified and isolated as the sodium salt.

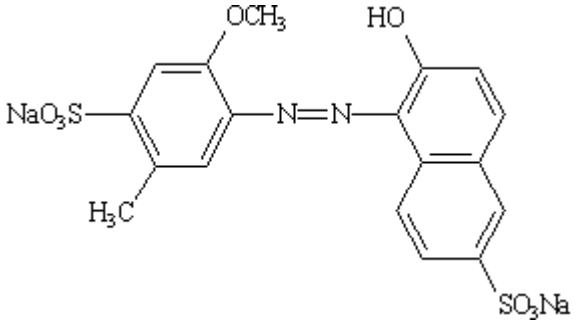
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* applies.

Chemical names Disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonatophenylazo)-2-naphthalenesulfonate

C.A.S. number 25956-17-6

Chemical formula $C_{18}H_{14}N_2Na_2O_8S_2$

Structural formula



Formula weight 496.43

Assay Not less than 85% total colouring matters

DESCRIPTION Dark red powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility Freely soluble in water, insoluble in ethanol

Spectrophotometry (Vol. 4)	Maximum wavelength approximately 501 nm. Determine the UV-visible absorption spectrum of the sample solution dissolved in water.
PURITY	
Loss on drying, chloride and sulfate as sodium salts (Vol. 4)	Not more than 15% as total amount Determine according to chloride as sodium chloride, sulfate as sodium sulfate, and water content (loss on drying at 135 °C) in Volume 4 (under “Specific Methods, Food Colours”).
Water insoluble matter (Vol. 4)	Not more than 0.2%
Subsidiary colouring matters	Not more than 3% See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.3% of sodium 6-hydroxy-2-naphthalenesulfonate Not more than 0.2% of 4-amino-5-methoxy-2-methylbenzenesulfonic acid Not more than 1.0% of disodium 6,6'-oxybis(2-naphthalenesulfonate) See description under TESTS
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline
Ether extractable matter (Vol. 4)	Not more than 0.2%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Subsidiary colouring matters

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

Column: C18 (250 mm x 4.6 mm i.d., 5 μm particle size)
Eluent A: 0.05 M ammonium acetate
Eluent B: methanol
Injection volume: 20 μl
Detector: UV-visible/PDA at 500 nm
Flow rate: 1 ml/min

Gradient:

Min	%A	%B
0	95	5
3	95	5
19	25	75
20	0	100
25	0	100

Standards:

Higher sulfonated subsidiary colors:
3-Hydroxy-4-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2,7-naphthalenedisulfonic acid, trisodium salt – Wako, Cat. No. 037-23311 or equivalent
7-Hydroxy-8-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-1,3-naphthalenedisulfonic acid, trisodium salt – Wako, Cat. No. 034-23321 or equivalent

Lower sulfonated subsidiary color:
4-[(2-Hydroxy-1-naphthalenyl)azo]-5-methoxy-2-methylbenzenesulfonic acid, sodium salt – Wako, Cat. No. 030-23301 or equivalent

Sample preparation: Dissolve 150 mg of sample in 100 ml of 0.05 M ammonium acetate.

Organic compounds other than colouring matters (Vol. 4)

Determine sodium 6-hydroxy-2-naphthalenesulfonate, 4-amino-5-methoxy-2-methylbenzenesulfonic acid, and disodium 6,6'-oxybis(2-naphthalenesulfonate) by reversed-phase HPLC (under "Specific Methods, Food Colours") using the following conditions:

Column: C18 (250 mm x 4.6 mm i.d., 5 μm particle size)
Eluent A: 0.05 M ammonium acetate
Eluent B: methanol
Injection volume: 20 μl
Detector: UV-visible/PDA at 235 and 254 nm
Flow rate: 1 ml/min

Gradient:

Min	%A	%B
0	95	5
3	95	5
19	25	75
20	0	100
25	0	100

Note: A general gradient for the separation of organic compounds other than colouring matters in food colours is given in Vol. 4. Analyst may use above gradient for the analytes in Allura Red AC.
Standards:
Sodium 6-hydroxy-2-naphthalenesulfonate – Wako, Cat. No. 010-25141 or equivalent
4-Amino-5-methoxy-2-methylbenzenesulfonic acid – Wako, Cat. No. 196-17301 or equivalent
Disodium 6,6'-oxybis(2-naphthalenesulfonate) – Santa Cruz Biotechnology, Cat. No. 210553 or equivalent

Sample preparation:
Dissolve 150 mg of sample in 100 ml of 0.05 M ammonium acetate.

METHOD OF ASSAY

Determine total colouring matters content by spectrophotometry using Procedure 1 in Volume 4 (under "Specific Methods, Food Colours") and an appropriate solvent.
Using water as the solvent: absorptivity (a) = 54.0 l/(g·cm) and wavelength of maximum absorbance = 501 nm.

ASPARTAME

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016) superseding specifications prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001)
An ADI of 0-40 mg/kg bw was established at the 25th JECFA (1981)

SYNONYMS Aspartyl phenylalanine methyl ester: APM; INS No. 951

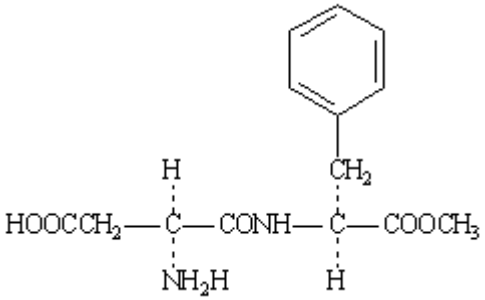
DEFINITION

Chemical names 3-Amino-N-(alpha-carbomethoxy-phenethyl)-succinamic acid, N-L-alpha-aspartyl-L-phenylalanine-1-methyl ester

C.A.S. number 22839-47-0

Chemical formula $C_{14}H_{18}N_2O_5$

Structural formula



Formula weight 294.31

Assay Not less than 98% and not more than 102% on the dried basis

DESCRIPTION White, odourless, crystalline powder

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Slightly soluble in water and practically insoluble or insoluble in ethanol

Test for amine group Dissolve 2 g of ninhydrin in 75 ml of dimethylsulfoxide, add 62 mg of hydrindantin, dilute to 100 ml with 4 M lithium acetate buffer solution (pH 9), and filter. Transfer about 10 mg of the sample to a test tube, add 2 ml of the reagent solution, and heat. A dark purple colour is formed.

Test for ester Dissolve about 20 mg in 1 ml of methanol, add 0.5 ml of methanol saturated with hydroxylamine hydrochloride, mix, then add 0.3 ml of 5 M potassium hydroxide in methanol. Heat the mixture to boiling, then cool, adjust the pH to between 1 and 1.5 with hydrochloric acid TS, and add 0.1 ml of ferric chloride TS. A burgundy colour is produced.

PURITY

Loss on drying (Vol. 4)	Not more than 4.5% (105°, 4 h)
pH (Vol. 4)	4.5 - 6.0 (1 in 125 soln)
Specific rotation (Vol. 4)	[alpha] 20, D: Between + 14.5 and + 16.5° (4% solution in 15 M formic acid; determine within 30 min after preparation of the sample solution)
Spectrophotometry (Vol. 4)	The transmittance of a 1 in 100, 2 M hydrochloric acid solution, determined in a 1-cm cell at 430 nm with a suitable spectrophotometer, using 2 M hydrochloric acid as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.
Sulfated ash (Vol. 4)	Not more than 0.2% Test 5 g of the sample (Method I)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”
5-Benzyl-3,6-dioxo-2-piperazineacetic acid (DKP)	Not more than 1.5% See description under TESTS
Other optical isomers	Not more than 0.02% (As sum of L-alpha-aspartyl-D-phenylalanine methyl ester (L,D-APM) and D-alpha-aspartyl-L-phenylalanine methyl ester (D,L-APM)) See description under TESTS

TESTS

PURITY TESTS

DKP

Apparatus

High performance liquid chromatograph equipped with a UV detector.

Reagents and solutions

*Mobile phase:*Dissolve 5.6 g of potassium dihydrogen phosphate in 820 ml of water before adjusting the pH to 4.3 with 10% phosphoric acid solution. Add 180 ml of methanol to 820 ml of this solution and mix well.

Standard Preparation

Standard stock solution: Dissolve 25 mg DKP Reference Standard (available from The United States Pharmacopeial Convention, Inc.) in 10 ml of methanol and dilute to 100 ml with water.

Standard solutions: Dilute the standard stock solution with 10% methanol to concentrations of 100, 75, 50, 25 and 5.0 µg/ml.

Sample Preparation

Accurately weigh 100 mg of the sample and dissolve in 10% methanol to make exactly 20 ml (5 mg/ml). This solution can be used for the purity test of other optical isomers.

Procedure

HPLC conditions:

Column: L-column2 ODS column (4.6 mm I.D. × 150 mm, particle size: 5 µm, Chemical Evaluation and Research

Institute, Japan) or equivalent.
Column temperature: 40°
Mobile phase: Mixture of phosphate buffer solution (0.05 mol/l, pH 4.3) and methanol (82:18 v/v)
Flow rate: 1.0 ml/min
Injection volume: 20 µl
Detector: UV at 210 nm
Run Time: 50 min

Inject the sample and read the concentration of the sample from the standard curve.

Calculation
Calculate the content (%) of DKP using the following formula:
Content (wt%) = (C x V x 0.1)/W
where
C is the concentration of DKP in the sample solution (µg/ml);
V is the volume of the sample solution (20 ml);
W is the weight of the sample (mg);

Other optical isomers

Apparatus

High performance liquid chromatograph equipped with a UV detector.

Reagents and solutions
Mobile phase:
Mobile phase A: (Mixture of 0.05 mol/l phosphate buffer solution and acetonitrile (87:13 v/v)): Dissolve 3.0 g of sodium dihydrogen phosphate and 3.55 g of disodium hydrogen phosphate in 1000 ml of water. Add 130 ml of acetonitrile to 870 ml of this solution and mix well.
Mobile phase B: (Mixture of 0.05 mol/l phosphate buffer solution and acetonitrile (80:20 v/v)): Dissolve 3.0 g of sodium dihydrogen phosphate and 3.55 g of disodium hydrogen phosphate in 1000 ml of water. Add 200 ml of acetonitrile to 800 ml of this solution and mix well.

Standard Preparation
Standard stock solution: Accurately weigh 20 mg of L,D-APM (Available from Wako Pure Chemical Industries, Ltd., Japan) and dissolve in 10% methanol to make exactly 50 ml (400 µg/ml). (L,D-APM alone is used as the standard since L,D-APM and D,L-APM (enantiomers) have the same retention time and molar absorbance coefficient)
Standard solutions: Dilute the standard stock solution with 10% methanol to concentrations of 10, 5.0, 2.0, 1.0 and 0.5 µg/ml.
Sample Preparation
Accurately weigh 100 mg of the sample and dissolve in 10% methanol to make exactly 20 ml (5 mg/ml). This solution can be used for the purity test of DKP.

Procedure
HPLC conditions:
Column: L-column2 ODS column (4.6 mm I.D. × 250 mm,
particle size: 5 µm, Chemical Evaluation and Research Institute, Japan) or equivalent.
Column temp.: 40°
Mobile phase:
Mobile phase A: Mixture of 0.05 mol/l phosphate buffer solution and acetonitrile (87:13 v/v)

Mobile phase B: Mixture of 0.05 mol/l phosphate buffer solution and acetonitrile (80:20 v/v)
Flow rate: 0.8 ml/min
Injection volume: 10 µl
Detector: UV at 220 nm
Run Time: 40 min

Gradient program:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	100	0
25.00	100	0
25.01	0	100
40.00	0	100

Inject the sample and read the concentration of the sample from the standard curve.

Calculation
Calculate the content (%) of L,D-APM using the following formula:
Content (wt%) = (C x V x 0.1)/W
where
C is the concentration of L,D-APM in the sample solution (µg/ml);
V is the volume of the sample solution (20 ml);
W is the weight of the sample (mg);

METHOD OF ASSAY Weigh accurately about 150 mg of the sample, previously dried at 105° for 4 h dissolve in 35 ml of dimethylformamide, add 5 drops of thymol blue TS, and titrate with a microburette to a dark blue end-point with 0.1 M lithium methoxide. Perform a blank determination and make any necessary correction. Each ml of 0.1 M lithium methoxide is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.
Caution: Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminium foil while dissolving the sample and during the titration.

CAROB BEAN GUM

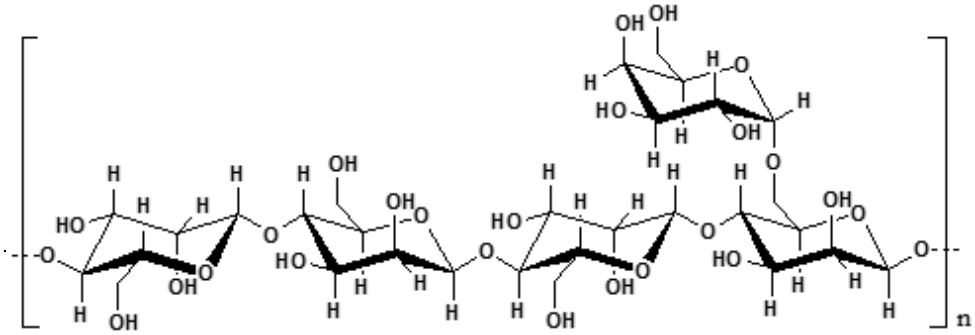
Prepared at the 82nd JECFA and published in JECFA Monograph 19 (2016) superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008), superseding tentative specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). An ADI "not specified" was established at the 25th JECFA (1981) for carob bean gum.

SYNONYMS Locust bean gum clarified, INS No. 410

DEFINITION Carob bean gum, also known as locust bean gum, is a galactomannan polysaccharide obtained from the seeds of *Ceratonia siliqua* (L.) Taub. (Fam. *Leguminosae*). The ground endosperm consists mainly of high molecular weight (approximately 50,000-3,000,000) polysaccharides composed of galactomannans with a mannose:galactose ratio of about 4:1. The seeds are dehusked by treating the seeds with dilute sulfuric acid or with thermal mechanical treatments, elimination of the germ, followed by milling and screening of the endosperm to obtain native carob bean gum.
The gum is clarified by dispersing in hot water, filtration and precipitation with ethanol or isopropanol, filtering, drying and milling. The clarified carob bean gum does not contain cell wall materials. Clarified carob bean gum in the market is normally standardized with sugars for viscosity and reactivity.

C.A.S. number 9000-40-2

Structural formula



DESCRIPTION White to yellowish white, nearly odourless powder

FUNCTIONAL USES Stabilizer, thickener, emulsifier, gelling agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol

Gel formation Add small amounts of sodium borate TS to an aqueous dispersion of the sample; a gel is formed.

Viscosity Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with about 4 ml of isopropanol. Add 200 ml of water with vigorous stirring until the gum is completely and uniformly dissolved. An opalescent, slightly

	viscous solution is formed. Transfer 100 ml of this solution into another 400-ml beaker. Heat the mixture in a boiling water bath for about 10 min and cool to room temperature. There is an appreciable increase in viscosity (differentiating carob bean gums from guar gums).
Gum constituents (Vol. 4)	Proceed as directed under Gum Constituents Identification using 100 mg of the sample instead of 200 mg and 1 to 10 µl of the hydrolysate instead of 1 to 5 µl. Use galactose and mannose as reference standards. These constituents should be present.
PURITY	
Loss on drying (Vol. 4)	Not more than 14% (105º, 5 h)
Total ash (Vol. 4)	Not more than 1.2% (800º, 3-4 h)
Acid-insoluble matter (Vol. 4)	Not more than 3.5%
Protein (Vol. 4)	Not more than 1.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method) in Volume 4 (under "General Methods, Inorganic components"). The percentage of nitrogen determined multiplied by 6.25 gives the percentage of protein in the sample.
Starch	To a 1 in 10 solution of the sample add a few drops of iodine TS; no blue colour is produced
Residual solvents	Not more than 1% of ethanol or isopropanol, singly or in combination 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 Volume 4 (under "General Methods, Metallic Impurities").
Arsenic (Vol. 4)	Not more than 3 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender. Total (aerobic) plate count: Not more than 5,000 CFU/g *E. coli:* Negative in 1 g *Salmonella*: Negative in 25 g Yeasts and moulds: Not more than 500 CFU/g

TESTS

PURITY TESTS

Residual solvents

Determine residual solvents using headspace gas chromatography (Method I)

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

Standard solution: Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 µl ethanol and weigh to within 0.01mg. Inject 15 µl isopropanol through the septum and reweigh the vial.

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

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

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

Follow the procedure described in Vol. 4.

CASSIA GUM
(TENTATIVE)

Revised and made tentative at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications prepared at the 73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010). An ADI "not specified" was established at the 71st JECFA (2009).

Information required:

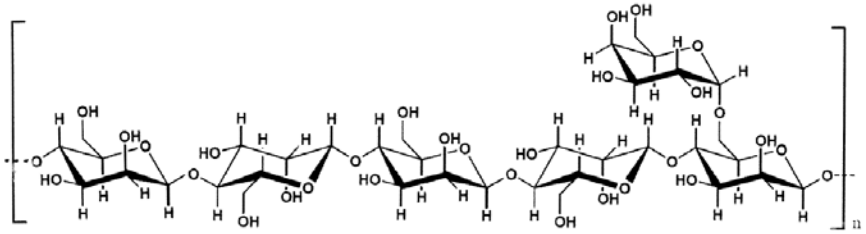
- *Suitable method for the determination of anthraquinones, including:*
  - *the use of standard (reference) materials*
  - *the extraction efficiency of the initial steps*
  - *the recovery of the analytes in question*
  - *performance data for the method*
  - *results of analysis by an accredited laboratory of a minimum of five batches of material*

The tentative specifications will be withdrawn unless the requested information is submitted before 31st December 2017.

SYNONYMS INS 427

DEFINITION Primarily the ground purified endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia*, (Fam. *Leguminsae*) containing less than 0.05% of Cassia occidentalis. It consists mainly of high molecular weight (approximately 200,000-300,000) polysaccharides composed of galactomannans; the mannose:galactose ratio is about 5:1. The structural formula for cassia gum galactomannan is given below. The seeds are dehusked and degermed by thermal mechanical treatment followed by milling and screening of the endosperm. The ground endosperm is further purified by extraction with isopropanol.

Structural formula



Assay Not less than 75% of galactomannans

DESCRIPTION Pale yellow to off-white, odourless free-flowing powder

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

CHARACTERISTICS

IDENTIFICATION

Solubility Insoluble in ethanol
Disperses well in cold water forming colloidal solutions.

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

Gel formation with xanthan gum	Passes test See description under tests
Gum constituents (Vol. 4)	Proceed as directed under Gum Constituents Identification (Vol. 4) using 100 mg of sample instead of 200 mg and 1-10 µl of the hydrolysate instead of 1-5 µl. Use galactose and mannose as reference standards. These constituents should be present.
Viscosity	Less than 500 mPas (25º, 2h) (1% solution) See description under TESTS
pH (Vol. 4)	5.5-8.0 (1%)
PURITY	
Loss on drying (Vol. 4)	Not more than 12% (105º , 5 h)
Total ash (Vol. 4)	Not more than 1.2%
Acid-insoluble matter (Vol. 4)	Not more than 2.0%
Protein (Vol. 4)	Not more than 7.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method; Vol. 4). The percent of nitrogen in the sample multiplied by 6.25 gives the percent of protein in the sample.
Crude fat	Not more than 1% See description under TESTS
Starch	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.
Anthraquinones	Not more than 0.5 mg/kg See description under TESTS
Residual solvents (Vol. 4)	Isopropanol: Not more than 1.0% See description under Tests
Lead (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").
Microbiological criteria (Vol. 4)	Total plate count: Not more than 5,000 cfu/g Yeast and mould: Not more than 100 cfu/g *E. coli*: Negative in 1 g *Salmonella*: Negative in 25 g

TESTS

IDENTIFICATION TESTS	
Gel formation with xanthan gum	Weigh 1.5 g of the sample and 1.5 g of xanthan gum and blend them. Add this blend with (rapid stirring) into 300 ml water at 80º in a 400 ml

beaker. Stir until the mixture is dissolved and continue stirring for an extra 30 min after dissolution (maintain the temperature above 60º during the stirring process). Discontinue stirring and allow the mixture to cool at room temperature for at least 2 h.

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

Viscosity

Weigh 5 g of the sample in a plastic dish and 495 g of distilled water at 20º in a 1000 ml beaker. Add a magnetic bar and place the beaker on the agitation plate. Adjust the speed of agitation to 750 rpm. Introduce quickly the 5 g of sample in the water and cover the beaker with a watch glass. Keep the temperature at 90º for 15 min. Cool the solution at 25º (the cooling must be ±1.5º) in a water bath and measure the viscosity after 2 h at 25º using a RVT Brookfield Spindle 1, speed 20 rpm. Repeat the procedure with a sample of 5 g of carob (locust) bean gum.

(Note: The viscosity of the cassia gum (150 - 500 mPas) must be less than 50% that of carob bean gum (2000 - 3500 mPas))

PURITY TESTS

Residual solvents

Determine residual solvents using headspace gas chromatography (Vol. 4; Method I)

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

Standard solution: Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 µl ethanol and weigh to within 0.01mg. Inject 15 µl isopropanol through the septum and reweigh the vial.

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

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

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

Follow the procedure described in Vol. 4.

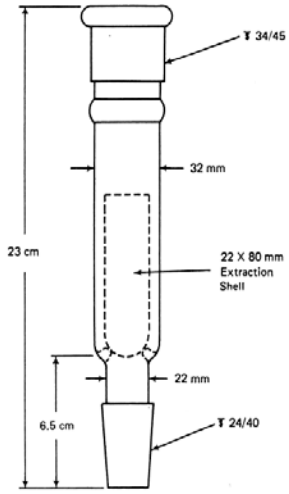
Crude fat

Apparatus

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

Procedure

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



Butt-Type Extractor for fat determination.

NOTE: The method for crude fat is referenced from the Food Chemicals Codex, 6th Edition, 2008, p. 1163.
Reprinted with permission from the US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

Anthraquinones — *A validated method for determination of anthraquinones at the specified maximum level is requested*

METHOD OF ASSAY The difference between 100 and the sum of the percent Loss on Drying, Total Ash, Acid-Insoluble Matter, Protein and Crude Fat represents the percent *Galactomannans*.

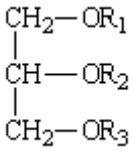
CITRIC and FATTY ACID ESTERS of GLYCEROL

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications prepared at the 79th JECFA (2014), and 35th JECFA (1989) published in FNP 49 (1990) and in FNP 52 (1992). An ADI 'not limited' was established at the 17th JECFA (1973)

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

DEFINITION Obtained by esterification of glycerol with citric acid and edible fatty acids, or by reaction of a mixture of mono- and diglycerides of edible fatty acid, with citric acid; consists of mixed esters of citric acid and edible fatty acids with glycerol; may contain minor parts of free fatty acids, free glycerol, free citric acid and mono- and diglycerides; may be wholly or partially neutralized with sodium hydroxide or potassium hydroxide (as declared on the label).

Structural formula



Where at least one of R_1, R_2 or R_3 represents a citric acid moiety, one represents a fatty acid moiety and the remainder may represent citric acid, fatty acid or hydrogen.

DESCRIPTION White to ivory coloured, oily to waxy material.

FUNCTIONAL USES Stabilizer, emulsifier, dough conditioner, antioxidant synergist

CHARACTERISTICS

IDENTIFICATION

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

Test for fatty acids (Vol. 4) Passes test

Test for citric acid (Vol. 4) Passes test

Test for glycerol (Vol. 4) Passes test

PURITY

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

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

Total glycerol	8-33% See description under TESTS
Total citric acid	13-50% See description under TESTS
Total fatty acid	37-81% See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg. (Not more than 0.1 mg/kg for use in infant formula and formula for special medical purposes intended for infants). Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Total glycerol

Principle: Determined by oxidation of glycerol by sodium periodate in a strongly acid medium and subsequent periodate titration.

Procedure:
Weigh to the nearest 0.1 mg about 2 g of the sample into a saponification flask, add 50 ml of 0.5 M ethanolic potassium hydroxide, and reflux for 30 min.

To a 1-liter volumetric flask add 99 ml ± 0.2 ml (from a buret) of chloroform.

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

Pipet 50 ml of acetic periodic acid TS into a series of 400-ml beakers. Prepare two blanks by adding 50 ml of water to each. Pipet 50 ml of the aqueous layer into one of the 400-ml beakers containing 50 ml of acetic periodic acid TS; shake gently to mix; cover with watch glass, and allow to stand 30 min. but no longer than 1.5 h. Add 20 ml of 15% potassium iodide solution, shake gently to mix, and allow to stand at least 1 min. but not more than 5 min. Do not allow to stand in bright or direct sunlight. Add 200 ml of water and titrate with 0.1 N sodium thiosulfate. Use a variable speed electric stirrer to keep the solution thoroughly mixed. Continue the titration to the disappearance of the brown iodine colour from the aqueous layer. Add 2 ml of starch TS and continue the titration to the disappearance of iodine from the tiny chloroform layer separated during titration and the disappearance of the blue iodine-starch complex colour from the aqueous layer. Read the buret to the nearest 0.01 ml. Treat the blanks in the same way as the sample.

Calculation

% total glycerol = [(B - S) x N x 2.302]/W

where
B is volume (ml) of 0.1 N sodium thiosulfate used for the blank
S = is volume (ml) of 0.1 N sodium thiosulfate used for the sample
N = exact normality of 0.1 N sodium thiosulfate
W = weight of sample used for the analysis
i.e. W = [a x b]/900
where a = weight in g of sample, b = volume of aqueous sample layer used

Total citric acid

Principle
The sample is saponified with alcoholic potassium hydroxide solution and the fatty acids removed by extraction. The citric acid is converted to trimethylsilyl (TMS) derivatives and analyzed by *gas liquid chromatography.*

Reagents: Tartaric acid, Citric acid, potassium hydroxide TS, ethanolic, hydrochloric acid, heptane, pyridine, trimethyl-chlorosilane, hexamethyl-disilazane, N-methyl-N-trimethylsilyl-tri-fluoroacetamide

Preparation of solutions:

Internal Standard solution: 1mg/mL Tartaric acid solution

Standard Stock solution: 3 mg/ml Citric acid solution in water

Procedure:

Saponification of Sample:
Weigh accurately about 1 g of the sample into a round bottomed flask, add 25 ml of 0.5 M potassium hydroxide TS, ethanolic, and reflux for 30 min. Acidify the mixture with hydrochloric acid and evaporate in rotary evaporator or by other suitable method.

Extraction of sample
Quantitatively transfer the content of the flask to a separator, using not more than 50 ml of water and extract with three 50-ml portions of heptane, discarding the extracts. Transfer the aqueous layer to a 100-ml volumetric flask, neutralize, dilute to volume with water, and mix.

Derivatization of sample
Pipette 1 ml of this solution and 1 ml of tartaric acid solution (1 mg/ml in water) into a 10 ml cappable round bottom flask and evaporate to dryness. Add 1 ml of pyridine, 0.2 ml of trimethyl-chlorosilane (TMCS), 0.4 ml of hexamethyl- disilazane (HMDS), 0.1 ml of N-methyl-N-trimethylsilyl-tri- fluoroacetamide (MSTFA). Cap the flask tight and swirl carefully to obtain total dissolution. Heat the flask in an oven at 60º for 1h.

Gas chromatography
Any suitable gas chromatograph may be used fitted with a flame ionization detector and a column (glass 1.8 m x 2 mm i.d.) packed with 10% DC-200 on chromosorb Q (80/100 mesh), or equivalent. Recommended conditions are: oven temperature, 165º; injection block temperature, 240º; detector block temperature, 240º; nitrogen carrier gas flow rate, 24 ml/min, injection volume, 5 µl.

Procedure

Inject a 5 µl sample of the TMS derivative of sample. Measure each peak area by a suitable method, and calculate the percentage of citric acid in the sample taken. The retention time for tartaric acid is about 12 min. and the retention time for citric acid is about 27.6 min.

Repeat the procedure of saponification, extraction and derivitization as described above for sample using 1 ml of the standard stock solution (3 mg/ml citric acid) instead of 1 ml of sample solution. Perform the same Gas chromatography procedure.

Calculation

Measure each peak area by a suitable method.

% Total citric acid = R_s x 100 x R_o x 100 x (W_o/W_s)Rs here

R_s = peak area ratio of citric acid and tartaric acid for sample solution

R_o = peak area ratio of tartaric acid and citric acid forstandardsolution

W = sample weight, g

Wo = weight (g) of citric acid in standard solution

Total fatty acid

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

Procedure

Weigh accurately 5.000 g of the sample into a 250-ml round-bottomed flask, add 50 ml of potassium hydroxide, ethanolic, TS, and reflux for 1 h on a water bath.

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

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

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

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

Calculation:

$$\text{Total Fatty acids, \%} = \frac{\text{mass of fatty acids, g} \times 100}{\text{mass of sample, g}}$$

LUTEIN ESTERS FROM *TAGETES ERECTA*

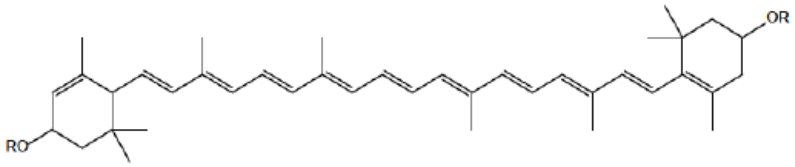
Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding tentative specifications for Lutein esters from Tagetes erecta prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). A Temporary ADI "not specified" was established at the 79th JECFA (2014).

SYNONYMS Xanthophyll esters

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

Chemical formula Lutein esters: R = $CH_3(CH_2)_{10}CO$, $CH_3(CH_2)_{12}CO$, $CH_3(CH_2)_{14}CO$ or $CH_3(CH_2)_{16}CO$

Structural formula



Formula weight 496.43

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

DESCRIPTION Dark orange brown solid

FUNCTIONAL USES Colour, nutrient

CHARACTERISTICS

IDENTIFICATION

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

Spectrophotometry (Vol. 4) A hexane solution of the sample shows a maximum absorption at about 444 nm

Test for carotenoids (Vol. 4) The colour of a solution of the sample in acetone disappears after successive addition of a 5% solution of sodium nitrite in 0.5 M sulfuric acid.

PURITY

Ash (Vol. 4) Not more than 1%

Zeaxanthin (Vol. 4) Not more than 10% of total carotenoids.

See description under TESTS.

Residual solvents (Vol. 4)	Determine using method (I) Hexane Methanol Ethanol 2-Propanol Acetone Methyl ethyl ketone	Not more than 50 mg/kg, singly or in combination
Waxes	Not more than 25%. See description under TESTS)	
Lead (Vol. 4)	Not more than 2 mg/kg. Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").."	

TESTS

PURITY TESTS

Zeaxanthin

Principle

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

Reagents

Extraction solvent: Hexane:acetone:toluene:absolute ethanol (10:7:7:6)
40% Methanolic potassium hydroxide: Dissolve 40 g of KOH in about 50 ml methanol. Dilute to 100 ml with methanol.
10% Sodium sulfate solution: Dissolve 10 g of sodium sulfate in 100 ml water.
Hexane, HPLC-grade
Ethyl acetate, HPLC-grade

Chromatography

Column: 250 mm x 4.6 mm i.d. beta-cyclodextrin (Cyclobond I or equivalent)
Mobile phase: Hexane/Ethyl acetate (75/25)
Filter through a 0.45µm membrane
Flow rate: 1.0 ml/min
Detector wavelength: UV/Vis or PDA at 445nm
Injection volume: 10 µl

Procedure

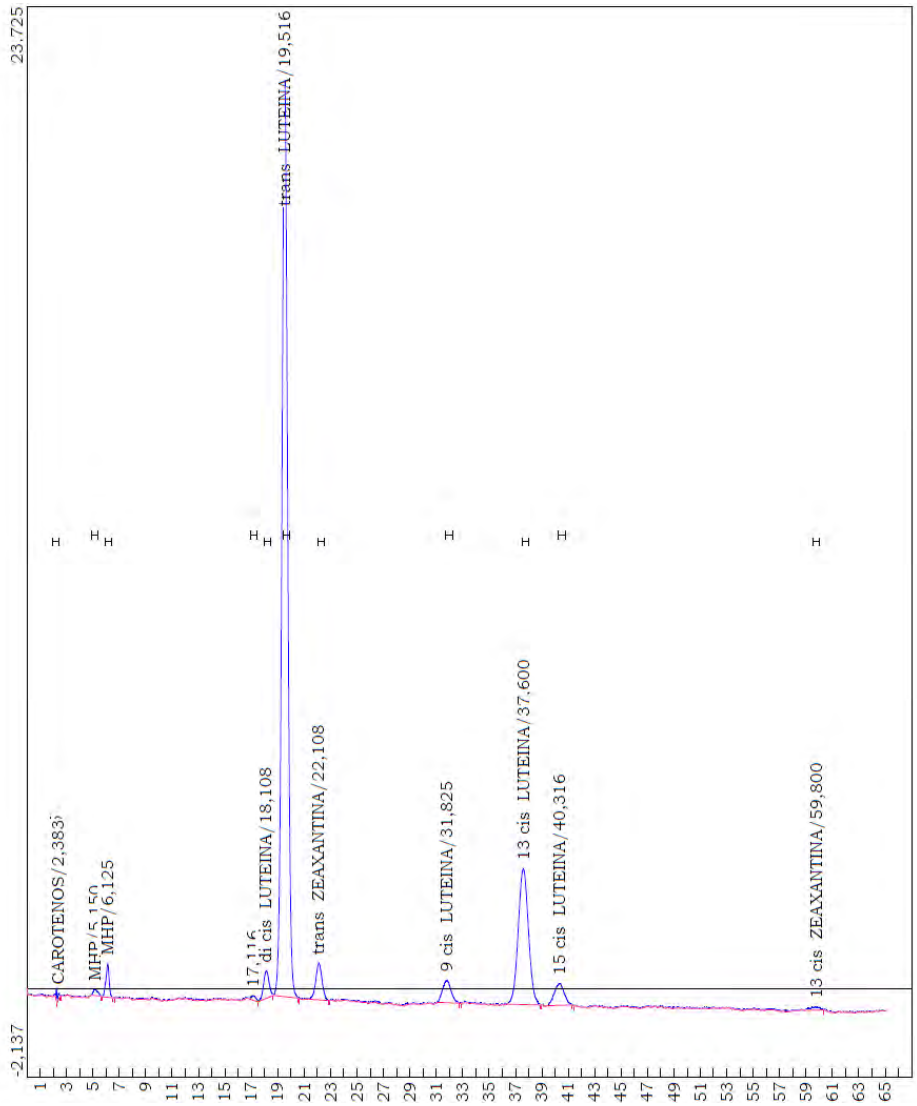
Weigh about 10 mg of sample and transfer into a 100 ml round bottom flask. Dissolve in 30 ml of extraction solvent. Add 2 ml methanolic potassium hydroxide solution. Attach a reflux condenser to the flask to prevent loss of solvent. Place flask in a 56° water bath for 20 min. Cool sample and transfer to a 100 ml volumetric flask using a small volume of extraction solvent. Let stand in the dark for 1 h. Add 30 ml of hexane, swirl for 1 min. Dilute with sodium sulfate solution up to the 100 ml mark. Shake 1 min. Let stand in the dark until organic layer (top layer) is clear (about 1 h).
Transfer 1 ml of organic layer to a scintillation vial. Evaporate to almost dry under vacuum at 45°. Dissolve residue with 1 ml mobile phase. Inject 10 µl of this solution into the chromatograph.

Results

A typical chromatograph should look as follows.

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

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



Waxes

Determine by gas chromatography using the following conditions:

Apparatus

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

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

Standards:
Hydrocarbons mixed standard: C25 to C46
Internal standard:
Hexatriancontane (C36)

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

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

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

Calculation:

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

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

METHOD OF ASSAY

Determine the total content of carotenoid esters as follows:

Apparatus:

UV/VIS spectrophotometer

1-cm cuvettes

Sample analysis:

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

Calculation:

$$\text{Total carotenoid ester content (\% w/w)} = \frac{\text{Abs} \times d \times 100}{A^{1\%}_{1cm\ \text{isosbestic}} \times W}$$

Where:

Abs is the measured absorbance

d is the dilution factor

$A^{1\%}_{1cm\ \text{isosbestic}}$ (specific absorbance of lutein ester at the wavelength of the isosbestic point) = 898

W is the weight of sample (g)

MODIFIED STARCHES

ACETYLATED DISTARCH ADIPATE
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Acetylated distarch adipate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *A suitable test for identification of the adipate groups*
- *Levels of free adipic acid*

SYNONYMS INS No. 1422

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

Acetylated distarch adipate is a modified starch. It is obtained by esterification of food starch with acetic anhydride and esterification/cross-linking with adipic anhydride, in accordance with good manufacturing practice. Acetylation results in substitution of hydroxyl groups with acetyl esters. In cases of cross-linking, where adipic anhydride, connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = CO-(CH2)4-CO and Starch refers to the linear and/or branched structure.

Acetylated distarch adipate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 63798-35-6
63055-36-7 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain	Passes test See description under TESTS
Copper reduction	Passes test See description under TESTS
Specific reaction for acetyl groups	Passes test See description under TESTS
Ester groups	Passes test See description under TESTS
Test for adipate groups	*Information Required*

PURITY

Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Acetyl groups	Not more than 2.5% on the dried basis See description under TESTS
Adipate groups	Not more than 0.135% on the dried basis See description under TESTS
Free Adipic Acid	*Information Required* See description under TESTS
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of

the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed

Iodine stain — Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red

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

Specific reaction for acetyl groups

Principle

Acetate is liberated upon saponification of acetylated starch. After concentration the acetate is converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure

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

Ester groups — The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm-1 which is an indication for ester groups. The limit of detection is about 0.5% acetyl or adipyl groups in the product.

Test for adipate groups — *Information Required*

PURITY TESTS

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

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

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

where

M is the molarity of hydrochloric acid solution; and
W is the weight of sample, In grams.

Adipate groups and Free Adipic Acid

Reagents and Solutions

N,N-Bis-trimethylsilyltrifluoroacetamide (BSTFA): Macherey-Nagel, D 5160 Dueren, Germany or equivalent.
Glutaric acid solution: Dissolve 1.00 g of glutaric acid (Merck or equivalent) in water and dilute to 1000 ml.
Adipic acid solution: Dissolve 1.00 g of adipic acid (UCB, Brussels, Belgium or equivalent) in 900 ml of warm water, cool to room temperature, dilute to 1000 ml and mix.

Apparatus

Chromatograph: Hewlett Packard Model 7620A gas chromatograph or equivalent equipped with flame ionization detector and Model 3370Aintegrator. (Hewlett-Packard Model 7620A, with integrator Model 3370A or equivalent)

Column parameters: 2-m stainless steel, 1.83 mm id, packed with 5% OV-17 on 80-100 mesh Chromosorb GAW-DMCS (Alltech Europe, Inc., B 9731 Eke, Belgium); precondition column 24 h at 350° with nitrogen carrier gas at 40 ml/min. Operating gas flow rates (ml/min): nitrogen carrier 30, hydrogen 40, air 400. Temperature: injection 280°, detector 250°, column 140°. Retention times (min): glutaric acid 2.83, adipic acid 4.50.

Calibration

Weigh 1.0 g waxy corn starch into each of four 250-ml Erlenmeyer flasks. To each flask add 50 ml water and 1.0 ml of an aqueous solution containing 1.0 mg glutaric acid/ml. Add, to one flask, 0.25 ml of an aqueous solution containing 1.0 mg adipic acid per ml; to the other three, add 0.50 ml, 0.75 ml, and 1.0 ml, respectively. Each flask then contains 1.0 mg glutaric acid and, respectively, 0.25, 0.50, 0.75 and 1.0 mg adipic acid. Agitate flasks manually to disperse the starch fully and add 50 ml 4N sodium hydroxide. Continue agitation another 5 min, place each flask in water bath at ambient temperature, and carefully add 20 ml 12 N hydrochloric acid to each. When each flask is cool quantitatively transfer contents to 250 ml separatory funnel. Extract with 100 ml reagent grade ethyl acetate. Drain bottom aqueous layer into beaker and collect upper organic layer in 500-ml Erlenmeyer flask containing 20 g anhydrous sodium sulphate. Transfer aqueous portion back to separatory funnel and repeat ethyl acetate extraction twice more. Shake flasks periodically during 10 min and then filter contents through Whatman No. 1 paper into 1-litre round-bottom flasks. Rinse flasks and insoluble residues in filters twice with 50 ml of ethyl acetate. Under vacuum, (50 mm Hg) at temperature not exceeding 40°, evaporate total organic extraction and washings of each flask until completely dry.

The evaporation of ethyl acetate should be effected as quickly as possible because some hydrolysis takes place on standing. The products of hydrolysis cause deterioration in the resolution of adipic acid in the chromatographic separation.

Successively add 2 ml pyridine and 1 ml N,N-bis-trimethylsilyltrifluoro-acetamide to the dry contents. Close each of the round-bottom flasks with stopper and rinse internal surfaces thoroughly by swirling. Let flasks stand 1 h; then transfer ca 2 ml from each to small glass vials and immediately seal. Inject 4 µl into gas chromatograph.

Calculations

Establish retention times for each acid and determine peak height for glutaric acid and for each level of adipic acid represented. A plot of peak height ratio of adipic acid to glutaric acid against amount of adipic acid is linear. This calibration curve may be used, but it is simpler to use a response factor (RF):

$$RF = \frac{H_I \times W_S}{H_S}$$

where

H_S and H_I is the peak heights of the standard adipic acid and glutaric acid, respectively; and

W_S is the weight of the standard adipic acid.

RF should be verified weekly.

Total adipate

Accurately weigh about 1.0 g of the sample into a 250 ml Erlenmeyer flask, and add 50 ml water and 1.0 ml of an aqueous solution containing 1.0 mg glutaric acid/ml. Proceed as in Calibration, beginning "Agitate flasks manually...".

Free adipic acid

Accurately weigh about 5.0 g of the sample into a 250 ml Erlenmeyer flask, add 100 ml water and 1.0 ml of the glutaric acid solution. Agitate for 1 h, filter through a 0.45 µm Millipore filter, add 1 ml concentrated hydrochloric acid to the filtrate and transfer it quantitatively to a 250-ml separating funnel. Proceed as in Calibration, beginning "Extract with 100 ml..."

Calculation

For both preparations ("Total adipate content" and "Free adipic acid content") record peak heights for adipic acid and glutaric acid (internal standard). Calculate the amounts of total adipate and free adipic acid, respectively, contained in the sample as follows:

$$A = \frac{H_X \times RF}{H_{IX} \times S \times 10}$$

where

A is the content of total adipate or free adipic acid respectively (%);

H_X is the peak height of adipic acid in the actual sample preparation;

H_{IX} is the peak height of glutaric acid in the actual sample preparation;

RF is the response factor for adipic acid; and

S is the weight of sample in the actual preparation (g).

Adipate groups (%) is equal to content of total adipate (%) - content of free adipic acid (%).

ACETYLATED DISTARCH PHOSPHATE
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Acetylated distarch phosphate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 17 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *A suitable test for identification of the phosphate groups and of crosslinking.*

SYNONYMS INS No. 1414

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
Acetylated distarch phosphate is a modified starch. It is obtained by esterification/cross-linking of food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate in accordance with good manufacturing practice. Acetylation results in substitution of hydroxyl groups with acetyl esters. In cases of cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure.

Acetylated distarchphosphate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 9067-33-8
68130-14-3
113894-91-0 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction	Passes test See description under TESTS
Phosphate groups	*Information required*
Crosslinking	*Information required*
Specific reaction for acetyl groups	Passes TEST See description under TESTS
Ester groups	Passes TEST See description under TESTS

PURITY

Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Acetyl groups	Not more than 2.5% on the dried basis See description under TESTS
Phosphate (calculated as phosphorus) (Vol. 4)	Not more than 0.14% on the dried basis for potato and wheat starch Not more than 0.04% on the dried basis for other starches
Vinyl acetate	Not more than 0.1 mg/kg See description under TESTS
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic

observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed

Iodine stain

Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red

Copper reduction

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

Specific reaction for acetyl groups

Principle

Acetate is liberated upon saponification of acetylated starch. After concentration the acetate is converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure

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

Ester groups

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

PURITY TESTS

Acetyl groups

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

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

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

where

M is the molarity of hydrochloric acid solution; and
W is the weight of sample, In grams.

Vinyl acetate - Headspace Gas Chromatographic method

Chromatographic system

Use a gas chromatograph equipped with a 2 m x 2 mm (i.d.) glass column containing Porapak Q, 80-100 mesh (or equivalent) fitted with a flame ionization detector, under the following conditions:

- Carrier gas flow (nitrogen): 20 ml/min
- injection port temperature: 200°
- column temperature: 50
- detector temperature: 200°

Standard preparation: Accurately weigh 150 mg vinyl acetate (reagent grade) into a 100 ml volumetric flask. Dissolve and make up to volume with distilled water. Place 1 ml of this solution in a 10-ml volumetric flask and make up to volume with distilled water. Add 1 ml of this dilute solution to 30 g unmodified starch of the same botanical origin as the test substance in a 100-ml flask with a septum-liner. Seal the flask immediately with the septum-liner. This provides a standard starch preparation with a vinyl acetate content of 5 mg/kg.

Procedure

Weigh 30 g of the test substance into a 100-ml flask with a septum-liner. Seal the flask. Place the flask containing the test substance and the flask containing the standard preparation in a constant temperature water bath at 70° for 30 min. Withdraw 2.0 ml from the headspace volume of the flask containing the standard preparation using a gas-tight syringe, inject directly into the injection port of the gas chromatograph and record the peak height of the chromatogram. Similarly inject 2.0 ml of the headspace volume from the flask containing the test substance into the chromatograph. Calculate the content of vinyl acetate in the test substance from a comparison of the peak heights of the two chromatograms.

ACETYLATED OXIDIZED STARCH

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Acetylated oxidized starch included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 57th JECFA (2001).

SYNONYMS

INS No. 1451

DEFINITION

Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

Acetylated oxidized starch is a modified starch. It is obtained by treatment of food starch with sodium hypochlorite followed by esterification with acetic anhydride in accordance with good manufacturing practice. Oxidation involves the deliberate production of carboxyl groups. Acetylation results in substitution of hydroxyl groups with acetyl esters.

Acetylated oxidized starch may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number

68187-08-6

DESCRIPTION

White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES

Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

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

Microscopy

Passes test
See description under TESTS

Iodine stain

Passes test
See description under TESTS

Copper reduction

Passes test
See description under TESTS

Hypochlorite oxidized starch

Passes test
See description under TESTS

Specific reaction for acetyl groups	Passes test See description under TESTS
Ester groups	Passes test See description under TESTS
PURITY	
Loss on drying	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Acetyl groups	Not more than 2.5 % on the dried basis See description under TESTS
Carboxyl groups (Vol. 4)	Not more than 1.3 % on the dried basis
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

IDENTIFICATION TESTS	
Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced
Hypochlorite oxidized starch	Principle Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such

as methylene blue. The test is not suitable for acetylated slightly oxidized potato starch due to the presence of phosphate groups.

Procedure

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

Specific reaction for acetyl groups

Principle

Acetate is liberated upon saponification of acetylated starch. After concentration the acetate is converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure

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

Ester groups

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

PURITY TESTS

Acetyl groups

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

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

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

where

M is the molarity of hydrochloric acid solution; and
W is the weight of sample, In grams.

ACID TREATED STARCH
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Acid treated starch included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *A suitable method for the Dispersion or Reducing Sugars Distinguishing Test*

SYNONYMS INS No. 1401

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

Acid treated starch is a modified starch. It is obtained by treatment of food starch with hydrochloric acid or ortho-phosphoric acid or sulfuric acid, in accordance with good manufacturing practice. The alteration of the starch is a minor fragmentation.

Acid treated starch may additionally be subjected to bleaching, in accordance with good manufacturing practices.

C.A.S number 65996-63-6
68909-37-5 (Acid treated amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Dispersion test or Reducing sugars distinguishing test	*Information required*
PURITY	
pH	4.8-7.0 See description under TESTS
Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS	
Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

PURITY TESTS

pH (Vol. 4)	Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (In the case of pre-gelatinized starches, 3 g should be suspended in 97 ml of water).

ALKALINE TREATED STARCH
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Alkaline treated starch included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *A suitable method for the Dispersion or Reducing Sugars Distinguishing Test*

SYNONYMS INS No. 1402

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

Alkaline treated starch is a modified starch. It is obtained by treatment of food starches with sodium hydroxide or potassium hydroxide in accordance with good manufacturing practice. The alteration of the starch is a minor fragmentation.

Alkaline treated starch may additionally be subjected to bleaching, in accordance with good manufacturing practices.

C.A.S number 9005-84-9

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Dispersion test or Reducing sugars distinguishing test	*Information required*
PURITY	
pH	5.0-7.5
Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS	
Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced
PURITY TESTS	
pH (Vol. 4)	Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (In the case of pre-gelatinized starches, 3 g should be suspended in 97 ml of water).

BLEACHED STARCH
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Bleached starch included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *Typical levels of residual reagents or byproducts*

SYNONYMS INS No. 1403

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
Bleached starch is a modified starch. It is obtained by treatment of food starch, in accordance with good manufacturing practice, with peracetic acid and/or hydrogen peroxide, or sodium hypochlorite or sodium chlorite, or sulfur dioxide or alternative permitted forms of sulfites, or potassium permanganate or ammonium persulfate. The change is essentially in the colour only. Reagents are either removed or limited to technically unavoidable levels.

Bleached starch may additionally be subjected to acid, alkali or enzyme treatments in accordance with good manufacturing practices.

C.A.S number 977075-42-5

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

PURITY

Loss on drying	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
[Residual reagents]	*Information required*
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

DEXTRIN ROASTED STARCH
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016),, superseding specifications for Dextrin roasted starch included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *A suitable method for the Dispersion or Reducing Sugars Distinguishing Test*

SYNONYMS INS No. 1400

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
Dextrin roasted starch is a modified starch. It is obtained by dry heat treatment with hydrochloric acid or ortho-phosphoric acid of food starch, in accordance with good manufacturing practice. The alteration of the starch is a minor fragmentation.

Dextrin roasted starch may additionally be subjected to bleaching, in accordance with good manufacturing practices.

C.A.S number 9004-53-9

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Dispersion test or Reducing sugars distinguishing test *Information required*

PURITY

Loss on drying	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
pH	2.5-7.0 See description under TESTS
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

PURITY TESTS

pH (Vol. 4)	Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (In the case of pre-gelatinized starches, 3 g should be suspended in 97 ml of water).

DISTARCH PHOSPHATE
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Distarch phosphate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:

- *A suitable test for identification of the phosphate groups and of crosslinking.*

SYNONYMS Modified starch, INS No. 1412

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
Distarch phosphate is a modified starch. It is obtained by esterification of food starch with sodium trimetaphosphate or phosphorus oxychloride in accordance with good manufacturing practice. This treatment results in cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains. The structure can be represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure.

Distarch phosphate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 55963-33-2
63055-37-8 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction	Passes test See description under TESTS
Phosphate groups	*Information required*
Crosslinking	*Information required*

PURITY

Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Phosphate (calculated as phosphorus) (Vol.4)	Not more than 0.5% on the dried basis for potato and wheat starch Not more than 0.4% on the dried basis for other starches
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

ENZYME-TREATED STARCH
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Enzyme-treated starch included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).
Information is required on:

- *A suitable method for the Dispersion or Reducing Sugars Distinguishing Test*

SYNONYMS INS No. 1405

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

Enzyme-treated starch is a modified starch. It is obtained by treatment in accordance with good manufacturing practice, of food starch in an aqueous solution at a temperature below the gelatinization point with one or more food-grade amyolyticenzymes.The alteration is a minor fragmentation.

Enzyme-treated starch may additionally be subjected to bleaching, in accordance with good manufacturing practices.

C.A.S number 65996-64-7
100 1439-91-3 (Enzyme-treated amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Dispersion test or Reducing sugars distinguishing test	*Information Required*
PURITY	
Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS	
Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

HYDROXYPROPYL DISTARCH PHOSPHATE
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Hydroxypropyldistarch phosphate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *A suitable method for the determination of propylene chlorohydrin*
- *A suitable test for identification of the phosphate groups*

SYNONYMS INS No. 1442

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose)and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
Hydroxypropyldistarch phosphate is a modified starch. It is obtained in accordance with good manufacturing practice by esterification of food starch with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide.Hydroxypropylation results in substitution of hydroxyl groups with 2-hydroxypropyl ether. In cases of cross-linking, where phosphorus oxychloride, connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure.

Hydroxypropyldistarch phosphate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 53124-00-8
113894-92-1 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain	Passes test See description under TESTS
Copper reduction	Passes test See description under TESTS
Phosphate groups	*Information required*
Hydroxypropyl ether groups	Passes test See description under TESTS

PURITY

Loss on drying	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Hydroxypropyl groups	Not more than 7.0%(calculated on dry substance) See description under TESTS
Propylene chlorohydrin	Not more than 1 mg/kg See description under TESTS
Phosphate (calculated as phosphorus) (Vol. 4)	Not more than 0.14% on the dried basis for potato and wheat starch Not more than 0.04% on the dried basis for other starches
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed

Iodine stain

Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red

Copper reduction

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

Hydroxypropyl ether groups[1]

Ninhydrin reagent
A 3% solution of 1,2,3-triketohydrindene crystals in 4.55% aqueous sodium bisulfite solution.

Procedure
Weigh 100 mg of the sample into a 100-ml volumetric flask and add 12.5 ml of 2 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake).
A violet colour develops only in the modified sample within 5 min due to the presence of hydroxypropyl groups (starch ether).For all other non-hydroxypropyl treated starches a light pink colour is observed.

PURITY TESTS

Phosphorus (Vol. 4)

Reagents

- Ammonium Molybdate Solution (5%): Dissolve 50 g of ammonium molybdatetetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 900 ml of warm water, cool to room temperature, dilute to 1000 ml with water, and mix.
- Ammonium Vanadate Solution (0.25%): Dissolve 2.5 g of ammonium metavanadate, NH4VO3, in 600 ml of boiling water, cool to 60 - 70o, and add 20 ml of nitric acid. Cool to room temperature, dilute to 1000 ml with water, and mix.
- Zinc Acetate Solution (10%): Dissolve 120 g of zinc acetate dihydrate, Zn(C2H3O2)2·2H2O, in 880 ml of water, and filter through Whatman No. 2V or equivalent filter paper before use.
- Nitric Acid Solution (29%): Add 300 ml of nitric acid (sp. gr 1.42) to 600 ml of water, and mix.
- Standard Phosphorus Solution: (100 µg P in 1 ml): Dissolve 438.7 mg of monobasic potassium phosphate, KH2PO4, in water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

[1]USP29-NF34: U.S. Pharmacopeial Convention, Hydroxylpropyl corn starch monograph, 2015. Reproduced with permission.

Standard Curve

Pipet 5.0, 10.0, and 15.0 ml of the Standard Phosphorus Solution into separate 100-ml volumetric flasks. To each of these flasks, and to a fourth blank flask, add in the order stated 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg P per 100 ml.

Sample pre-treatment

Place 20 to 25 g of the starch sample in a 250-ml beaker, add 200 ml of a 7 to 3 methanol-water mixture, disperse the sample, and agitate mechanically for 15 min. Recover the starch by vacuum filtration in a 150 ml medium-porosity fritted-glass or Buchner funnel, and wash the wet cake with 200 ml of the methanol-water mixture. Reslurry the wet cake in the solvent, and wash it a second time in the same manner. Dry the filter cake in an air oven at a temperature below 50°, then grind the sample to 20-mesh or finer, and blend thoroughly. Determine the amount of dry substance by drying a 5 g portion in a vacuum oven, not exceeding 100 mm of Hg, at 120° for 5 h. (NOTE: The treatment outlined above is satisfactory for starch products that are insoluble in cold water.

For pregelatinized starch and other water-soluble starches, prepare a 1% to 2% aqueous paste, place it in a cellophane tube, and dialyze against running distilled water for 30 to 40 h. Precipitate the starch by pouring the solution into 4 volumes of acetone per volume of paste, while stirring. Recover the starch by vacuum filtration in a medium-porosity fritted-glass or Buchner funnel, and wash the filter cake with absolute ethanol. Dry the filter cake, and determine the amount of dry substance as directed for water-insoluble starches).

Sample preparation

Transfer about 10 g of the Treated Sample, calculated on the dry-substance and accurately weighed, into a Vycor dish, and add 10 ml of Zinc Acetate Solution in a fine stream, distributing the solution uniformly in the sample. Carefully evaporate to dryness on a hot plate, then increase the heat, and carbonize the sample on the hot plate or over a gas flame. Ignite in a muffle furnace at 550° until the ash is free from carbon (about 1 to 2 h), and cool. Wet the ash with 15 ml of water and wash slowly down the sides of the dish with 5 ml of Nitric Acid Solution. Heat to boiling, cool, and quantitatively transfer the mixture into a 200-ml volumetric flask, rinsing the dish with three 20-ml portions of water and adding the rinsings to the flask. Dilute to volume with water, and mix. Transfer an accurately measured aliquot (V, in ml) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-ml volumetric flask and add 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min.

Procedure

Determine the absorbance of the Sample Preparation in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the

instrument at zero. From the Standard Curve, determine the mg of phosphorus in the aliquot taken, recording this value as a. Calculate the amount in mg/kg of Phosphorus (P) in the original sample by the formula:

$$\frac{a \times 200 \times 1000}{V \times W}$$

where

W is the weight of the sample taken, in g.

Hydroxypropyl groups

Ninhydrin reagent

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

Procedure

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

Calculation

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

where

C is the amount of propylene glycol in the sample solution read from the calibration curve (µg/ml);

F is the dilution factor (if a further dilution has been necessary); and

W is the weight of sample (mg).

Propylene chlorohydrin

Information Required

HYDROXYPROPYL STARCH
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Hydroxypropyl starch included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *A suitable method for the determination of propylene chlorohydrin*

SYNONYMS INS No. 1440

DEFINITION Starch is a carbohydrate polymer consisting of alarge number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose)and branchedthrough alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
Hydroxypropyl starch is a modified starch. It is obtained by etherification of food starch with propylene oxide, in accordance with good manufacturing practice. Hydroxypropylation results in substitution of hydroxyl groups with 2-hydroxypropyl ether.

Hydroxypropyl starch may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 9049-76-7
74315-67-6 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Hydroxypropyl ether groups	Passes test See description under TEST
PURITY	
Loss on drying	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Hydroxypropyl groups	Not more than 7.0% on the dried basis See description under TESTS
Propylene chlorohydrin	Not more than 1 mg/kg See description under TESTS
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS	
Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

Hydroxypropyl ether groups[2]

Ninhydrin reagent
A 3% solution of 1,2,3-triketohydrindene crystals in 4.55% aqueous sodium bisulfite solution.

Procedure
Weigh 100 mg of the sample into a 100-ml volumetric flask and add 12.5 ml of 2 N sulfuric acid. Prepare a sample of unmodified starch of the same source (i.e. corn or potato) in the same manner. Place the flasks in a boiling water bath and heat until the samples are in solution. Cool and dilute the contents to 100 ml with water. Pipet 1 ml of the solutions into 25-ml graduated test tubes with glass stoppers and, with the tubes immersed in cold water, add dropwise 8 ml of concentrated sulfuric acid to each. Mix well and place the tubes in a boiling water bath for exactly 3 min. Immediately transfer the tubes to an ice bath until the solution is chilled. Add 0.6 ml of ninhydrin reagent, carefully allowing the reagent to run down the walls of the test tubes. Immediately shake well, and place the tubes in a 25° water bath for 100 min. Adjust the volume in each tube to 25 ml with concentrated sulfuric acid and mix by inverting the tubes several times. (Do not shake).
A violet colour develops only in the modified sample within 5 min due to the presence of hydroxypropyl groups (starch ether).For all other non-hydroxypropyl treated starches a light pink colour is observed.

PURITY TESTS

Hydroxypropyl groups

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

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

[2] USP29-NF34: U.S. Pharmacopeial Convention, Hydroxylpropyl corn starch monograph, 2015. Reproduced with permission.

Calculation

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

where

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

Propylene chlorohydrin *Information required*

MONOSTARCH PHOSPHATE
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monographs 19 (2016), superseding specifications for Monostarch phosphate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:

- *A suitable test for identification of the phosphate groups*

SYNONYMS INS No. 1410

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

Monostarch phosphate is a modified starch. It is obtained by esterification of food starch with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate in accordance with good manufacturing practice. This treatment results in partial substitution in the 2, 3- or 6- position of the anhydroglucose unit unless the 6-position is occupied for branching.

Monostarch phosphate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 11120-02-8
63055-37-8 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Phosphate groups *Information required*

PURITY

Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Phosphate (calculated as phosphorus) (Vol. 4)	Not more than 0.5% on the dried basis for potato or wheat starches Not more than 0.4% on the dried basis for other starches
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced.

OXIDIZED STARCH

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Oxidized starch included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

SYNONYMS INS No. 1404

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

Oxidized starch is a modified starch. It is obtained by treatment of food starch in accordance with good manufacturing practice with sodium hypochlorite. Oxidation involves the deliberate production of carboxyl groups.

Oxidized starch may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 65996-62-5
113894-86-3 (Oxidized amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Test for hypochlorite oxidized starch Passes test
See description under TESTS

PURITY

Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Carboxyl groups (Vol. 4)	Not more than 1.3% on the dried basis
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed.
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red.
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced.
Test for hypochlorite oxidized starch	Principle Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue. The test is not suitable for slightly oxidized potato starch due to the presence of phosphate groups. Procedure 50 mg of the sample are kept in suspension for 5-10 min in 25 ml of a 1% aqueous dye solution and stirred occasionally. After decantation of the excess solution, the starch is washed with distilled water. Microscopic inspection clearly shows colouring, if the sample is hypochlorite-oxidized starch. By this test hypochlorite-oxidized starch is distinguished from native and acid modified starch of the same botanical origin.

PHOSPHATED DISTARCH PHOSPHATE
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Phosphated distarch phosphate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014).An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:

- *A suitable test for identification of the phosphate groups and of crosslinking.*

SYNONYMS INS No. 1413

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
Phosphated distarch phosphate is a modified starch. It is obtained by esterification/cross-linking of food starch with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate, in accordance with good manufacturing practice. The esterification results in partial substitution in the 2, 3- or 6- position of the anhydroglucose unit unless the 6-position is occupied for branching. In the case of cross-linking, where a polyfunctional substituting agent, such as phosphorus oxychloride, connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = cross-linking group and Starch refers to the linear and/or branched structure.

Phosphated distarch phosphate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 11120-02-8
63055-37-8 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy	Passes test See description under TESTS
Iodine stain	Passes test See description under TESTS
Copper reduction	Passes test See description under TESTS
Phosphate groups	*Information required*
Crosslinking	*Information required*

PURITY

Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Phosphate (calculated as phosphorus) (Vol. 4)	Not more than 0.5% on the dried basis for potato and wheat starch Not more than 0.4% on the dried basis for other starches
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red

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

STARCH ACETATE

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Starch acetate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

SYNONYMS INS No. 1420

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.

Starch acetate is a modified starch. It is obtained by esterification of food starches with acetic anhydride or vinyl acetate in accordance with good manufacturing practice. The esterification/acetylation results in substitution of hydroxyl groups with acetyl esters.

Starch acetate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 9045-28-7
60164-73-0 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Specific reaction for acetyl groups Passes test
See description under TESTS

Ester groups Passes test
See description under TESTS

PURITY

Loss on drying (Vol. 4)	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Acetyl groups	Not more than 2.5% on the dried basis See description under TESTS
Vinyl acetate	Not more than 0.1 mg/kg See description under TESTS
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

Specific reaction for acetyl groups

Principle

Acetate is liberated upon saponification of acetylated starch. After concentration the acetate is converted to acetone by heating with calcium hydroxide. The acetone thus produced stains blue with o-nitrobenzaldehyde.

Procedure

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

Ester groups

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

PURITY TESTS

Acetyl groups

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

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

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

where

M is the molarity of hydrochloric acid solution; and
W is the weight of sample, In grams.

Vinyl acetate

- Headspace Gas Chromatographic method

Chromatographic system

Use a gas chromatograph equipped with a 2 m x 2 mm (i.d.) glass column containing Porapak Q, 80-100 mesh (or equivalent) fitted with a flame ionization detector, under the following conditions:

- Carrier gas flow (nitrogen): 20 ml/min
- injection port temperature: 200°
- column temperature: 50
- detector temperature: 200°

Standard preparation: Accurately weigh 150 mg vinyl acetate (reagent grade) into a 100 ml volumetric flask. Dissolve and make up to volume with distilled water. Place 1 ml of this solution in a 10-ml volumetric flask and make up to volume with distilled water. Add 1 ml of this dilute solution to 30 g unmodified starch of the same botanical origin as the test substance in a 100-ml flask with a septum-liner. Seal the flask immediately with the septum-liner. This provides a standard starch preparation with a vinyl acetate content of 5 mg/kg.

Procedure

Weigh 30 g of the test substance into a 100-ml flask with a septum-liner. Seal the flask. Place the flask containing the test substance and the flask containing the standard preparation in a constant temperature water bath at 70° for 30 min. Withdraw 2.0 ml from the headspace volume of the flask containing the standard preparation using a gas-tight syringe, inject directly into the injection port of the gas chromatograph and record the peak height of the chromatogram. Similarly inject 2.0 ml of the headspace volume from the flask containing the test substance into the chromatograph. Calculate the content of vinyl acetate in the test substance from a comparison of the peak heights of the two chromatograms.

STARCH SODIUM OCTENYLSUCCINATE
(TENTATIVE)

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding specifications for Starch sodium octenylsuccinate included in the specifications for Modified starches prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 26th JECFA (1982).

Information is required on:
- *A suitable test for identification of the octenylsuccinate groups*

SYNONYMS INS No. 1450

DEFINITION Starch is a carbohydrate polymer consisting of a large number of glucose units linked together primarily by alpha 1-4 glucosidic bonds. The starch polymers come in two forms: linear (amylose) and branched through alpha 1-6 glucosidic bonds (amylopectin), with each glucose unit possessing a maximum of three hydroxyls that can undergo chemical substitution.
.
Starch sodium octenylsuccinate is a modified starch. It is obtained by esterification of food starch with octenylsuccinic anhydride, and neutralisation with either sodium hydroxide or sodium carbonate as a pH buffer, in accordance with good manufacturing practice.

Starch sodium octenylsuccinate may additionally be subjected to acid, alkali, enzyme, or bleaching treatment in accordance with good manufacturing practice.

C.A.S number 66829-29-6
52906-93-1
125109-81-1 (modified amylopectin)

DESCRIPTION White or nearly white powder or granules or (if pregelatinized) flakes, or amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in cold water (if not pre-gelatinized); forming typical colloidal solutions with viscous properties in hot water; insoluble in ethanol.

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Ester groups	Passes test See description under TESTS
Octenylsuccinate groups	*Information required*
PURITY	
Loss on drying	Cereal starch: not more than 15.0% Potato starch: not more than 21.0% Other starches: not more than 18.0% (120°, 4 h, vacuum not exceeding 100 mm Hg)
Octenylsuccinyl groups	Not more than 3% on the dried basis See description under TEST
Residual octenylsuccinic acid	Not more than 0.3% on the dried basis See description under tests
Sulfur dioxide (Vol. 4)	Not more than 50 mg/kg on the dried basis for modified cereal starches Not more than 10 mg/kg on the dried basis for other modified starches
Lead (Vol. 4)	Not more than 2 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Manganese (Vol. 4)	Not more than 50 mg/kg on the dried basis Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Carboxyl groups (Vol. 4)	Not more than 0.1% on the dried basis

TESTS

IDENTIFICATION TESTS

Microscopy	Modified starches which have not been pre-gelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under cross nicol prisms the typical polarization cross will be observed
Iodine stain	Add a few drops of 0.1 N potassium tri-iodide to an aqueous suspension of the sample. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red
Copper reduction	Place about 2.5 g of the sample previously washed with water, in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about three hours and cool. Add 0.5 ml of the

resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced

Ester groups

The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm-1 which is an indication for ester groups.
The limit of detection is about 0.5% octylsuccinyl groups in the product.

Octenylsuccinyl groups

Information required

PURITY TESTS

Octenylsuccinyl groups in starch sodium octenyl succinate

Principle

The sample is equilibrated with mineral acid to convert octenyl succinate salts to the acid form. Cations and excess acid are removed by thorough washing with 90% isopropanol in water. The washed sample is titrated with standard alkali.

Procedure

Weigh accurately about 5.000 g of sample into a 150-ml beaker and wet the sample with a few ml of isopropanol. Add 25 ml of 2.5 M hydrochloric acid in isopropanol, allowing the acid to wash down any sample on the sides of the beaker. Stir the mixture with a magnetic stirrer for 30 min. Using a graduated measuring cylinder, add 100 ml of 90% isopropanol in water and stir the contents for another 10 min. Filter through a Buchner funnel and wash the filter cake with 90% isopropanol in water until the filtrate is negative for chloride (check using 0.1 M silver nitrate). Quantitatively transfer the filter cake into a 600-ml beaker using distilled water and, making sure to rinse the Buchner funnel to wash any starch into the beaker.Bring to about 300-ml using distilled water. Place the beaker on a boiling water bath for 10 min with stirring. Titrate, while hot, with 0.1 M sodium hydroxide using phenolphthalein TS as an indicator. Repeat the titration procedure with native unmodified starch of the same origin as the OSA starch sample, as a blank.

Calculation

$$\text{Octenylsuccinyl groups (\%)} = \frac{21.1 \times M \times [V_{sample} - V_{blank}]}{W}$$

where

V_{sample} is the titration volume of sodium hydroxide for the sample, ml
$V_{blank}$is the titration volume of sodium hydroxide for the blank, ml
M is the molarity of sodium hydroxide
W is the dry weight of sample, g

Residual octenyl succinic acid in starch Sodium octenyl succinate

Determine by HPLC on the 2-bromoacetophenone-derivatised methanolic extract of the sample.

Extraction and Preparation of Sample Solution

Accurately weigh 500 mg (to nearest 0.1 mg) of the sample in a 25 ml Erlenmeyer flask, add 15 ml of methanol, stopper the flask and shake it on a shaker overnight. Filter the extract using a filter paper, wash the residue, three times with 7 ml portions of methanol and combine the filtrate (about 80% of the OSA residues is extracted by this procedure). Add 1 ml of 0.16 M KOH in methanol to the combined filtrate. Dry the extract using a flash evaporator at 30° and dissolve the residue in 2 ml of methanol. Pipette 0.5 ml of this solution into a reaction vial, add 0.5 ml of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and

0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml CH_3CN]. Add 2 ml CH_3CN to the reaction vial, cap the vial and heat at 80° for 30 min. Allow the vial to reach room temperature and analyse by HPLC within 24 h.

HPLC Conditions:
Column: µ-Bondapack C18 or equivalent
Mobile Phase: Methanol and Water with gradient elution: 70% to 80% of methanol in water in 5 min
Flow rate: 1.5 ml/min
Detector: UV at 254 nm
Injection volume: 5 µl

Preparation of Standard Curve
Prepare a 105.14 mg/ml solution of octenylsuccinic acid anhydride (available from Milliken Chemicals) in methanol (Solution A). Using a syringe draw 0.25 ml of Solution A, transfer into a 25-ml volumetric flask and dilute to mark with methanol (Solution B).

Prepare three working standards (Solution C1, C2 and C3) by transferring 0.5, 1 and 2 ml each of Solution B into three 50-ml round bottom flasks, add 1 ml of 0.16 M KOH in methanol to each flask, dry the solution using a flash evaporator at 30° and dissolve the residue in 2.0 ml of methanol. To 0.5 ml each of these solutions in reaction vials, add 0.5 ml each of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml of CH_3CN]. Add 2 ml of CH_3CN to each vial; cap the vials and heat for 30 min at 80°. Allow the vials to reach room temperature and analyze by HPLC immediately. The amount of octenyl succinic acid in each 5-µl injection is as follows:

Solution C1: 0.2375 µg
Solution C2: 0.4750 µg
Solution C3: 0.9500 µg

Construct the standard curve using peak height against the amount of standard in the injection.

Inject 5-µl of prepared sample solution and read the amount of octenyl succinic acid in the injection from the standard curve.

Calculation

$$\% \text{ Residual octenyl succinic acid} = \frac{300 \times V}{W}$$

where
V is the amount of OSA in the injected volume; and
W is the weight of the sample (mg).

NOTE: The formula is corrected to 100% recovery by dividing with 0.80, so that 240/0.80 = 300.

OCTANOIC ACID

Prepared at the 82nd JECFA (2016), published in FAO JECFA Monograph 19 (2016), superseding specifications prepared at the 63rd JECFA (2004) and published in FNP 52 Add 12 (2004). Small residues of octanoic acid on food (which has been treated with antimicrobial washing solutions) at the time of consumption would not pose a safety concern (63rd JECFA, 2004).

SYNONYMS Caprylic acid

DEFINITION Octanoic acid is manufactured from vegetable oils (coconut, palm, kernel, or palm stearin) by first refining the oil, followed by methyl transesterification and separation by distillation. The separated methyl octanoate is saponified and acidified to give octanoic acid.

Chemical name Octanoic acid

C.A.S. number 124-07-2

Chemical formula $CH_3(CH_2)_6CO_2H$

Formula weight 144.21

Assay Not less than 95%

DESCRIPTION Colourless oily liquid

FUNCTIONAL USES Antifoaming agent, surfactant, flavouring agent (see JECFA No. 99)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Slightly soluble in water; soluble in most organic solvents

frared spectrum (Vol. 4) The infrared spectrum of a liquid film of a sample corresponds to the reference infrared spectrum in the Annex.
Hold 1 to 2 drops of a sample between two optical plates and examine the liquid film of a sample between the plates using an infrared spectrometer. The optical plates are usually made by sodium chloride or potassium bromide.

Acid value (Vol. 4) Between 366 and 396

PURITY

Water (Vol. 4) Not more than 0.4% (Karl Fischer Method)

Sulfated ash (Vol. 4) Not more than 0.1%
Test 10 g of the sample (Method II)

Unsaponifiable matter	Not more than 0.2% See description under TESTS
Iodine value (Vol. 4)	Not more than 2.0
Decanoic acid	Not more than 3% See description under METHOD OF ASSAY
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Unsaponifiable matter

Weigh accurately 5.0 g of the sample into a 250 ml flask, add a solution of 2 g of potassium hydroxide in 40 ml of ethanol, and boil gently under a reflux condenser for 1 h or until saponification is complete. Transfer the contents of the flask to a glass-stoppered graduated extraction cylinder. Wash the flask with sufficient ethanol to make a volume of 40 ml in the cylinder, and complete the transfer with warm and then cold water until the total volume is 80 ml. Finally, wash the flask with a few ml of petroleum ether, add the washings to the cylinder, cool the contents of the cylinder to room temperature, and add 50 ml of petroleum ether.

Insert the stopper, shake the cylinder vigorously for at least 1 min, and allow both layers to become clear. Siphon the upper layer as completely as possible without removing any of the lower layer, collecting the ether fraction in a 500-ml separator. Repeat the extraction and siphoning at least six times with 50-ml portions of petroleum ether, shaking vigorously each time. Wash the combined extracts, with vigorous shaking, with 25-ml portions of 10% ethanol until the wash water is neutral to phenolphthalein, and discard the washings. Transfer the petroleum ether extract to a tared beaker, and rinse the separator with 10 ml of petroleum ether, adding the rinsings to the beaker. Evaporate the solvent on a steam bath just to dryness, and dry the residue to constant weight, preferably at 75° to 80° under a vacuum of not more than 200 mm of Hg, or at 100° for 30 min. Cool in a desiccator, and weigh to obtain the uncorrected weight of unsaponifiable matter.

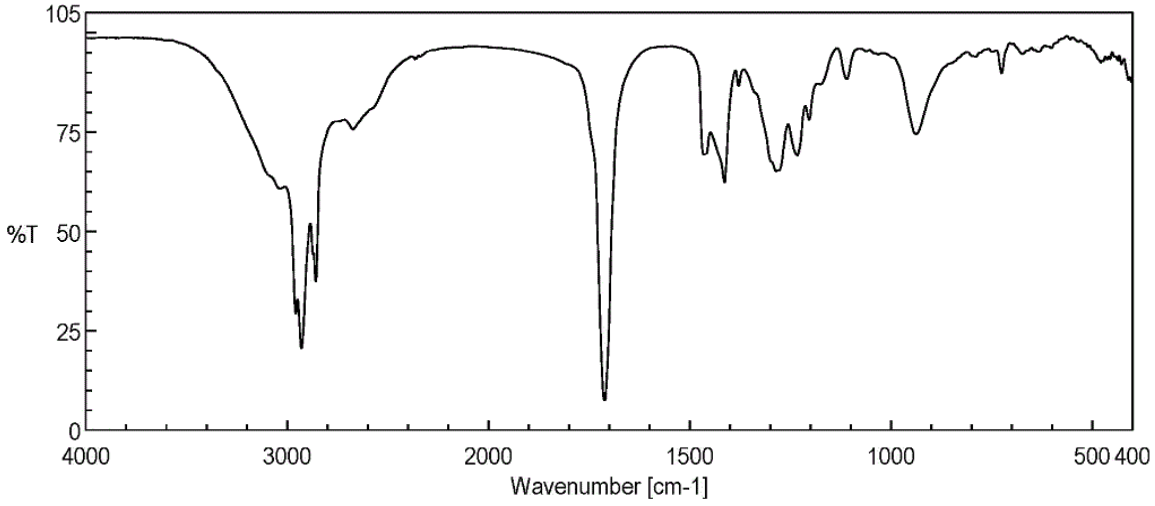
Determine the quantity of fatty acids in the residue as follows: Dissolve the residue in 50 ml of warm ethanol (containing phenolphthalein TS and previously neutralized with sodium hydroxide to a faint pink colour), and titrate with 0.02 M sodium hydroxide to the same color. Each ml of 0.02 M sodium hydroxide is equivalent to 5.659 mg of fatty acids, calculated as oleic acid.

Subtract the calculated weight of fatty acids from the weight of the residue to obtain the corrected weight of unsaponifiable matter in the sample.

METHOD OF ASSAY Determine using an appropriate gas chromatographic technique. The selection of sample size and method of sample preparation may be based on AOCS Method Ce 1-62 and Ce 1j-07 and follow the principles of the method described in Vol. 4 (under Analytical Techniques, Gas Chromatography). The percentage of octanoic acid is the area percent of the methyl octanoate peak.

Decanoic acid may be determined using the same gas chromatographic technique.

Appendix Infrared spectrum of octanoic acid standard



OCTENYL SUCCINIC ACID MODIFIED GUM ARABIC

Prepared at the 82nd JECFA (2016) and published in the FAO JECFA Monographs 19 (2016), superseding tentative specifications prepared at the 79th JECFA (2014) and published in the FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 82nd JECFA (2016)

SYNONYMS Gum arabic hydrogen octenylbutandioate; Gum arabic hydrogen octenylsuccinate; OSA modified gum arabic; OSA modified gum acacia; INS No. 423

DEFINITION Octenyl succinic acid modified gum arabic is produced by esterifying gum arabic (*Acacia seyal*), or gum arabic (*Acacia senegal*) in aqueous solution with octenyl succinic acid anhydride. The modified gum, containing not more than 3% octenyl succinate on a weight basis is subsequently spray dried.

C.A.S. number 455885-22-0

DESCRIPTION Off-white to light tan, free flowing powder

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water; insoluble in ethanol

^{1}H-NMR spectrum The ^{1}H-NMR spectrum of the sample obtained using the procedure described in Tests under "Esterified octenyl succinic acid" corresponds to the reference ^{1}H-NMR spectrum in the Appendix.

pH (Vol. 4) 3.5 to 6.5 (5% solution)

Viscosity Not more than 30 cP (5% solution, 25°)
Add 95 ml of water to a beaker. Place a magnetic stir bar into the water and while stirring add 5 g of the sample. Stir on medium speed for 2 h. Measure viscosity on Brookfield LV viscometer, or equivalent, using spindle number 3 at 30 rpm (factor = 40).

PURITY

Esterified octenyl succinic acid Not more than 3%
See description under TESTS

Loss on drying (Vol.4) Not more than 15% (105°, 5h)

Total ash (Vol.4) Not more than 10% (530°)

Acid-insoluble ash (Vol.4) Not more than 0.5%

Water-insoluble matter (Vol. 4)	Not more than 1.0%
Starch or dextrin	Boil a 1 in 50 aqueous solution of the sample, add about 0.1 ml iodine TS. No bluish or reddish colour should be produced.
Tannin-bearing gums	To 10 ml of a 1 in 50 aqueous solution of the sample add about 0.1 ml ferric chloride TS. No blackish coloration or blackish precipitate should be formed.
Residual octenyl succinic acid	Not more than 0.3% See description under TESTS
Microbiological criteria (Vol. 4)	*Salmonella* species: absent in 25 g *Escherichia coli*: absent in 1 g
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Esterified octenyl succinic acid

Principle: Determine using ^{1}H-NMR method to measure remaining octenyl succinic groups present in product following extraction of residual octenyl succinic acid (OSA).

Procedure

Extraction of residual OSA

Accurately weigh 500 mg (to nearest 0.1 mg) of the sample in a 25 ml Erlenmeyer flask, add 15 ml of methanol, stopper the flask and shake it on a shaker overnight. Filter the extract using a filter paper and wash the solid residue, three times with 7 ml portions of methanol. Allow the methanol to evaporate from the filter cake in the hood and dry the filter cake in a forced air oven at 40° overnight. Grind the dried cake using mortar and pestle.

^{1}H NMR Solvent system A

Dimethyl sulfoxide-d_6 (DMSO-d_6 containing 1.88% w/w deuterated trifluoroacetic acid (TFA-d_1) and 0.42% w/w recrystallized 1,4-bis(trichloromethyl)benzene (BTCMB, recrystallized from hexane). In the solvent system, BTCMB is an internal standard.

^{1}H NMR Solvent system B

Dimethyl sulfoxide-d_6 (DMSO-d_6 containing 1.88% w/w deuterated trifluoroacetic acid (TFA-d_1) and 1.25 mg/ml OSA standard.

System validation

Dissolve 15 mg dried purified non-modified gum Arabic in total 750 µl NMR solvent system mixture A & B prepared according to the following Table. Heat the solution to fully solubilize the purified gum Arabic. Transfer the solution into a 5 mm NMR tube.

Volume A (µl)	Volume B (µl)	BTCMB (mg)	OSA (mg)	OSA % (theoritecal)
750	0	3.75	0	0
620	130	3.1	0.16	1.08
500	250	2.50	0.31	2.08
400	350	1.75	0.44	2.92

Sample preparation
Dissolve 15 mg dried purified OSA-modified gum Arabic in 750 µl ^{1}H NMR solvent system A. Heat the solution to fully solubilize the purified OSA-modified gum Arabic. Transfer the solution into a 5 mm NMR tube.

Determine percent of esterified OSA in OSA-modified gum Arabic by ^{1}H-NMR using a 400 MHz NMR spectrometer.
Experimental conditions:
Temperature: 85°.
17.8 µs 90° pulse, 32 second relaxation delay, 1.37 second acquisition time, 8192 data points, 5,973.8 Hz sweep width, 0.5 Hz exponential apodization line broadening, 16 scans.

Calculation

Use the OSA methyl proton peak at 0.8-0.89 ppm and BTCMB peak at 8.1 ppm to calculate the percent of esterified OSA in the sample.

$$\% \text{ OSA} = \frac{I_{\text{OSA-Me}}}{I_{\text{BTCMB}}} \times \frac{4}{3} \times \frac{210.27}{312.84} \times \frac{W_{\text{BTCMB}}}{W_{\text{MGA}}} \times 100$$

where:

$I_{\text{OSA-Me}}$ is integrated peak area of the OSA methyl proton peak;
I_{BTCMB} is integrated peak area of the BTCMB internal standard proton peak;
W_{BTCMB} is the weight of BTCMB internal standard in mg (4.2 mg/ml x 0.750 ml) and W_{MGA} is the weight of modified gum Arabic present in solution in the NMR experiment tube in mg.

NOTE:

1: The signal intensity for OSA-Me comes from 3 protons/molecule, therefore, $I_{\text{OSA-Me}}/3$ corresponds to the number of molecules of OSA. The signal intensity for BTCMB comes from 4 protons/molecule and so $I_{\text{BTCMB}}/4$ corresponds to the number of molecules of BTCMB. 210.27 and 312.84 are the molecular weights of OSA and BTCMB, respectively.

2. Plot the % OSA theoretically calculated in the Table above vs the % OSA calculated by the NMR measurement, using the weight of the unmodified gum arabic in the place of W_{MGA}. (A linear correlation should be obtained with correlation coefficient >0.99, slope close to 1 and low intercept).

3. Use the correlation slope and intercept to correct the calculated amount in the sample.

Residual octenyl succinic acid

Determine by HPLC on the 2-bromoacetophenone-derivatised methanolic extract of the sample.

Extraction and Preparation of Sample Solution

Accurately weigh 500 mg (to nearest 0.1 mg) of the sample in a 25 ml Erlenmeyer flask, add 15 ml of methanol, stopper the flask and shake it on a shaker overnight. Filter the extract using a filter paper, wash the residue, three times with 7 ml portions of methanol and combine the filtrate (about 80% of the OSA residues is extracted by this procedure). Add 1 ml of 0.16 N KOH in methanol to the combined filtrate. Dry the extract using a flash evaporator at 30° and dissolve the residue in 2 ml of methanol. Pipette 0.5 ml of this solution into a reaction vial, add 0.5 ml of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml CH_3CN]. Add 2 ml CH_3CN to the reaction vial, cap the vial and heat at 80° for 30 min. Allow the vial to reach room temperature and analyse the reaction product by HPLC within 24 h.

HPLC Conditions:

Column: µ-Bondapack C18 or equivalent
Mobile Phase: Methanol and Water with gradient elution: 70% to 80% of methanol in water in 5 min
Flow rate: 1.5 ml/min
Detector: UV at 254 nm
Injection volume: 5 µl

Preparation of Standard Curve

Prepare a 105.14 mg/ml solution of octenylsuccinic acid anhydride (available from Milliken Chemicals) in methanol (Solution A). Using a syringe draw 0.25 ml of Solution A, transfer into a 25-ml volumetric flask and dilute to mark with methanol (Solution B).

Prepare three working standard (Solution C1, C2 and C3) by transferring 0.5, 1 and 2 ml each of Solution B into three 50-ml round bottom flasks, add 1 ml of 0.16 N KOH in methanol to each flask, dry the solution using a flash evaporator at 30° and dissolve the residue in 2.0 ml of methanol. To 0.5 ml each of these solutions in reaction vials, add 0.5 ml each of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml of CH_3CN]. Add 2 ml of CH_3CN to each vial, cap the vials and heat for 30 min at 80°. Allow the vials to reach room temperature and analyze by HPLC immediately. The amount of octenyl succinic acid in each 5-µl injection is as follows:

Solution C1: 0.2375 µg
Solution C2: 0.4750 µg
Solution C3: 0.9500 µg

Construct the standard curve using peak area against the amount of standard in the injected volume.

Inject 5-µl of prepared sample solution and read the amount of octenyl succinic acid in the injection from the standard curve.

Calculation

$$\% \text{ Residual octenyl succinic acid} = \frac{300 \times V}{W}$$

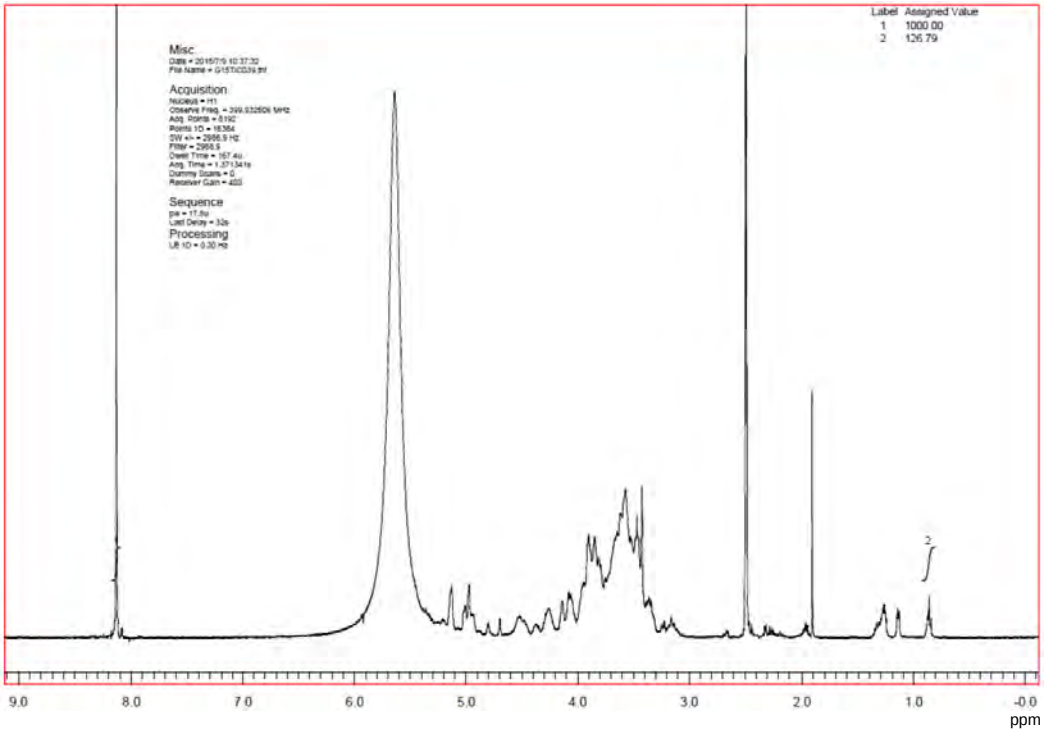
where

V is the amount of OSA (µg) in the injected volume; and

W is the weight of the sample (mg).

NOTE: The formula is corrected to 100% recovery by dividing with 0.80, so that 240/0.80 = 300.

Appendix Representative ^{1}H NMR spectrum of OSA modified gum Arabic



PECTINS

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016) superseding specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A group ADI "not specified" was established for pectins and amidated pectins, singly or in combination at the 25th JECFA (1981).

SYNONYMS INS No. 440

DEFINITION Consists mainly of the partial methyl esters of polygalacturonic acid and their sodium, potassium, calcium and ammonium salts; obtained by extraction in an aqueous medium of appropriate edible plant material, usually citrus fruits or apples; no organic precipitants shall be used other than methanol, ethanol and isopropanol; in some types a portion of the methyl esters may have been converted to primary amides by treatment with ammonia under alkaline conditions. Sulfur dioxide may be added as a preservative.

The commercial product is normally diluted with sugars for standardization purposes. In addition to sugars, pectins may be mixed with suitable food-grade buffer salts required for pH control and desirable setting characteristics. The article of commerce may be further specified as to pH value, gel strength, viscosity, degree of esterification, and setting characteristics.

C.A.S. number 9000-69-5

DESCRIPTION White, yellowish, light greyish or light brownish powder

FUNCTIONAL USES Gelling agent, thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Test for pectins — Passes test
See description under TESTS

Test for amide group — Passes test (amidated pectins only)
Add 2 ml of concentrated hydrochloric acid and 50 ml of 60% ethanol to 0.5 g of the sample, and stir well for 20 min. Transfer to a fritted glass filter tube wash with six 10 ml portions of the HCl-60% ethanol mixture. Dissolve in 100 ml distilled water; it may be necessary to add a few drops 0.1 mol/L sodium hydroxide to achieve solution. Transfer 4 ml of this solution into a test tube (recommended dimensions 15.5 mm inner diameter and 146 mm length). Add 1 ml 5 mol/L sodium hydroxide and mix. The mixture will form a gel. Fill a small glass tube (recommended dimensions 7.8 mm inner diameter and 79 mm length) with 2.5 ml boric acid TS and let glide into the test tube. Close with parafilm and incubate overnight at 30°. In case of presence of amide groups the indicator changes its colour from red to green, due to release of ammonia.

PURITY

Loss on drying (Vol. 4)	Not more than 12% (105°, 2 h)
Sulfur dioxide	Not more than 50 mg/kg See description under TESTS
Residual solvents (Vol. 4)	Not more than 1% methanol, ethanol and isopropanol, singly or in combination See description under TESTS
Acid-insoluble ash Vol. 4)	Not more than 1%
Total insolubles	Not more than 3% See description under TESTS
Nitrogen content (Vol. 4)	Not more than 2.5% after washing with acid and ethanol
Galacturonic acid	Not less than 65% calculated on the ash-free and dried basis See description under TESTS
Degree of amidation	Not more than 25% of total carboxyl groups of pectin See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities.")

TESTS

IDENTIFICATION TESTS

Test for Pectins

Moisten 0.05 g of the sample with 2-propanol. Add 50 ml of water on a magnetic stirrer. Adjust pH to 12 using 0.5 mol/l sodium hydroxide and let the solution remain without stirring for 15 min. Reduce pH to 7.0 with 0.5 mol/l hydrochloric acid. Adjust to 100.0 ml with water. Make up samples in 1 cm quartz cuvettes as follows:

	Buffer pH 7.0 *)	Sample soln	Water	Enzyme soln **)
Enzyme blank	0.5 ml	1.0 ml	1.0 ml	-
Sample blank	0.5 ml	-	1.5 ml	0.5 ml
Sample	0.5 ml	1.0 ml	0.5 ml	0.5 ml

*) Dissolve 6.055 g of tris(hydroxymethyl)aminomethane (e.g. TRIZMA Base, Sigma) and 0.147 g of calcium chloride dihydrate in water to 1 l. Adjust pH to 7.0 with 1 mol/l hydrochloric acid

**) Dilute pure pectate lyase 1:100 with buffer pH 7.0
Shake the solutions well, and measure the absorbance at 235 nm at 0 and 10 min.

Calculations

A_0 = absorbance at 0 min = Sample - (enzyme blank + sample blank)
A_{10} = absorbance at 10 min = Sample - (enzyme blank + sample blank)

The amount of unsaturated product produced is proportional to the change in absorbance (A_{10} - A_0). This value should be greater than 0.023. This distinguishes pectins from other gums, which show essentially no change.

PURITY TESTS

Sulfur dioxide

Suspend 100 g of the sample in 500 ml of methanol in a 1000-ml round-bottom flask, which is provided with a gas inlet tube reaching almost the bottom and connected to the neck with a reflux condenser. Prepare a glass joint connection from the condenser to an absorption flask or U-tube containing 10 ml of 3% hydrogen peroxide solution neutralized to methyl red TS. Connect the gas inlet tube with an oxygen-free source of carbon dioxide or nitrogen, and maintain a gas stream so as to cause steady bubbling. As soon as the apparatus is flushed free of air, pour 30 ml of hydrochloric acid solution (10 ml conc. HCl + 20 ml H_2O) into the reflux condenser, and immediately connect the absorption flask or U-tube. Heat slowly until methanol starts refluxing, and reflux gently for 2 h. Disconnect the apparatus and titrate the hydrogen peroxide solution against methyl red TS with 0.01 mol/l sodium hydroxide. Each ml of 0.01 mol/l sodium hydroxide corresponds to 0.32 mg of SO_2.

Total insolubles

Dry a 70 mm glass fiber filter paper (GF/B (Whatman code 1821 070) in an oven with fan set at 105° for about 1 h. Transfer the filter paper to a desiccator containing silica gel and allow to cool. Weigh the paper (M_1). Weigh about 1 g (= S) of the sample into a 250-ml beaker. Add 5 ml of 2-propanol to disperse the sample. While stirring magnetically, add 100 ml of 0.03 mol/l sodium hydroxide containing 0.1% (w/w) ethylene diamine tetra-acetic acid (Na salt), which has been filtered through GF/B paper. Stir for about 30 min at room temperature, then heat to boiling (remove heat if excessive foaming occurs). Filter the hot solution through the glass fiber paper under vacuum using, e.g. a vacuum filtration kit with 3 piece Hartley funnel (70 cm), with heat resistant plate. Rinse the beaker five times and filter the rinsings with 100 ml of warm (about 50°) water that has been filtered through GF/B paper. Dry the filter paper with the residue at 105° for 1 h. Transfer to desiccator containing silica gel and leave to cool. Weigh the paper (M_2). Calculate the percentage of total insolubles from

$$\text{Total insolubles (\%)} = [(M_2 - M_1)/S] \times 100$$

Galacturonic acid and Degree of amidation

Weigh 5 g of the sample to the nearest 0.1 mg, and transfer to a suitable beaker. Stir for 10 min with a mixture of 5 ml of hydrochloric acid TS, and 100 ml of 60% ethanol. Transfer to a fritted-glass filter tube (30 to 60 ml capacity) and wash with six 15-ml portions of the HCl-60% ethanol mixture, followed by 60% ethanol until the filtrate is free of chlorides. Finally wash with 20 ml of ethanol, dry for 2.5 h in an oven at 105°, cool and weigh. Transfer exactly one-tenth of the total net weight of the dried sample (representing 0.5 g of the original unwashed sample) to a 250-ml conical flask and moisten the sample with 2 ml of ethanol TS. Add 100 ml of recently boiled and cooled distilled water, stopper and swirl occasionally until a complete solution is formed. Add

5 drops of phenolphthalein TS, titrate with 0.1 mol/l sodium hydroxide and record the results as the initial titre (V_1).

Add exactly 20 ml of 0.5 mol/l sodium hydroxide TS, stopper, shake vigorously and let stand for 15 min. Add exactly 20 ml of 0.5 mol/l hydrochloric acid and shake until the pink colour disappears. Titrate with 0.1 mol/l sodium hydroxide to a faint pink colour which persists after vigorous shaking; record this value as the saponification titre (V_2). Quantitatively transfer the contents of the conical flask into a 500-ml distillation flask fitted with a Kjeldahl trap and a water-cooled condenser, the delivery tube of which extends well beneath the surface of a mixture of 150 ml of carbon dioxide-free water and 20.0 ml of 0.1 mol/L hydrochloric acid in a receiving flask. To the distillation flask add 20 ml of a 1-in-10 sodium hydroxide solution, seal the connections, and then begin heating carefully to avoid excessive foaming. Continue heating until 80-120 ml of distillate has been collected. Add a few drops of methyl red TS to the receiving flask, and titrate the excess acid with 0.1 mol/l sodium hydroxide recording the volume required, in ml, as S. Perform a blank determination on 20.0 ml of 0.1 mol/l hydrochloric acid, and record the volume required, in ml, as B. The amide titre is (B - S).

Transfer exactly one-tenth of total net weight of the dried sample (representing 0.5 g of the original unwashed sample) and wet with about 2 ml ethanol in a 50-ml beaker. Dissolve the pectin in 30 ml of 0.1 mol/l sodium hydroxide. Let the solution stand for 1 h with agitation at room temperature. Transfer quantitatively the saponified pectin solution to a 50-ml measuring flask and dilute to the mark with distilled water. Transfer 25 ml of the diluted pectin solution to a distillation apparatus and add 20 ml of Clark's solution, which consists of 100 g of magnesium sulfate heptahydrate and 0.8 ml of concentrated sulphuric acid and distilled water to a total of 180 ml. This apparatus consists of a steam generator connected to a round-bottom flask to which a condenser is attached. Both steam generator and round-bottom flask are equipped with heating mantles.

Start the distillation by heating the round-bottom flask containing the sample. Collect the first 15 ml of distillate separately in a measuring cylinder. Then start the steam supply and continue distillation until 150 ml of distillate have been collected in a 200-ml beaker. Add quantitatively the first 15 ml distillate and titrate with 0.05 mol/l sodium hydroxide to pH 8.5 and record volume required, in ml, as A.

Perform a blank determination on 25 ml distilled water. Record the required volume, in ml, as A_0. The acetate ester titre is ($A - A_0$). Calculate degree of amidation (as % of total carboxyl groups) by the formula:

$$100 \times \frac{B - S}{V_1 + V_2 + (B - S) - (A - A_0)}$$

Calculate mg of galacturonic acid by the formula:

$$19.41 \times [V_1 + V_2 + (B - S) - (A - A_0)]$$

The mg of galacturonic acid obtained in this way is the content of one-tenth of the weight of the washed and dried sample. To calculate %

galacturonic acid on a moisture- and ash-free basis, multiply the number of mg obtained by 1000/x, x being the weight in mg of the washed and dried sample.

NOTE 1: If the pectin is known to be of the nonamidated type, only V1 and V2 need to be determined and (B - S) may be regarded as zero.
NOTE 2: For pectins from apple or citrus ($A - A_0$) is usually insignificant in calculating galacturonic acid and degree of amidation.
NOTE 3: If desired, calculate degree of esterification (as % of total carboxyl groups) by the formula:

$$100 \times \frac{V_2 - (A - A_0)}{V_1 + V_2 + (B - S) - (A - A_0)}$$

NOTE 4: If desired, calculate degree of acetate ester (as % of total carboxylic groups from galacturonic acid) by the formula:

$$100 \times \frac{A - A_0}{V_1 + V_2 + (B - S) - (A - A_0)}$$

Residual solvents (Vol. 4)

Determine residual solvents using headspace gas chromatography (Method I)

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

Standard solution: Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15 µl ethanol and weigh to within 0.01mg. Inject 15 µl isopropanol through the septum and reweigh the vial.

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

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

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

Follow the procedure described in Vol. 4.

QUINOLINE YELLOW

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016), superseding tentative specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011). A temporary ADI of 0-5 mg/kg bw was established at the 74th JECFA (2011).

SYNONYMS INS No. 104; CI Food Yellow 13; CI (1982) No. 47005

DEFINITION Quinoline Yellow is manufactured by sulfonating 2-(2-quinolyl)-1,3-indandione. It consists predominantly of sodium salts of disulfonates of 2-(2-quinolyl)-1,3-indandione with smaller amounts of monosulfonates and trisulfonates; and subsidiary colouring matters, sodium chloride and/or sodium sulfate.

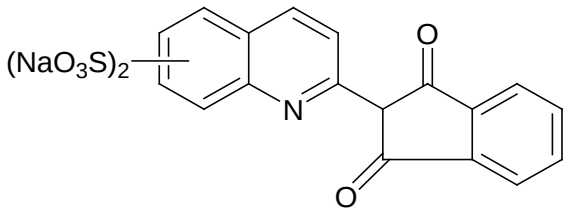
Quinoline Yellow may be converted to the corresponding aluminium lake, in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical name Disodium 2-(2-quinolyl)indan-1,3-dionedisulphonate (principal component)

C.A.S. number 80583-08-0 (principal component)

Chemical formula $C_{18}H_9NO_8S_2Na_2$ (principal component)

Structural formula (Principal component)



Formula weight 477.38 (Principal component)

Assay Not less than 70% total colouring matters.
Of the total colouring matters present:
- not less than 80% of disodium 2-(2-quinolyl)-indan-1,3-dione-disulfonates;
- not more than 15% of sodium 2-(2-quinolyl)-indan-1,3-dione-monosulfonates;
- not more than 7% of trisodium 2-(2-quinolyl)-indan-1,3-dione-trisulfonate

DESCRIPTION Yellow powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in water; sparingly soluble in ethanol
Spectrophotometry (Vol.4)	The UV-visible absorption spectrum of an aqueous solution of the sample shows a maximum wavelength approximately at 414 nm.

PURITY

Loss on drying at 135°, chloride and sulfate as sodium salts (Vol. 4)	Not more than 30% Determine according to Loss on drying in Volume 4 (under "Specific Methods, Food Colours").
Water-insoluble matter (Vol. 4)	Not more than 0.2%
Subsidiary colouring matters	Not more than 4 mg/kg of 2-(2-quinolyl)-1,3-indandione and 2-[2-(6-methylquinolyl)]-1,3-indandione See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5%, sum of 2-methylquinoline, 2-methylquinolinesulfonic acid and phthalic acid See description under TESTS
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline
Ether-extractable matter (Vol. 4)	Not more than 0.2%
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").
Zinc (Vol. 4)	Not more than 50 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Subsidiary colouring matters	Determine 2-(2-quinolyl)-1,3-indandione and 2-[2-(6-methyl-quinolyl)]-1,3-indandione by reversed-phase HPLC (Vol.4) using the following conditions: Solvent A: 0.05 M ammonium acetate Solvent B; acetonitrile

Injection volume: 100 µl
Column: C18 (250 mm x 4.6 mm i.d., 5 µm particle size)
Detector: UV-Vis/PDA at 436 nm
Flow rate: 1 ml/min

Gradient:

Min	%A	%B
0	40	60
19.9	40	60
20.0	0	100
35.0	0	100

Standards: 2-(2-quinolyl)-1,3-indandione, Sigma 01354 or equivalent; 2-[2-(6-methyl-quinolyl)]-1,3-indandione, Chemos GmbH or equivalent.

Sample preparation: Dissolve 1 g of sample in 10 ml of hot water. Allow solution to cool to room temperature. Extract analytes using chloroform, evaporate the solvent and dissolve the residue in acetonitrile.

Organic compounds other than colouring matters
(Vol. 4)

Determine sum of 2-methylquinoline, 2-methylquinolinesulfonic acid and phthalic acid by reversed-phase HPLC (under “Specific Methods, Food Colours”) using the following conditions:
Solvent A: 0.2 M ammonium acetate in water/methanol (95:5, v/v)
Solvent B: methanol
Injection volume: 20 µl
Detector wavelength: UV/PDA at 254 nm
Flow rate: 1 ml/min

Gradient:

Min	%A	%B
0	100	0
8.8	80	20
9.0	0	100
12.0	0	100

Note: A general gradient for the separation of organic compounds other than colouring matters in several food colours is given in Vol. 4. The above gradient may be use for the analytes in Quinoline Yellow.

Standard solution: 0.1% of quinaldine-6-sulfonic acid, quinaldine-6,8-disulfonic acid, trisodium salt of 4-sulfophthalic acid and 3-sulfophthalic acid in Solvent A
Sample solution: 100 mg (± 5 mg) of sample in 10 ml of water

METHOD OF ASSAY

Determine total colouring matters using Procedure 1 in Volume 4 (under “Specific Methods, Food Colours”) and mono-, di- and trisulfonates of 2-(2-quinolyl)-indan-1,3-dione by reversed-phase HPLC (Vol.4).

Total colouring matters
Solvent: Water
Absorptivity (a) = 87.9 L/(g·cm)
Wavelength of maximum absorbance = 414 nm

Mono-, di- and trisulfonates
Determine sodium salts of mono-, di- and trisulfonates of 2-(2-quinolyl)-indan-1,3-dione by reversed-phase HPLC using the following conditions:

Solvent A: 0.1 M ammonium acetate in water/methanol (95:5, v/v)
Solvent B: methanol
Injection volume: 20 µl
Column: Hypersil RP C8 (250 mm x 4.6 mm i.d.,5 µm particle size) or equivalent.
Column temperature: 25 º
Detector: UV-Vis/PDA at 414 nm
Flow rate: 1 ml/min

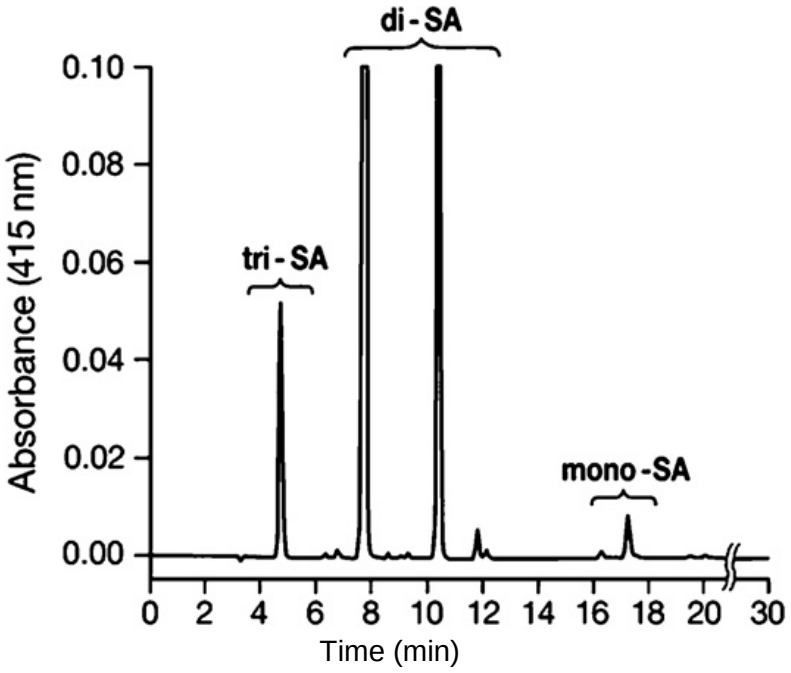
Gradient:

Min	%A	%B
0	90	10
30	0	100

Standard solution (for retention times): 10 mg of Quinoline Yellow containing mono-, di-, and trisulfonates in 100 ml of water/methanol (75:25).
Sample solution: 10 mg of sample in 100 ml of water/methanol (75:25).

Calculation: Express the results as percentage of the peak area of each components/the peak area of total peaks on the chromatogram.

Appendix Typical HPLC chromatogram of Quinoline Yellow



mono-SA: sodium 2-(2-quinolyl)-indan-1,3-dione-monosulfonates
di-SA: disodium 2-(2-quinolyl)-indan-1,3-dione-disulfonates
tri-SA: trisodium 2-(2-quinolyl)-indan-1,3-dione-trisulfonate

[Reference] A. Weisz, E. P. Mazzola, and Y. Ito, “Preparative Separation of Di- and Trisulfonated Components of Quinoline Yellow Using Affinity-Ligand pH-Zone-Refining Counter-Current Chromatography,” J. Chromatography A, 1216, 4161-4168 (2009).

REBAUDIOSIDE A FROM MULTIPLE GENE DONORS EXPRESSED IN *YARROWIA LIPOLYTICA*

New Specifications prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016). ADI for steviol glycosides of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).

SYNONYMS

DEFINITION

Rebaudioside A is a steviol glycoside composed predominantly of rebaudioside A with minor amounts of other steviol glycosides such as rebaudioside B, rebaudioside D, rebaudioside M, and stevioside.

Rebaudioside A is obtained from the fermentation of a non-toxigenic non-pathogenic strain of *Yarrowia lipolytica* that is genetically modified with heterologous genes from multiple donor organisms to overexpress steviol glycosides. After removal of the biomass by solid-liquid separation and heat treatment, the process involves concentration of the steviol glycosides (e.g. by resin adsorption), followed by purification of the rebaudioside A by crystallization and drying. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Chemical names

13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester

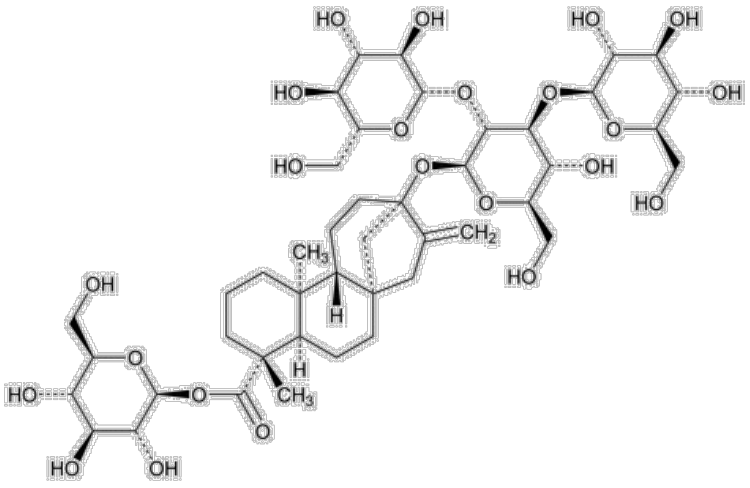
C.A.S. number

58543-16-1

Chemical formula

$C_{44}H_{70}O_{23}$

Structural Formula[3]



Formula weight

967.01

Assay

Not less than 95% calculated on the anhydrous basis

[3] FCC10-: U.S. Pharmacopeial Convention, Rebaudioside A Monograph, 2016. Reproduced with permission.

DESCRIPTION White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION[4]

Solubility (Vol. 4)	Freely soluble in ethanol:water 50/50 (v/v), sparingly soluble in water, and sparingly soluble in ethanol.
HPLC	The main peak in the Sample chromatogram obtained by following the procedure in METHOD OF ASSAY corresponds to rebaudioside A.
pH (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)
Infrared spectrum	The infrared spectrum of the sample using attenuated total reflectance (ATR) analysis corresponds to that of the standard. Rebaudioside A standards available from Wako Pure Chemical Industries, Ltd. Japan, US Pharmacopeia, USA, and ChromaDex, USA

PURITY

Related Steviol Glycosides	The sum of the percentages for stevioside, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside is NMT 5% on the anhydrous basis. See description under TESTS.
Total ash (Vol. 4)	Not more than 1%
Water (Vol. 4)[4]	Not more than 6% Determination using the Karl Fischer Titrimetric Method.
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
Lead (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY

Solution A, Solution B, Mobile phase, Diluent, System suitability solution, Sample, Sample solution, Standard solution 2, HPLC

[4] FCC10-: U.S. Pharmacopeial Convention, Rebaudioside A Monograph, 2016. Reproduced with permission.

Related steviol glycosides

conditions, and System suitability: Prepare as directed in METHOD OF ASSAY.

Analysis
Separately inject equal volumes of the System suitability solution, Standard solution 2, and Sample solution into the chromatograph. Calculate the percentages of rebaudioside D, stevioside, rebaudioside F, rebaudioside C, dulcoside A, rubusoside, rebaudioside B, and steviolbioside in the portion of the sample taken as follows, which takes into account the UV response factors between the analytes and rebaudioside A:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak area for the analyte in the Sample solution
r_S = peak area for rebaudioside A in Standard solution
C_S = concentration of rebaudioside A in Standard solution 2, corrected for purity and water content (mg/ml)
C_U = concentration of the sample in the Sample solution corrected for water content (mg/ml)
F = relative response factor (see Table 2)

Add together the individual percentages of the eight measured steviol glycoside impurities corrected for water.

METHOD OF ASSAY[5]

Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions.

Mobile phase
Solution A: 5 mM potassium phosphate buffer, pH 3.0. Prepare by dissolving 1.36 g of potassium dihydrogen phosphate (KH_2PO_4) in water in a 2-L volumetric flask, adjusting with phosphoric acid to a pH of 3.0, diluting to volume with water, and passing through a 0.45-µm filter.

Solution B: Acetonitrile

Diluent
Solution A and Solution B (65:35 v/v)

Standard solution 1
1.2 mg/ml of rebaudioside A in Diluent. Prepare by dissolving in 80% volume of Diluent, sonicating until dissolved (2–5 min), and diluting to volume. High purity rebaudioside A standards available from Wako Pure Chemical Industries, Ltd. Japan, US Pharmacopeia, USA, and ChromaDex, USA.

Standard solution 2
0.03 mg/ml of rebaudioside A in Diluent. Prepare by dissolving in 80% volume of Diluent, sonicating until dissolved (2–5 min), and diluting to volume.

System suitability solution
Prepare a solution containing all nine named steviol glycosides for peak identification and system suitability as follows. 1.2 mg/ml of USP Steviol

[5] FCC10-: U.S. Pharmacopeial Convention, Rebaudioside A Monograph, 2016. reproduced with permission.

Glycosides System Suitability RS (available from US Pharmacopeia, USA) in Diluent. Prepare by dissolving in 80% volume of Diluent, sonicating until dissolved (2–5 min), and diluting to volume.

Alternative preparation: Prepare a solution for peak identification and system suitability containing 0.6 mg/ml each of rebaudioside D, rebaudioside A, stevioside, rebaudioside F, rebaudioside C, dulcoside A, rubusoside, rebaudioside B, and steviolbioside in Diluent (standards available from Wako Pure Chemical Industries, Ltd. Japan, US Pharmacopeia, USA, and ChromaDex, USA).

Sample
Rebaudioside A is hygroscopic, and accurate quantitative analysis requires moisture equilibration before analysis. Equilibrate sample specimens in the lab no less than 24 h before weighing by spreading into a thin layer no more than 1/4 in. Intermittent stirring will ensure uniform moisture absorption. The water content of the equilibrated samples should be determined at the time of weighing using Water Determination (Karl Fischer Titrimetric Method) (Vol. 4)

Sample solution
1.2 mg/ml of the Sample in Diluent. Prepare by dissolving in 80% volume of Diluent, sonicating until dissolved (2–5 min), and diluting to volume.

HPLC Conditions
Detector: UV 210 nm
Column: 25-cm × 4.6-mm, packed with 5-µm reversed phase C18 silica stationary phase (YMC-Pack ODS-AQ, YMC America Inc.), or equivalent
Column temperature: 32
Flow rate: 0.5 ml/min
Injection volume: 15 µl
Gradient program: See Table 1

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
10	65	35
25	65	35
35	25	75
50	25	75
52	90	10
67	90	10

System suitability
Sample: System suitability solution
Suitability requirements
Suitability requirement 1: The relative standard deviation of the rubusoside peak area is no more than 2.5% for five replicate injections.
Suitability requirement 2: The resolution, R, between rebaudioside A and stevioside is no less than 1.7.
Suitability requirement 3: The relative standard deviation of the rebaudioside A peak area is no more than 1.5% for five replicate injections.

Analysis

Separately inject equal volumes of the System suitability solution, Standard solution 1, and Sample solution into the chromatograph. Use the chromatogram of the Standard solution to identify the rebaudioside A peak, and the chromatogram of the System suitability solution to identify the peaks corresponding to the other eight steviol glycosides listed in Table 2.

Table 2: Chromatographic Profile

Compound	Relative Retention Time	Relative Response Factor
Rebaudioside D	0.80	0.83
Rebaudioside A	1.00	1.00
Stevioside	1.03	1.22
Rebaudioside F	1.10	1.02
Rebaudioside C	1.15	0.98
Dulcoside A	1.21	1.16
Rubusoside	1.44	1.06
Rebaudioside B	1.59	1.07
Steviolbioside	1.61	1.38

Calculate the percentage of rebaudioside A in the portion of the sample taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area for the analyte in the Sample solution
r_S = peak area for rebaudioside A in Standard solution 1
C_S = concentration of rebaudioside A in Standard solution 1, corrected for purity andwater content (mg/ml)
C_U = concentration of the sample in the Sample solution corrected for water content (mg/ml)

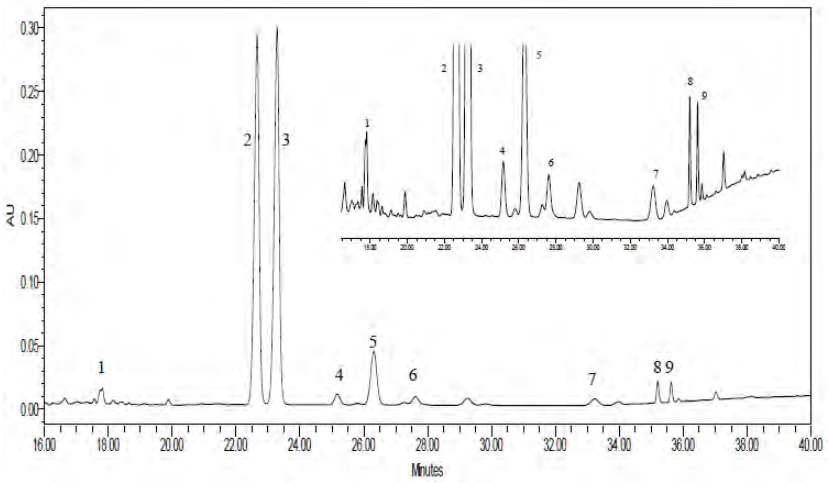


Figure. Chromatogram of System suitability solution
Conditions: 1.2 mg/ml USP Steviol Glycosides System Suitability RS

ROSEMARY EXTRACT
(TENTATIVE)

New specifications prepared at the 82nd JECFA (2016), published in FAO JECFA Monograph 19 (2016). A temporary ADI of 0-0.3 mg/kg bw was established at the 82nd JECFA (2016). .
Information required:
Validation data for residual solvents using Vol 4 Method "Determination of residual solvents in annatto extracts (solvent extracted bixin and norbixin), tentative method (June 2013)".

SYNONYMS INS No. 392

DEFINITION Rosemary extract is obtained from ground dried leaves of *Rosmarinus officinalis* L using food-grade solvents, namely, acetone or ethanol. Solvent extraction is followed by filtration, solvent evaporation, drying and sieving to obtain a fine powder. Additional concentration and/or precipitation steps followed by deodorisation, decolourisation and standardisation using diluents and carriers of food grade quality maybe included to produce the final product. Rosemary extract is characterised by its content of phenolic diterpenes, carnosic acid and carnosol, the principal antioxidative agents. Other antioxidant components present include triterpenes and triterpenic acids. Rosemary extract is identified by the total content of carnosol and carnosic acid as a ratio of reference volatile compounds which are responsible for flavour.

The product of commerce can be standardized to a total carnosic acid and carnosol content up to 33%.

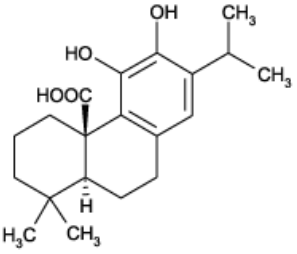
Chemical names Carnosic acid: 4a(2H)-Phenanthrenecarboxylic acid, 1,3,4,9,10,10a-hexahydro-5,6-dihydroxy-1,1-dimethyl-7-(1-methylethyl)-, (4aR-trans)-

Carnosol: 2H-9,4a-(Epoxymethano)phenanthren-12-one, 1,3,4,9,10,10a-hexahydro-5,6-dihydroxy-1,1-dimethyl-7(1- methylethyl), (4aR-(4aα,9α,10aβ))-

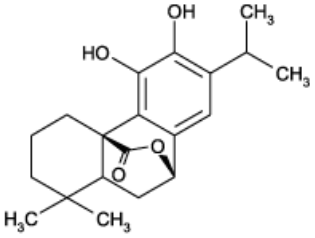
C.A.S. numbers Extract of rosemary: 84604-14-8
Carnosic Acid: 3650-09-7
Carnosol: 5957-80-2

Chemical formula Carnosic acid: $C_{20}H_{28}O_4$
Carnosol: $C_{20}H_{26}O_4$

Structural formula



Carnosic Acid



Carnosol

Formula weight Carnosic acid: 332.43
Carnosol: 330.42

Assay Not less than 5% of the total carnosic acid and carnosol.

DESCRIPTION Beige to light brown powder.

FUNCTIONAL USES Antioxidant

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in oil

Antioxidants/Reference Volatiles Ratio Total % of carnosic acid and carnosol /Total % of reference volatiles: (-)-borneol, (-)-bornyl acetate, (-)-camphor, 1,8-Cineole (eucalyptol) and verbenone: not less than 15
See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 5% (80° under vacuum, 4 hours). Test 1 g of sample

Residual solvents (Vol. 4) Acetone: Not more than 50 mg/kg
Ethanol: Not more than 500 mg/kg

Determine residual solvents following the method “Determination of residual solvents in annatto extracts (solvent extracted bixin and norbixin), tentative method (June 2013)”, or any other suitable method with similar performance characteristics.

Arsenic (Vol. 4) Not more than 3 mg/kg

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

TESTS

IDENTITY TESTS

Antioxidant/Reference Volatiles Ratio Antioxidant: Total % of carnosic acid and carnosol (comes from METHOD OF ASSAY)
Reference Volatile Ratio: Total % w/w of (-)-borneol, (-)-bornyl acetate, (-)-camphor, 1,8-Cineole (eucalyptol) and verbenone is determined using GC-MSD

Equipment and Reagents:

Equipment
GC/MS chromatograph with autosampler

Solvent: Tetrahydrofuran (THF) from Carlo Erba, HPLC grade ref. 412452000 or equivalent

Standards
(-)-Borneol from Fluka (Sigma-Aldrich) ref. 15598 or equivalent
(-)-Bornyl acetate from Fluka (Sigma-Aldrich) ref. 45855 or equivalent
(-)-Camphor from Fluka (Sigma-Aldrich) ref. 21293 or equivalent
1,8-Cineole (Eucalyptol) (from Aldrich (Sigma-Aldrich) ref. C80601 or equivalent
Verbenone from Fluka (Sigma-Aldrich) ref. 94882

Internal Standard: Heptanon-4 from Fluka (Sigma-Aldrich) ref. 43570 or equivalent

Preparation of Internal Standard Solution (ISS)
Accurately weigh 20 mg of 4-heptanon in a 50 ml volumetric flask. Dilute to volume with THF and homogenise the solution. The concentration of the Internal Standard Solution is approximately 400 µg/ml.

Preparation of Sample Solution:
Accurately weigh 2.5 g of the sample in a 10 ml volumetric flask. Add 500 µl of the Internal Standard Solution, and dilute to volume with THF. Sonicate 5 min for liquid samples or 10 min for powder extracts. Filter an aliquot through 0.45 µm filter.

Preparation of Standard Solutions (SS): Accurately weigh 20 mg of each Standard into a 50 ml volumetric flask. Dilute to volume with THF and homogenise the solution. The concentration of each Standard in the Standard Solution is approximately 400 µg/ml.

Preparation of Standard solutions for standard Curve (WSS):

Standard	WSS, µg each/ml	SS, µl	ISS, µl	THF, µl	Total Volume, µl
Level 0	0	0	100	1900	2000
Level 1	Approx. 5	20	100	1880	2000
Level 2	Approx. 20	100	100	1800	2000
Level 3	Approx. 50	200	100	1700	2000
Level 4	Approx. 100	500	100	1400	2000
Level 5	Approx. 200	1000	100	900	2000

Procedure: Load the WSS and the Sample solution, onto the autosampler of the GC/MS using following conditions. Inject in duplicate 1 µl of WSS.

GC conditions:

Column: FactorFour Capillary column VF-5ms 30M x 0.25 mm Ft = 0.25.
Carrier gas: He; flow rate 1 mL/min with constant flow
Split: 100/1

Temperature Program:

Temperature [°]	Rate [°/min]	Hold [min]	Total [min]
70	0.0	1.00	1.00
130	5.0	0.00	12.00
240	10.0	1.00	25.00

Injector: 250°
Temperature: Manifold: 150°, Transfer line: 240°, Quad: 230°

Auto sampler specifications
Syringe: 10 µl
Injection volume: 1 µl
Rinse: pre-clean solvent: 5 times, pre-clean sample: 5 times, post-clean solvent: 5 times
Washing solvent: Tetrahydrofuran

MS Acquisition:

Segments / Names	Ionization Scan type	Target TIC [counts]	Running Time [min]	Ion [m/z]
1	Off	-	0.00 – 3.00	-
2 / Heptanon (IS)	EI - SIS	10000	3.00 – 3.50	43 71 114
3	Off	-	3.50 – 5.00	-
4 / 1,8-Cineole (Eucalyptol)	EI - SIS	10000	5.00-6.50	43 139 154
5	Off	-	6.50 – 8.00	-
6 / Camphor, Borneol, Verbenone	EI - SIS	10000	8.00 – 11.00	95 107 110 135 152
7 / Bornyl acetate	EI - SIS	10000	11.00 – 13.00	95 154 196

Calculation:

Calculate the calibration curve by linear regression analysis for each individual volatile standard using the equation

$$Area=a\times(c\times P)+b$$

where:

Area is Individual Volatile Standard peak area in WSS chromatogram
c is Concentration [µg/ml] of Individual Volatile Standard
a is Slope of the regression line for Individual Volatile Standard
b is y-intercept of the regression line for Individual Volatile Standard

P is Purity of Individual Volatile Standard given by certificate of analysis from Supplier

Calculate the concentration of the volatiles in the sample using the following formula:

$$[\text{Compound}],\ \text{mg/kg} = \frac{A_S\text{-}y}{m \times A_{IS}} \times \frac{V}{W} \times C_{IS}$$

where

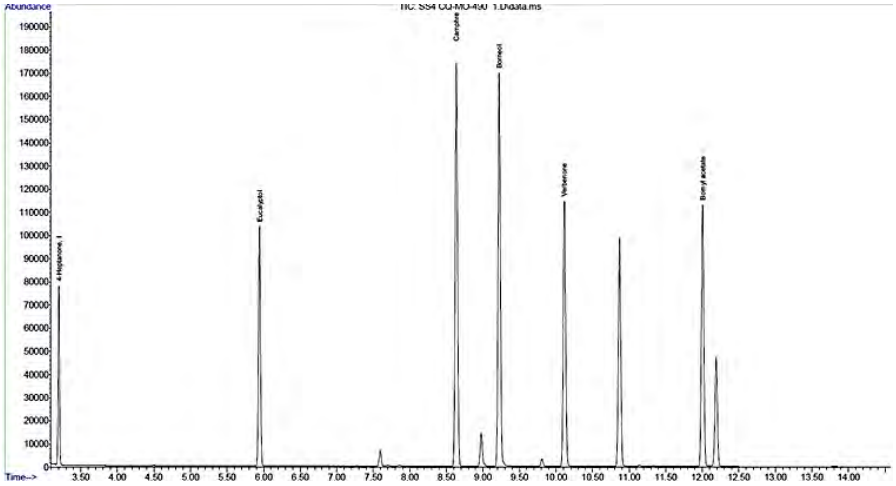
A_S is Individual Volatile peak area in Sample Solution chromatogram
y is y-intercept of Individual Volatile calibration curve
m is Slope of Individual Volatile calibration curve
A_{IS} is Peak area of Internal standard in Sample Solution chromatogram
V is Dilution volume (ml)
W is Weight of sample (g)
C_{IS} is Concentration of the Internal Standard Solution

With the software Varian MS Workstation version 6.9 Service Pack 1, report the following settings during the review:
Amount Standard is 1
Multiplier is Dilution volume [ml]
Divisor is Weight of sample [g]

The reported result, Total Volatiles, is the sum of each Individual Volatile result.

The limit of quantification (LOQ) is 20 ppm and the limit of detection (LOD) is 2 ppm.

A representative GC-MS analysis of the volatile standards is shown below



METHOD OF ASSAY Determine carnosic acid and carnosol content by HPLC using the following conditions:

HPLC conditions:
Detector: Ultraviolet (UV) 230 nm
Column: ZORBAX SB-C18 (Agilent Technologies) or equivalent; 4.6-mm x 250-mm containing 5-µm porous silica microparticles chemically bonded to octadecylsilane
Flow rate: 1.5 mL/min
Temperature: 25°
Injection size: 5 µl

Preparation of Mobile Phase:

Combine Acetonitrile with 0.5% phosphoric acid in water (v/v) at a ratio of 65:35.

Preparation of Solutions:

Preparation of Phosphoric Acid Solution: Dissolve 0.5 ml of phosphoric acid, ACS grade, in 100 ml of Methanol, HPLC grade.

Reference Standard Solution: Dissolve 200-500 µg/ml of USP Powdered Extract of Rosemary RS in Phosphoric Acid Solution. Sonicate for 5 min; filter through a 0.45-µm filter.

System Suitability Standard Solution: Dissolve 100 µg/ml of USP Carnosic Acid RS in Phosphoric Acid Solution. Sonicate for 5 min; filter through a 0.45-µm filter.

Sample Solution: Dissolve 500 µg/ml of the sample in Phosphoric Acid Solution. Sonicate for 5 min; filter through a 0.45-µm filter

Procedure: Separately inject the System Suitability Standard Solution, Reference Standard Solution and Sample Solution in duplicate, and record the HPLC UV outputs. Identify the peaks present in the chromatograms from the sample by comparison to the peaks from the Reference Standard chromatograms.

Calculations:

System Suitability Requirements:

Tailing Factor for the Carnosic Acid Peak in the chromatogram is 0.90 to 1.30

The RSD for the Carnosic Acid peak response on replicate injections is not more than 2%

% Carnosic Acid or Carnosol in sample:

$$\%\ \text{Carnosic Acid or Carnosol} = \frac{A_{Analyte}}{A_{Std}} \times \frac{C_{Std}}{C_u} \times F \times \frac{MW1}{MW2} \times 100$$

where

$A_{analyte}$ is peak area of the analyte of interest (carnosic Acid or Carnosol) obtained from the chromatogram of the Sample Solution

A_{Std} is peak area of carnosic acid obtained from the chromatogram of System Suitability Standard Solution

$C_{Carnosic\ Acid\text{-}SS}$ is concentration of carnosic acid in the System Suitability Standard Solution (µg/mL)

$C_{Carnosic\ Acid}$ is concentration of carnosic acid in Sample Solution (µg/mL)

F is Relative Response Factor of the analyte of interest (1.00 for carnosic acid; 0.92 for carnosol)

MW1 is molar weight of Carnosic acid (332.4 g/mol)

MW2 is molar weight of Carnosol (330.4 g/mol)

Add the individual percentages of Carnosic acid and Carnosol calculated using the above formula, and report the result as the total content of Carnosic acid and Carnosol in the sample taken.

STEVIOL GLYCOSIDES FROM *STEVIA REBAUDIANA* BERTONI
(TENTATIVE)

Tentative specifications prepared at the 82nd JECFA (2016) and published in FAO JECFA Monograph 19 (2016) superseding specifications prepared at the *73rd JECFA (2010) and published in FAO JECFA Monographs 10 (2010). An ADI of 0 - 4 mg/kg bw (expressed as steviol) was established at the 69th JECFA (2008).*

Information required by December 2017:

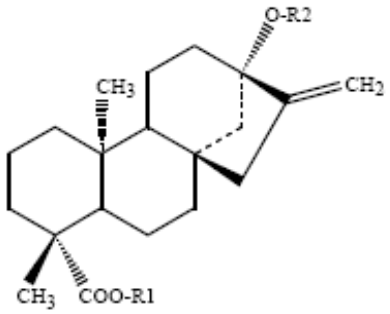
- *Method of Assay to replace the existing method and including as many steviol glycosides as possible (at least those listed in Appendix 1) in steviol glycoside mixtures, along with supporting validation information and chromatograms*
- *Analysis results from a minimum of 5 batches for commercial samples including supporting chromatograms*

SYNONYMS INS no. 960

DEFINITION Steviol glycosides consists of a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni including, glucose, rhamnose, xylose, fructose, and deoxyglucose. The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with a solvent alcohol to release the glycosides and the product is recrystallized from methanol or aqueous ethanol. Ion exchange resins may be used in the purification process. The final product may be spray-dried.

Chemical name, C.A.S. number, Chemical formula See Appendix 1

Structural formula



Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.
Glc, Rha, Fru, deoxyGlc and Xyl represent, respectively, glucose, rhamnose, fructose, deoxyglucose and xylose sugar moieties.

Assay Not less than 95% of total of steviol glycosides on the dried basis.

DESCRIPTION White to light yellow powder, odourless or having a slight characteristic odour. About 200 - 300 times sweeter than sucrose.

FUNCTIONAL USES Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Freely soluble in a mixture of ethanol and water (50:50)
HPLC chromatographic profile	The main peaks in the sample chromatogram obtained by following the procedure in METHOD OF ASSAY correspond to steviol glycoside compounds.
pH (Vol. 4)	Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4)	Not more than 1%
Loss on drying (Vol. 4)	Not more than 6% (105°, 2h)
Residual solvents (Vol. 4)	Not more than 200 mg/kg methanol and not more than 5000 mg/kg ethanol (Method I, General Methods, Organic Components, Residual Solvents)
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level (Use Method II to prepare the test (sample) solution). The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).
Lead (Vol. 4)	Not more than 1 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY Determine the percentages of the individual steviol glycosides by HPLC (Vol. 4) under the following conditions.

Reagents
Acetonitrile: more than 95% transmittance at 210 nm.

Standards
Stevioside: more than 99.0% purity on the dried basis.
Rebaudioside A: more than 99.0% purity on the dried basis.
Mixture of nine steviol glycosides standard solution: Containing stevioside, rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside. This solution is diluted with water-acetonitrile (7:3) accordingly and is used for the confirmation of retention times. Standards are available from Wako Pure Chemical Industries, Ltd. Japan and ChromaDex, USA.

Standard solution
Accurately weigh 50 mg of stevioside and rebaudioside A standard into each of two 50-ml volumetric flasks. Dissolve and make up to volume with water-acetonitrile (7:3).

Sample solution
Accurately weigh 50-100 mg of sample into a 50-ml volumetric flask. Dissolve and make up to volume with water-acetonitrile (7:3).

Procedure
Inject 5 µl of sample solution under the following conditions.
Column: Capcell pak C_{18} MG II (Shiseido Co.Ltd) or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)
Mobile phase: 32:68 mixture of acetonitrile and 10 mmol/L sodium phosphate buffer (pH 2.6)
Flow rate: 1.0 ml/min
Detector: UV at 210 nm
Column temperature: 40°
Record the chromatogram for about 30 min.

Identification of the peaks and Calculation
Identify the peaks from the sample solution by comparing the retention time with the peaks from the mixture of nine steviol glycosides standard solution (see under figure). Measure the peak areas for the nine steviol glycosides from the sample solution. Measure the peak area for stevioside and rebaudioside A from their standard solutions.
Calculate the percentage of each of the eight steviol glycosides except rebaudioside A in the sample from the formula:

$$\%X = [W_S/W] \times [f_X A_X/A_S] \times 100$$

Calculate the percentage of rebaudioside A in the sample from the formula:

$$\%\text{Rebaudioside A} = [W_R/W] \times [A_X/A_R] \times 100$$

where
X is each steviol glycoside;
W_S is the amount (mg) calculated on the dried basis of stevioside in the standard solution;
W_R is the amount (mg) calculated on the dried basis of rebaudioside A in the standard solution;
W is the amount (mg) calculated on the dried basis of sample in the sample solution;
A_S is the peak area for stevioside from the standard solution;
A_R is the peak area for rebaudioside from the standard solution;
A_X is the peak area of *X* for the sample solution; and
f_X is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 1.20 (rebaudioside A), 1.00 (rebaudioside B), 1.18 (rebaudioside C), 1.40 (rebaudioside D), 1.16 (rebaudioside F), 0.98 (dulcoside A), 0.80 (rubusoside) and 0.80 (steviolbioside).

Calculate the percentage of total steviol glycosides (sum the nine percentages).

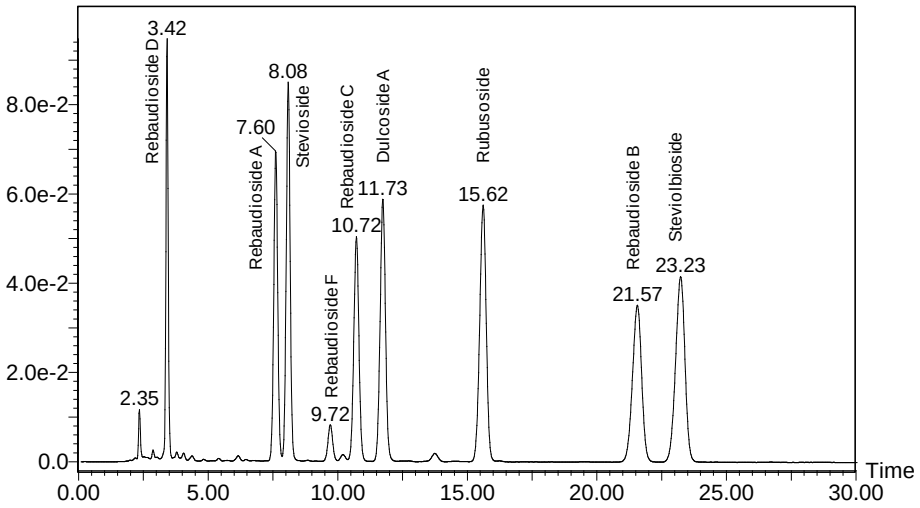


Figure. Chromatogram of mixture of nine steviol glycosides standard solution

Column: Capcell pak C_{18} MG II

Concentration: 0.5 mg/ml each except rebaudioside F (about 0.1 mg/ml)

Appendix 1: Chemical Information for Some Steviol Glycosides

Note: This list is not exhaustive - at least 30 steviol glycosides have been identified in stevia leaf extracts in literature.

Common Name	R_1	R_2	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Group 1: Steviol + Glucose (SvGn)						
Rubusoside	Glcβ1-	Glcβ1-	13-[(β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64849-39-4	$C_{32}H_{50}O_{13}$	642.73
Steviolbioside	H	Glcβ(1-2)Glcβ1-	13-[(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid	41093-60-1	$C_{32}H_{50}O_{13}$	642.73
Stevioside	Glcβ1-	Glcβ(1-2)Glcβ1-	13-[(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	57817-89-7	$C_{38}H_{60}O_{18}$	804.87
Rebaudioside *B*	H	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid	58543-17-2	$C_{38}H_{60}O_{18}$	804.87
Rebaudioside *E*	Glcβ(1-2)Glcβ1-	Glcβ(1-2)Glcβ1-	13-[(*O*-β- D-Glucoopyranosyl-(1,2)-*O*-[β- D-glucopyranosyl)-oxy]-kaur-16-en-18-oic acid (4')-*O*-β-D-glucopyranosyl-deoxy-(1,2)-*O*-[β-D-glucopyranosyl ester	63279-14-1	$C_{44}H_{70}O_{23}$	967.01
Rebaudioside *A*	Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	58543-16-1	$C_{44}H_{70}O_{23}$	967.01
Rebaudioside *D*	Glcβ(1-2)Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, 2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl ester	63279-13-0	$C_{50}H_{80}O_{28}$	1129.15

Common Name	R_1	R_2	Chemical Name	CAS Number	Chemical Formula	Formula Weight
Rebaudioside *M*	Glcβ(1-2)[Glcβ (1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(*O*-β- D-Glucopyranosyl-(1,2)-*O*-[β- D-glucopyranosyl-(1,3)]-β- D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-*O*-β- D-glucopyranosyl-(1,2)-*O*-[β- D-glucopyranosyl-(1,3)]-β- D-glucopyranosyl ester	1220616-44-3	$C_{56}H_{90}O_{33}$	1291.29
Group 2:Steviol + Rhamnose + Glucose (SvR1Gn)						
Dulcoside *A*	Glcβ1-	Rhaα(1-2)Glcβ1-	13-[(2-*O*-β-D-rhamnopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	64432-06-0	$C_{38}H_{60}O_{17}$	788.87
Rebaudioside *C*	Glcβ1-	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-*O*-β-D-rhamnopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	63550-99-2	$C_{44}H_{70}O_{22}$	951.01
Rebaudioside *N*	Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(*O*-β- D-Glucopyranosyl-(1,2)-*O*-[β- D-glucopyranosyl-(1,3)]-β- D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-*O*-6-deoxy-L-mannopyranosyl-(1,2)-*O*-[β- D-glucopyranosyl-(1,3)]-β- D-glucopyranosyl ester	1220616-46-5	$C_{56}H_{90}O_{32}$	1275.29
Rebaudioside *O*	Glcβ(1-3)Rhaα(1-2)[Glcβ(1-3)]Glcβ1-	Glcβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(*O*-β-D-Glucopyranosyl-(1,2)-*O*-[β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid (4')-*O*-β-D-glucopyranosyl-(1,3)-*O*-6- deoxy-L-mannopyranosyl-(1,2)-*O*-[β-D-glucopyranosyl-(1,3)]-β-D-glucopyranosyl ester	1220616-48-7	$C_{62}H_{100}O_{37}$	1437.44
Group 3: Steviol + Xylose + Glucose (SvX1Gn)						
Rebaudioside *F*	Glcβ1-	Xylβ(1-2)[Glcβ(1-3)]Glcβ1-	13-[(2-*O*-β-D-xylopyranosyl-3-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester	438045-89-7	$C_{43}H_{68}O_{22}$	936.99

Steviol (R1 = R2 = H) is the aglycone of the steviol glycosides.
Glc, Rha, Fru, deoxyGlc and Xyl represent, respectively, glucse, rhamnose, fructose, deoxyglucose and xylose sugar moieties

TARTRAZINE

Prepared at the 82nd JECFA and published in JECFA Monograph 19 (2016) superseding specifications 28th JECFA (1984), published in FNP 31/1 (1984) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-7.5 mg/kg was established at the 8th JECFA (1964).

SYNONYMS

INS No. 102, CI Food Yellow 4, CI (1975) No. 19140, FD&C Yellow No. 5

DEFINITION

Consists of trisodium 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)azo]-1H-pyrazole-3-carboxylate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components. It is manufactured by coupling diazotized 4-aminobenzenesulfonic acid with 5-oxo-1-(4-sulfophenyl)-2-pyrazoline-3-carboxylic acid or with the methyl ester, the ethyl ester, or a salt of this carboxylic acid. It also may be manufactured by condensing phenylhydrazine-4-sulfonic acid with dioxosuccinic acid or oxalacetic acid derivatives. The resulting dye is purified and isolated as the sodium salt.

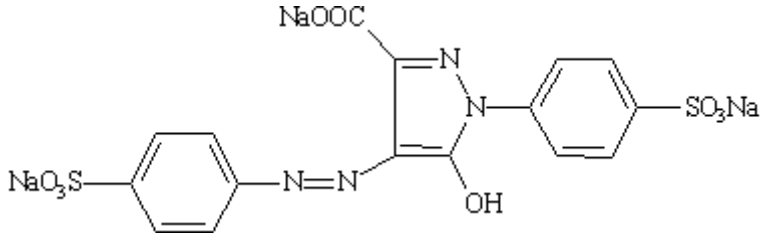
May be converted to the corresponding aluminium lake in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* applies.

Chemical name: Trisodium 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)azo]-1*H*-pyrazole-3-carboxylate

C.A.S. number: 1934-21-0

Chemical formula: $C_{16}H_9N_4Na_3O_9S_2$

Structural formula



Formula Weight: 534.37

Assay: Not less than 85% total colouring matters

DESCRIPTION

Light orange powder or granules

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water; sparingly soluble in ethanol

Spectrophotometry (Vol. 4): Maximum wavelength approximately 427 nm.
Determine the UV-visible absorption spectrum of the sample solution dissolved in water..

PURITY

Loss on drying, chloride and sulfate as sodium salts (Vol. 4)	Not more than 15% as total amount Determine according to chloride as sodium chloride, sulfate as sodium sulfate, and water content (loss on drying at 135 °C) in Volume 4 (under “Specific Methods, Food Colours”).
Water insoluble matter (Vol. 4)	Not more than 0.2%
Subsidiary colouring matters	Not more than 1% See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5% sum of 4-hydrazinobenzenesulfonic acid, 4-aminobenzenesulfonic acid, 5-oxo-1-(4-sulfophenyl)-2-pyrazoline-3-carboxylic acid, 4,4'-(diazoamino)dibenzenesulfonic acid, tetrahydroxysuccinic acid See description under TESTS
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline
Ether extractable matter (Vol. 4)	Not more than 0.2%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”)

TESTS

PURITY TESTS

Subsidiary colouring matters

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

Column: C18 (250 mm x 4.6 mm i.d., 5 µm particle size)
Eluent A: 0.2 M ammonium acetate
Eluent B: methanol
Injection volume: 20 µl
Detector: UV-visible/PDA at 254 nm
Flow rate: 1 ml/min
Gradient:

Min	%A	%B
0	100	0
5	90	10
15	75	25
35	60	40

Standards (all synthesized materials):
4,4'-[4,5-Dihydro-5-oxo-4-[(4-sulfophenyl)hydrazono]-1H-pyrazol-1,3-diyl]bis[benzenesulfonic acid], trisodium salt
4-[(4',5-Disulfo[1,1'-biphenyl]-2-yl)hydrazono]-4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole-3-carboxylic acid, tetrasodium salt
Ethyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)hydrazono]-1H-pyrazole-3-carboxylate, disodium salt
Methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)hydrazono]-1H-pyrazole-3-carboxylate, disodium salt
4,5-Dihydro-5-oxo-1-phenyl-4-[(4-sulfophenyl)azo]-1H-pyrazole-3-carboxylic acid, disodium salt
4,5-dihydro-5-oxo-4-(phenylazo)-1-(4-sulfophenyl)-1H-pyrazole-3-carboxylic acid, disodium salt

Sample solution:
Dissolve 500 mg of sample in 100 ml of 0.2 M ammonium acetate.

Organic compounds other than colouring matters (Vol. 4)

Determine sum of 4-hydrazinobenzenesulfonic acid, 4-aminobenzenesulfonic acid, 5-oxo-1-(4-sulfophenyl)-2-pyrazoline-3-carboxylic acid, 4,4'-diazoaminodi(benzenesulfonic acid), and tetrahydroxysuccinic acid by reversed-phase HPLC (Vol. 4) using the following conditions:

Column: C18 (250 mm x 4.6 mm i.d., 5 µm particle size)
Eluent A: 0.2 M ammonium acetate
Eluent B: methanol
Injection volume: 20 µl
Detector: UV-visible/PDA at 254 nm and 358 nm
Flow rate: 1 ml/min

Gradient:

Min	%A	%B
0	100	0
5	90	10
15	75	25
35	60	40

Note: A general gradient for the separation of organic compounds other than colouring matters in food colours is given in Vol. 4. Analyst may use above gradient for the analytes in Tartrazine.

Standards:
4-Hydrazinobenzenesulfonic acid – Wako, Cat. No. 081-09891 or

equivalent
4-Aminobenzenesulfonic acid – Sigma-Aldrich, Cat. No. 251917 or equivalent
5-Oxo-1-(4-sulfophenyl)-2-pyrazoline-3-carboxylic acid – Chemos, Cat. No. 119264 or equivalent
4,4'-(Diazoamino)dibenzenesulfonic acid, disodium salt – Wako, Cat. No. 040-33231 or equivalent
Tetrahydroxysuccinic acid – Chemos, Cat. No. 287405 or equivalent

Sample solution:
Dissolve 500 mg of sample in 100 ml of 0.2 M ammonium acetate.

METHOD OF ASSAY

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

Using water as the solvent: absorptivity (a) is 53.0 l/(g·cm) and wavelength of maximum absorption is approximately 427 nm.

XANTHAN GUM

Prepared at the 82nd JECFA (2016) and published in FAO JECFA Monographs 19 (2016), superseding specifications prepared at the 53rd JECFA (1999) and published in FNP Add 7 (1999). An ADI "not specified" was established at the 30th JECFA (1986).

SYNONYMS INS No. 415

DEFINITION Xanthan gum is a polysaccharide gum with high molecular weight (of the order of 1,000 kDa.) containing D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid and pyruvic acid. It is produced by fermentation of a carbohydrate in a pure-culture of *Xanthomonas campestris*, recovered from the fermentation broth by precipitation with ethanol or isopropanol, dried and milled. The final product is manufactured in the form of a sodium, potassium or calcium salt and its solutions are neutral.

C.A.S. number 11138-66-2

Assay Yields, on the dried basis, not less than 4.2% and not more than 5.4% of carbon dioxide (CO_2), corresponding to between 91.0% and 117.0% respectively of xanthan gum.

DESCRIPTION Cream-coloured powder

FUNCTIONAL USES Thickener, stabiliser, emulsifier, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water; insoluble in ethanol

Gel formation To 300 ml of water, previously heated to 80° and stirred rapidly with mechanical stirrer in a 400-ml beaker, add, at the point of maximum agitation, a dry blend of 1.5 g of the sample and 1.5 g of carob bean gum. Stir until the mixture goes into solution, and then continue stirring for 30 min longer. Do not allow the water temperature to drop below 60° during stirring. Discontinue stirring, and allow the mixture to cool at room temperature for at least 2 h. A firm rubbery gel forms after the temperature drops below 40°, but no such gel forms in a 1% control solution of the sample prepared in the same manner but omitting the carob bean gum.

PURITY

Loss on drying (Vol. 4) Not more than 15% (105°, 2.5 h)

Ash (total) (Vol. 4) Not more than 16% after drying

Pyruvic acid Not less than 1.5%
See description under TESTS

Nitrogen (Vol. 4) Not more than 1.5%
Proceed according to the Kjeldahl method

Residual Solvents (Vol. 4)

Not more than 500 mg/kg of ethanol and isopropanol either singly or in combination.
See description under TESTS

Lead (Vol. 4)

Not more than 2 mg/kg.
Not more than 0.5 mg/kg for use in infant formula and formula for special medical purposes intended for infants.

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

Microbiological criteria (Vol. 4)

Total plate count:	Not more than 5,000 cfu/g
E. coli:	Negative by test
Salmonella:	Negative by test
Yeasts and moulds:	Not more than 500 cfu/g

TESTS

See also description under TESTS

PURITY TESTS

Pyruvic acid

Sample preparation
Weigh 600 mg of the sample to the nearest 0.1 mg and dissolve in sufficient water to make 100 ml. Transfer 10.0 ml of the solution into a 50-ml glass-stoppered flask. Pipette 20 ml of N hydrochloric acid into the flask, weigh the flask, and reflux for 3 h, taking precautions to prevent loss of vapours. Cool to room temperature, and add water to make up for any weight loss during refluxing. Pipette 1.0 ml of a 1 in 200 solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid into a 30-ml separatory funnel, then add 2.0 ml of the sample solution, mix, and allow to stand at room temperature for 5 min. Extract the mixture with 5 ml of ethyl acetate, and discard the aqueous layer. Extract the hydrazone from the ethyl acetate with three 5-ml portions of sodium carbonate TS, collecting the extracts in a 50-ml volumetric flask. Dilute to volume with sodium carbonate TS and mix.

Standard preparation
Weigh 45 mg of pyruvic acid, to the nearest 0.1 mg, and transfer into a 500-ml volumetric flask. Dilute to volume with water, and mix. Transfer 10.0 ml of this solution into a 50-ml glass-stoppered flask, and continue as directed under "Sample preparation", beginning with "Pipette 20 ml of N hydrochloric acid into the flask".

Procedure
Determine the absorbance of each solution with a suitable spectrophotometer in 1-cm cells at the maximum of about 375 nm, using sodium carbonate TS as the blank. The absorbance of the "Sample preparation" is equal to or greater than that of the "Standard preparation".

Residual Solvents (Vol. 4)

Determine residual solvents using headspace gas chromatography (Method I)

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

Standard solution: Add 50.0 ml water to a 50 ml vial and seal. Accurately weigh and inject 15µl ethanol and weigh to within 0.01mg. Inject 15 µl isopropanol through the septum and reweigh the vial.

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

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

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

Follow the procedure described in Vol. 4.

Microbiological criteria (Vol. 4)

Total plate count: Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 12-15 ml of Plate Count Agar previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, allow the agar to solidify. Invert the plates and incubate for 48±2 h at 35±1°.
After incubation count the growing colonies visible on each plate and record the number of colonies. Take the average of both plates, and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 cfu/g.

E. coli:
Using aseptic technique, disperse 1 g of sample in 99 ml of Lactose broth using either a Stomacher, shaker or stirrer to fully dissolve the sample. Limit the dissolving time to about 15 min and then lightly seal the container and incubate the broth for 18-24 h at 35±1°. Using a sterile pipette, inoculate 1 ml of the incubate into a tube containing 10 ml GN broth. Incubate for 18-24 h and then streak any GN broths showing positive growth or gas production onto duplicate plates of Levine EMB agar. Incubate the plates for 24±2 h at 35±1° and then examine for colonies typical of *E. coli i.e.* showing strong purple growth with dark centre and a green metallic sheen sometimes spreading onto the agar. Record any typical *E. coli* colonies as presumptive positive, otherwise negative.
Streak any well isolated suspect colonies onto a plate of PCA and incubate for 18-24 h at 35±1°. Perform a Gram stain on any growth to confirm it is Gram negative. If so, disperse any colony growth into a small volume of 0.85% saline and perform chemical tests to confirm the identity of the bacterial growth. This can most conveniently be done by using API 20E or Micro ID strips or equivalent systems.

After completion of the tests, identify the organism from the Identification manual of the system used and record the final result.

Media
GN Broth (Gram Negative Broth)
Peptone 20.0 g
Dextrose 1.0 g
Mannitol 2.0 g
Sodium citrate 5.0 g
Sodium deoxycholate 0.5 g
Potassium phosphate (dibasic) 4.0 g
Potassium phosphate (monobasic) 1.5 g
Sodium chloride 5.0 g
Make up to 1 litre with distilled or de-ionised water, pH 7.0±0.2 at 25°

Salmonella:
Using aseptic technique, disperse 5 g of sample into 200 ml of sterile lactose broth using either a Stomacher, shaker or stirrer to maximise dissolution over a 15 min period. Loosely seal the container and incubate at 35±1° for 24±2 h.

Tighten lid and gently shake incubated sample mixture; transfer 1 ml mixture to 10 ml selenite cystine broth and another 1 ml mixture to 10 ml tetrathionate broth. Incubate 24±2 h at 35°. Mix (vortex, if tube) and streak 3-mm loopful incubated selenite cystine broth on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. (Prepare BS plates the day before streaking and store in dark at room temperature until streaked.) Repeat with 3-mm loopful of tetrathionate broth. Incubate plates 24±2 h at 35°. Continue as indicated on pages 221-226 of the Guide to Specifications, FAO Food and Nutrition Paper 5 Revision 2, Rome 1991, "Examine plates for presence of colonies".

Yeasts and moulds:
Using aseptic technique, disperse 1 g of sample into 99 ml of phosphate buffer and use a Stomacher, shaker or stirrer to fully dissolve. Limit dissolving time to about 10 min and then pipette 1 ml of the solution into separate, duplicate, appropriately marked petri dishes. Pour over the aliquot of sample in each petri dish 15-20 ml of Potato dextrose agar (either acidified or containing antibiotic) previously tempered to 44-46°. Mix well by alternate rotation and back and forth motion of the plates, and allow the agar to solidify. Invert the plates and incubate for 5 days at 20-25°.
After incubation, count the growing colonies visible on each plate using a colony counter and record the number of colonies. Separate the yeasts from the moulds according to their morphology and count them separately. Take the average of both plates and multiply by the sample dilution factor, 100. Where no colonies are visible, express the result as less than 100 cfu/g.

METHOD OF ASSAY (Vol. 4)

Proceed as directed in the test for Carbon Dioxide Determination by Decarboxylation (Vol. 4) using 1.2 g of the sample accurately weighed.

ANALYTICAL METHODS

Total colouring matters content (tentative since 74th meeting) (Vol. 4) was revised by amending Procedure 1 (water-soluble colouring matters) and Procedure 3 (lakes). Table 1 was revised to give spectrophotometric data for 17 synthetic colours, their aluminium lakes, cochineal extract and carmine dissolved in water and buffers. Reagents, solution preparations and sample preparation information were added. Equations shown in Procedures 1, 2 and 3 were edited. The tentative status of the method was removed.
Although data were not requested for Procedure 2 (organic solvent–soluble colouring matters), the Committee noted that chloroform is listed as a reagent in that procedure. The Committee was reminded of previous efforts to remove this reagent from test procedures and decided that efforts should be made to replace it.

TOTAL COLOURING MATTERS CONTENT (VOLUME 4)

Colouring Matters Content by Spectrophotometry

Three experimental procedures are described. Procedure 1 is used for water- soluble colouring matters. Procedure 2 is used for organic solvent-soluble colouring matters. Procedure 3 is used for lakes. The wavelength of maximum absorbance, absorptivity, and specific absorbance for each solvent used for the determination of total colouring matters content are included in Table 1.

Principle

The absorbance of a solution of the colouring matter is determined at its wavelength of maximum absorption and the total colouring matters content is calculated using the absorptivity or specific absorbance value provided in Table 1.

Apparatus

- UV-visible spectrophotometer capable of accurate (± 1% or better) measurement of absorbance in the region of 350-750 nm with an effective slit width of 10 nm or less
- Spectrophotometer cells, 1 cm path length

Procedure 1 – Colouring matters content of water-soluble colouring matters

Reagents

- Ammonium acetate, reagent grade
- Ammonium hydroxide, concentrated
- Hydrochloric acid, reagent grade
- Phosphoric acid, reagent grade
- Potassium dihydrogen phosphate, reagent grade
- Sodium hydroxide, reagent grade
- Water, purified

Preparation of solutions:

Ammonium acetate, 0.044 M: Weigh 3.40 g of ammonium acetate into a 1000 ml volumetric flask. Make up to volume with water, stopper, and shake to mix.
Hydrochloric acid, 2 M: Add approximately 50 ml of water to a 100 ml volumetric flask. Using a graduated cylinder, add 17 ml of concentrated hydrochloric acid. Make up to volume with water, stopper, and shake to mix.
Phosphoric acid, 1%: Pipet 1 ml of concentrated phosphoric acid into a 100 ml volumetric flask. Make up to volume with water, stopper, and shake to mix.
Sodium hydroxide, 1 M: Weigh 8 g of sodium hydroxide solution into a tared 200 ml volumetric flask with funnel. Add about 30 ml of water and swirl to mix. Allow the solution to cool to room temperature. Make up to volume with water, stopper, and shake to mix. Store in a polyethylene or polypropylene bottle.

Sodium hydroxide, 0.1 M: Using graduated cylinders, add 10 ml of the 1 M sodium hydroxide solution and 90 ml of water to a 200 ml polyethylene or polypropylene bottle. Cap the bottle and swirl to mix. Use until consumed.
Phosphate buffer, 0.11 M, pH 7: Weigh 15.2 g of potassium dihydrogen phosphate into a 1000 ml beaker and dissolve in about 900 ml of water. Gradually add about 90 ml of 1 M sodium hydroxide to approximately pH 7, measured using a pH meter. Adjust the pH to 7.0 using 0.1 M sodium hydroxide or diluted phosphoric acid. Transfer to a 1000 ml volumetric flask. Make up to volume with water, stopper, and shake to mix.
Ammonium hydroxide, 0.5%: Using a graduated cylinder, add 5 ml of concentrated ammonium hydroxide to a 100 ml volumetric flask. Make up to volume with water, stopper, and shake to mix.
Samples (except cochineal extract): Accurately weigh the quantity of sample (± 0.5 mg) given in Table 1 (W). Transfer to a 1000 ml volumetric flask. Add a solvent prescribed in Table 1 and swirl to dissolve. Make up to volume with the solvent, stopper, and mix. Dilute a 5 ml aliquot of the solution to 100 ml with the same solvent in order to obtain an absorbance of approximately 0.5. Measure the absorbance (A) at the wavelength of maximum absorption in a 1 cm path length cell, using the solvent as the blank.

Cochineal extract samples: Accurately weigh the quantity of sample (± 0.5 mg) given in Table 1 (W) into a 50 ml beaker. Add 3 ml of 2 M HCl preheated to just below boiling. Transfer to a 100 ml volumetric flask. Make up to volume with water. Dilute a 5 ml aliquot to 100 ml with water. The HCl concentration will be 0.06 M. Measure the absorbance (A) at the wavelength of maximum absorption in a 1 cm path length cell, using 0.06 M HCl as the blank.

Calculation

Calculate the total colouring matters content of the sample using either of the following equations:

$$\%\ \text{total colouring matters} = 100 \times \frac{A \times F}{a \times W}$$

$$\%\ \text{total colouring matters} = 1000 \times \frac{A \times F}{A\frac{1\%}{1\ \text{cm}} \times W}$$

where

A is the absorbance of the sample solution at the wavelength of maximum absorption;
a is the absorptivity in liter/(g·cm) given in Table 1;
$A\frac{1\%}{1\ \text{cm}}$ is the specific absorbance given in Table 1;
W is the weight of the sample in g; and
F is the dilution factor.

Procedure 2 – Colouring matters content of organic solvent-soluble colouring matters

Reagents

- Chloroform, reagent grade, acid free
- Cyclohexane, reagent grade

Accurately weigh 0.08 g (± 0.01 g) of the sample (W) into a 100 ml volumetric flask (V_1). Add 20 ml of chloroform and dissolve by swirling briefly. Make sure that the solution is clear. Make up to volume with cyclohexane and mix. Pipet 5.0 ml of the solution (v_1) into a second 100 ml volumetric flask (V_2)

and make up to volume with cyclohexane. Pipet 5.0 ml of this diluted solution (v_2) into the final 100 ml volumetric flask (V_3) and make up to volume with cyclohexane. Measure the absorbance (A) of the twice-diluted solution at the wavelength of maximum absorption in a 1 cm cell, using cyclohexane as the blank.

Perform this procedure promptly, avoiding exposure to air insofar as possible and undertaking all operations in the absence of direct sunlight.

Calculation

Calculate the total colouring matters content of the sample using either of the following equations:

$$\% \text{ total colouring matters} = 100 \times \frac{A \times V_1 \times V_2 \times V_3}{a \times 10 \times v_1 \times v_2 \times W}$$

$$\% \text{ total colouring matters} = 100 \times \frac{A \times V_1 \times V_2 \times V_3}{v_1 \times v_2 \times W \times A\frac{1\%}{1\ cm} \times 10W}$$

where

A is the absorbance of the sample solution at the wavelength of maximum absorption;
a is the absorptivity of the standard in liter/(g·cm);
$A\frac{1\%}{1\ cm}$ is the specific absorbance of the standard indicated in the specification monograph; and
V_1, V_2, and V_3 are the volumes of the three volumetric flasks (each 100 ml); v_1 and v_2 are the volumes of the two pipets (each 5 ml).

Procedure 3 – Colouring matters content of lakes

Samples (except Brown HT, Carmine, and Erythrosine lakes): Accurately weigh the quantity of lake (± 0.5 mg) given in Table 1. Transfer to a 250 ml beaker containing 10 ml hydrochloric acid previously diluted with water to approximately 50 ml. Heat with stirring as necessary to dissolve the lake, and then cool to ambient temperature. Transfer to a 100 ml volumetric flask, make up to volume with 0.11 M pH 7 phosphate buffer, and mix. Dilute a 5 ml aliquot to 100 ml with 0.11 M pH 7 phosphate buffer. Proceed as detailed in Procedure 1, above, and in the specification monograph, using the values for wavelength of maximum absorbance and absorptivity or specific absorbance included in Table 1, and using the pH 7 phosphate buffer as the spectrophotometric blank.

Brown HT lakes: Follow the above procedure except use 0.044 M ammonium acetate as the solvent and the spectrophotometric blank.

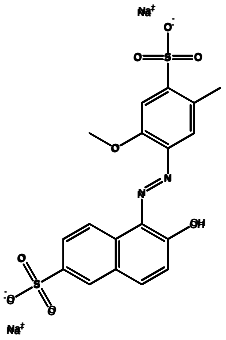
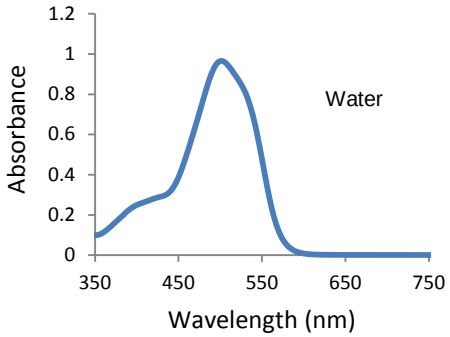
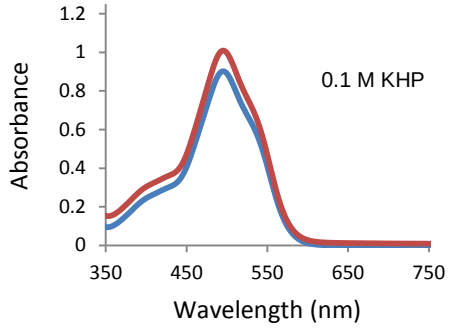
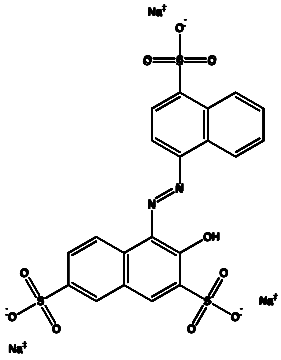
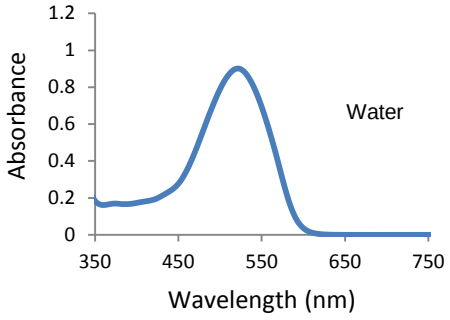
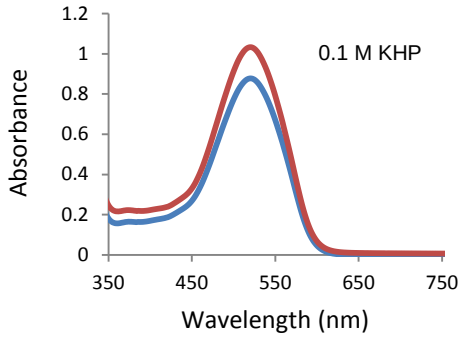
Carmine lakes: Accurately weigh the quantity of lake (± 0.5 mg) given in Table 1 (W) into a 50 ml beaker. Add 3 ml of 2 M HCl preheated to just below boiling. Transfer to a 100 ml volumetric flask. Make up to volume with water, stopper, and shake to mix. Dilute a 5 ml aliquot to 100 ml with water. The HCl concentration will be 0.06 M. Measure the absorbance (A) at the wavelength of maximum absorption in a 1 cm path length cell, using 0.06 M HCl as the blank.

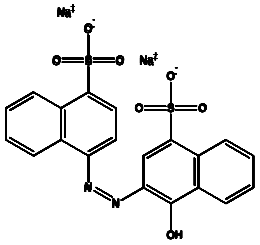
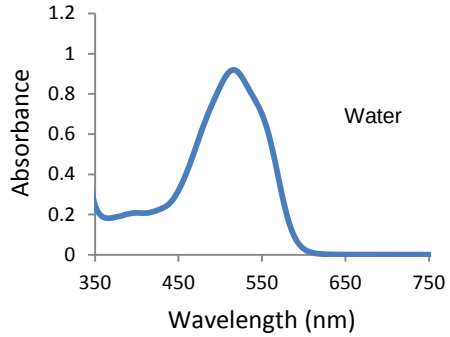
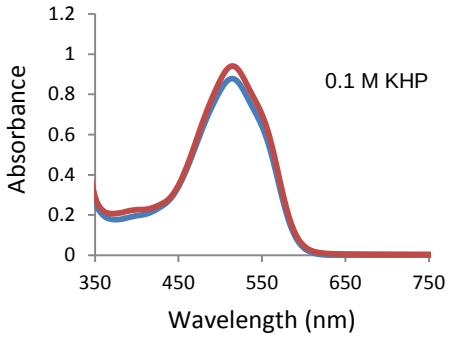
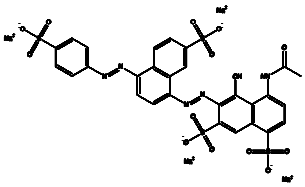
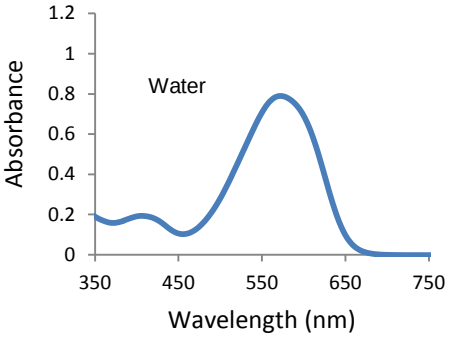
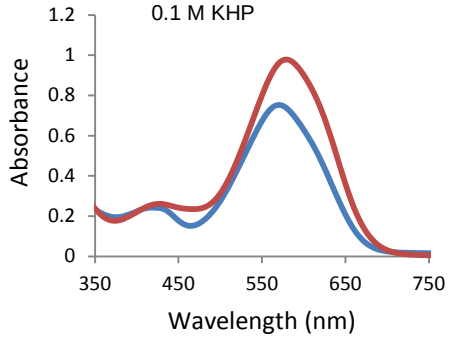
Erythrosine lakes: Accurately weigh the quantity of lake (± 0.5 mg) given in Table 1. Transfer to a 250 ml beaker containing 5 ml ammonium hydroxide previously diluted with water to approximately 50 ml. Heat with stirring to dissolve the lake, and then cool to ambient temperature. Transfer to a 100 ml volumetric flask, make up to volume with water, stopper, and shake to mix. Dilute a 5 ml aliquot to 100 ml with water. Proceed as detailed in Procedure 1, above, and in the specification monograph, using the values for wavelength of maximum absorbance and absorptivity or specific absorbance included in Table 1, and using 0.5% ammonium hydroxide as the spectrophotometric blank.

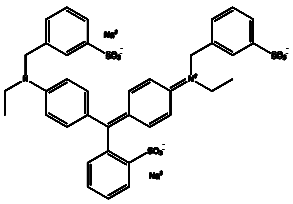
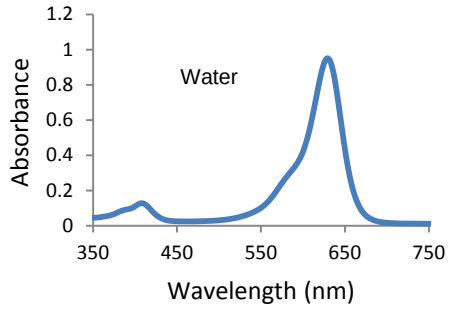
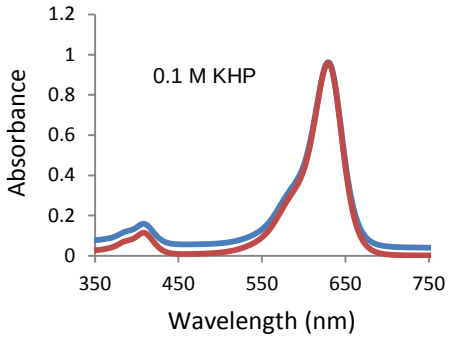
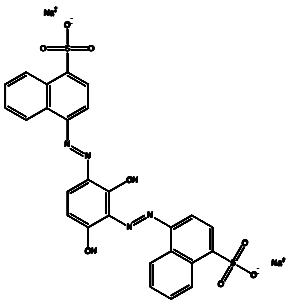
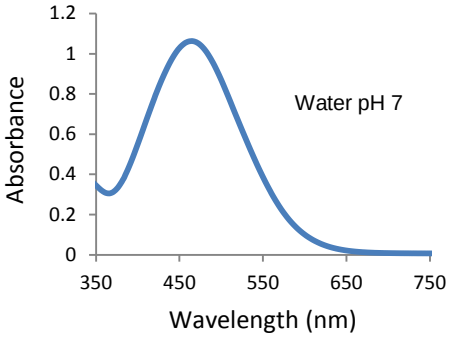
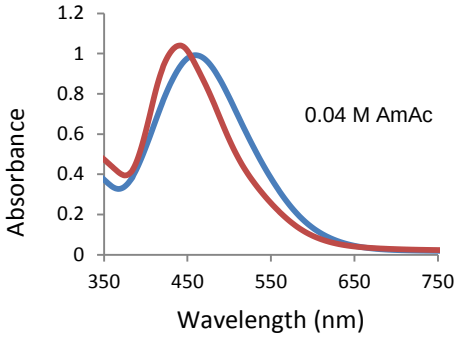
Table 1. Spectrophotometric data for Total Colouring Matters Content by Spectrophotometry (Vol. 4);

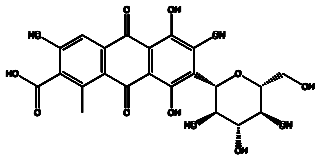
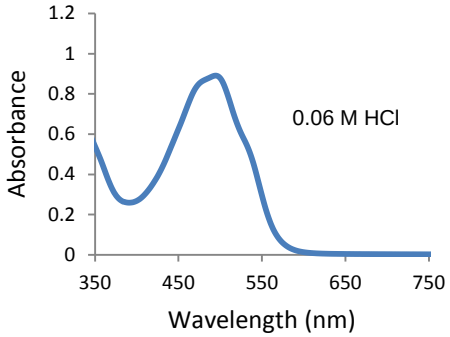
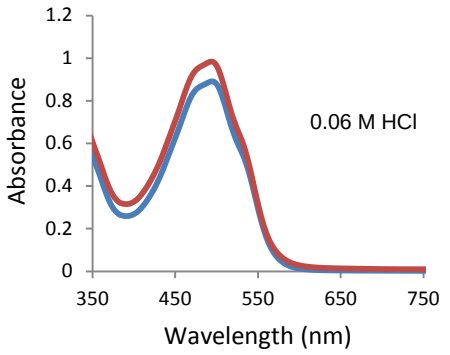
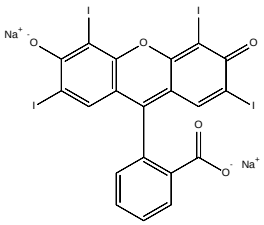
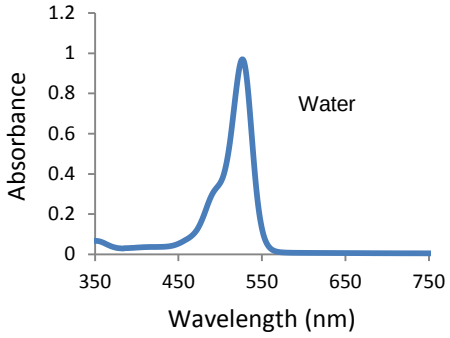
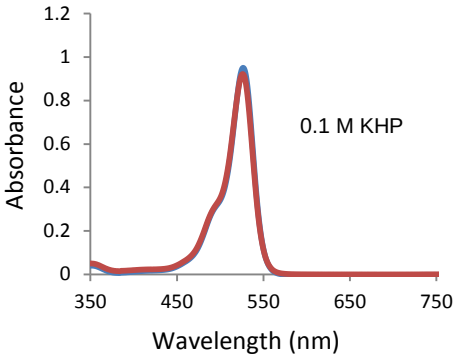
(A = absorbance; Spec abs = specific absorbance ($A\frac{1\%}{1\ cm}$); a = absorptivity (l/(g·cm)); KHP = potassium dihydrogen phosphate; AmAc = ammonium acetate)

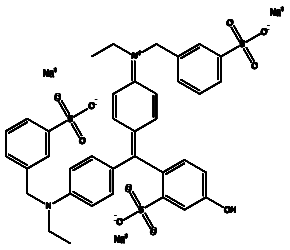
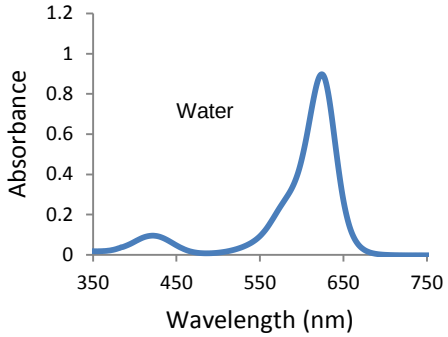
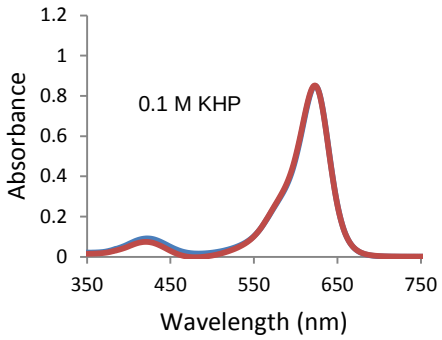
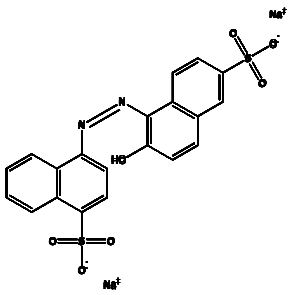
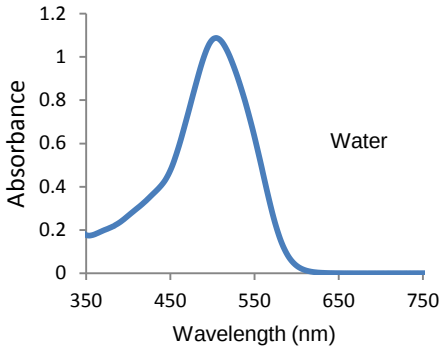
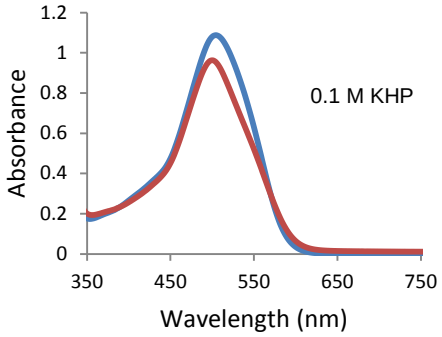
Table 1. Values for synthetic colours for use in performing test for Colouring Matters Content by Spectrophotometry

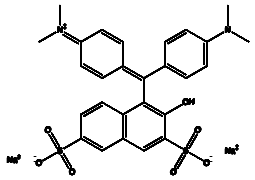
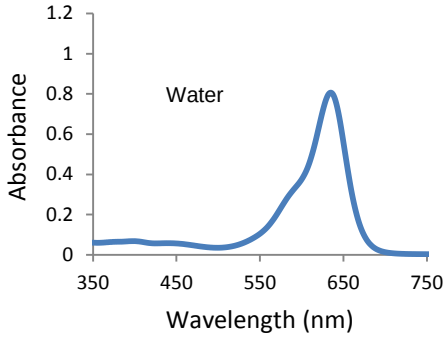
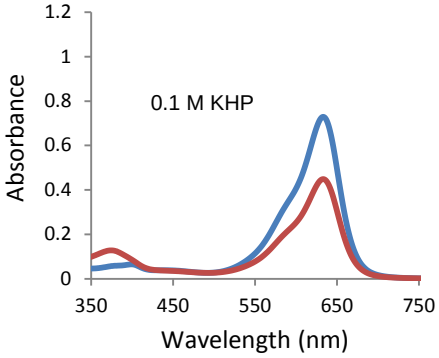
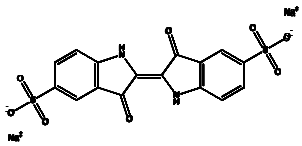
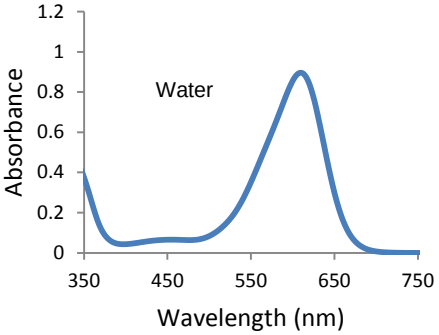
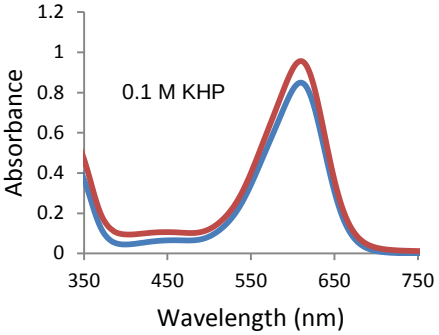
JECFA name	Sample weight	Structure	Spectral data	Visible absorption spectrum
Allura Red AC	195.3 mg		Water λ_{max} = 501 A = 0.9659 Spec abs = 540 a = 54.0 0.04 M AmAc λ_{max} = 497 A = 0.9315 Spec abs = 520 a = 52.0	
Allura Red AC Aluminium Lake	51.6 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 495 A = 0.9089 Lake (red) 0.1 M KHP λ_{max} = 495 A = 1.009	
Amaranth	229.3 mg		Water λ_{max} = 520 A = 0.9013 Spec abs = 440 a = 44.0 0.04 M AmAc λ_{max} = 521 A = 0.8957 Spec abs = 437 a = 43.7	
Amaranth Aluminium Lake	47.7 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 520 A = 0.8779 Spec abs = 429 (est.) a = 42.9 Lake (red) 0.1 M KHP λ_{max} = 520 A = 1.0330	

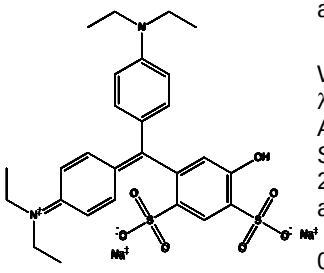
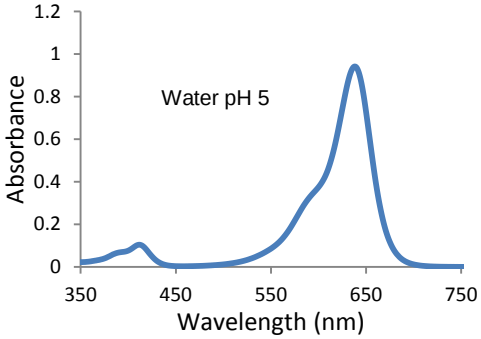
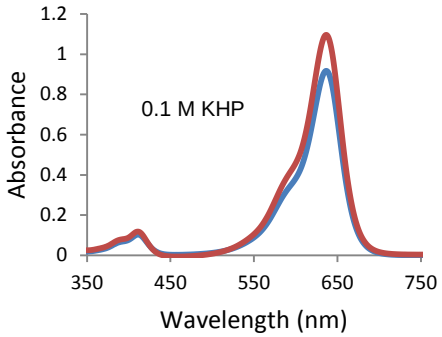
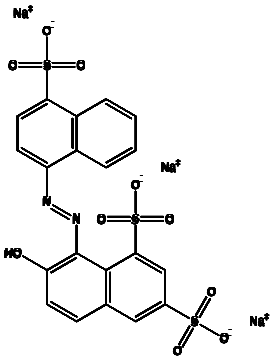
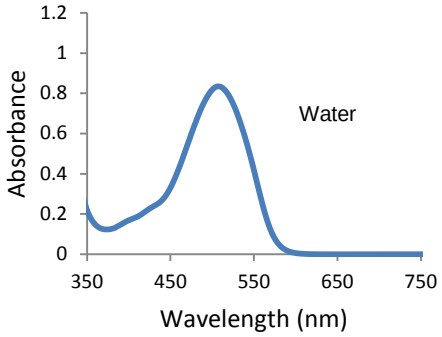
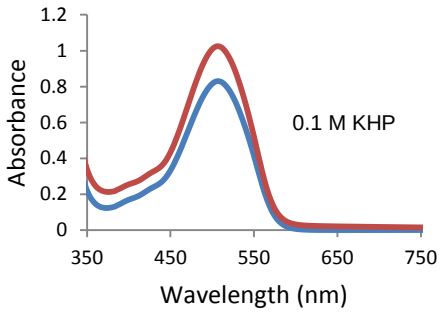
Azorubine	198.5 mg		Water λ_{max} = 516 A = 0.9196 Spec abs = 510 a = 51.0 0.04 M AmAc λ_{max} = 515 A = 0.8986 Spec abs = 498 a = 49.8	
Azorubine Aluminium Lake	40.9 mg		Straight colour 0.1 M KHP λ_{max} = 514 A = 0.8787 Spec abs = 487 a = 48.7 Lake 0.1 M KHP λ_{max} = 515 A = 0.9411	
Brilliant Black PN	187.1 mg		Water λ_{max} = 572 A = 0.7898 Spec abs = 530 a = 53.0 0.04 M AmAc λ_{max} = 571 A = 0.8127 Spec abs = 545 a = 54.5	
Brilliant Black PN Aluminium Lake	39.0 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 570 A = 0.7529 Spec abs = 505 a = 50.5 Lake (red) 0.1 M KHP λ_{max} = 579 A = 0.9787	

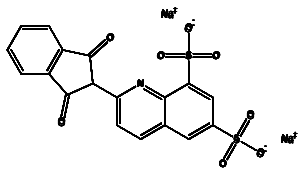
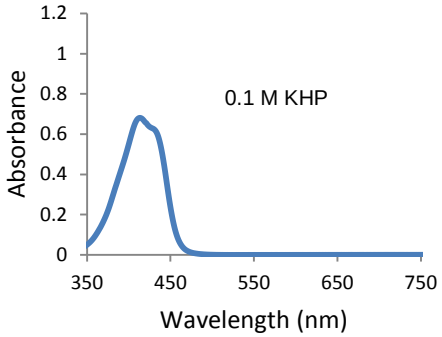
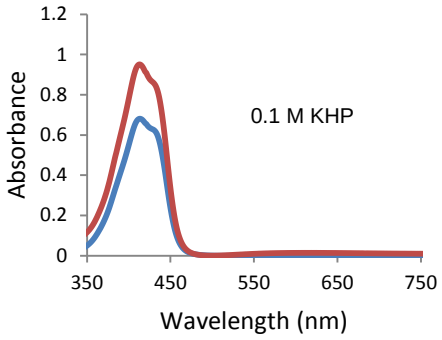
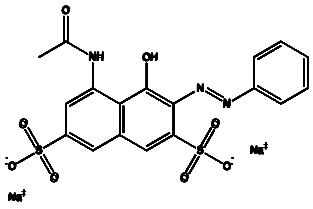
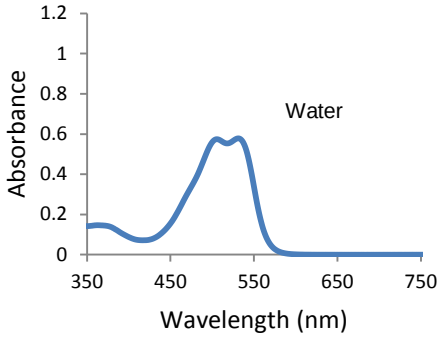
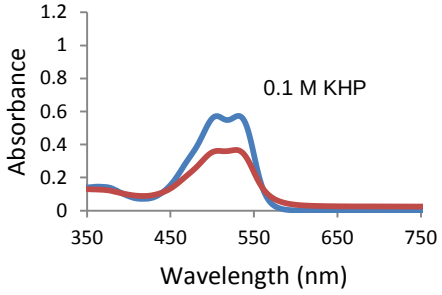
Brilliant Blue FCF	62.9 mg		Water λ_{max} = 629 A = 0.9512 Spec abs = 1630 0.04 M AmAc λ_{max} = 629 A = 0.9596 a = 164	
Brilliant Blue FCF Aluminium Lake	15.2 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 629 A = 0.9627 Lake (red) 0.1 M KHP λ_{max} = 629 A = 0.9599	
Brown HT	248.7 mg		Water, pH 7 λ_{max} = 465 A = 1.063 Spec abs = 403 a = 40.3 Water λ_{max} = 464 A = 1.068 Spec abs = 402 a = 40.2 0.04 M AmAc λ_{max} = 460 A = 0.9922 Spec abs = 376 a = 37.6	
Brown HT Aluminium Lake	50.8 mg		Straight colour (blue) 0.04 M AmAc λ_{max} = 456 A = 0.9922 Lake 0.04 M AmAc (red) λ_{max} = 442 A = 1.0399	

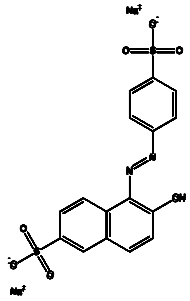
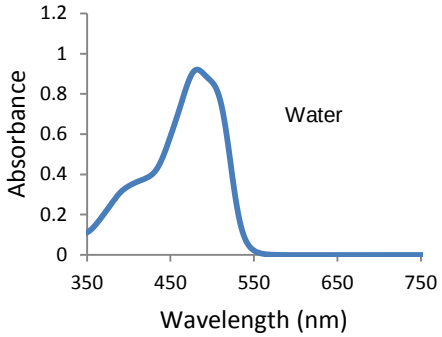
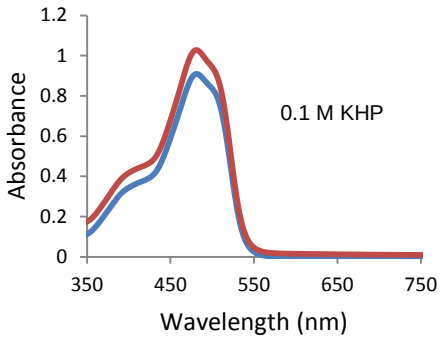
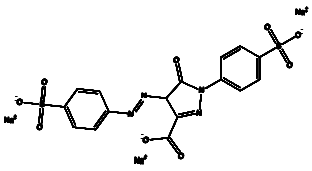
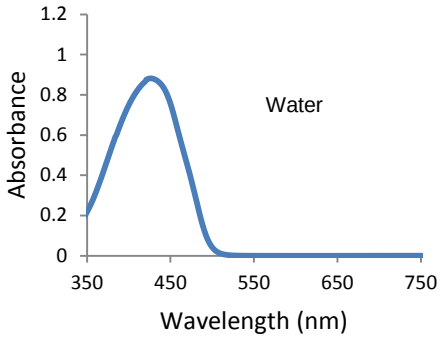
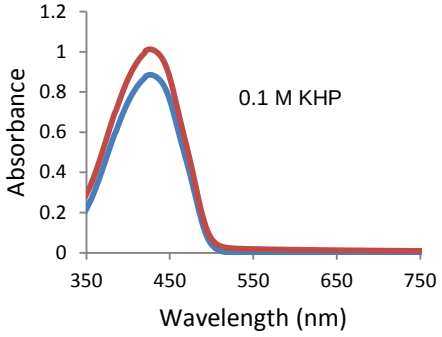
Cochineal extract	72.4 mg		0.06 M HCl λ_{max} = 495 A = 0.8909 Spec abs = 139 a = 13.9	
Carmine	145.0 mg		Straight colour (blue) 0.06 M HCl λ_{max} = 495 A = 0.8909 Lake (red) 0.06 M HCl λ_{max} = 494 A = 0.9838	
Erythrosine	91.9 mg		Water λ_{max} = 527 A = 0.9698 Spec abs = 1100 a = 110 0.5% NH_4OH λ_{max} = 527 A = 0.9554 Spec abs = 1100 a = 110	
Erythrosine Aluminium Lake	22.0 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 526 A = 0.9505 Spec abs = 1080 a = 108 Lake (red) 0.1 M KHP λ_{max} = 526 A = 0.9221 Lake 0.5% NH_4OH λ_{max} = 526 A = 0.9201	

Fast Green FCF	65.03 mg		Water λ_{max} = 624 A = 0.9112 Spec abs = 1610 a = 161 0.04 M AmAc λ_{max} = 624 A = 0.8824 Spec abs = 1560 a = 156	
Fast Green FCF Aluminium Lake	47.7 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 623 A = 0.8560 Lake (red) 0.1 M KHP λ_{max} = 624 A = 1.057	
Fast Red E	271.3 mg		Water λ_{max} = 504 A = 1.088 Spec abs = 467 a = 46.7 0.04 M AmAc λ_{max} = 502 A = 1.051 Spec abs = 465 a = 46.5	
Fast Red E Aluminium Lake	54.7 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 500 A = 1.025 Spec abs = 453 a = 45.3 Lake (red) 0.1 M KHP λ_{max} = 500 A = 0.9630	

Green S	58.7 mg		Water λ_{max} = 635 A = 0.8064 Spec abs = 1720 a = 172 0.04 M AmAc λ_{max} = 634 A = 0.7652 Spec abs = 1630 a = 16.3	
Green S Aluminium Lake	23.5 mg		Straight colour 0.1 M KHP λ_{max} = 633 A = 0.7289 Spec abs = 1550 a = 155 Lake 0.1 M KHP λ_{max} = 633 A = 0.4485	
Indigotine	209.7 mg		Water λ_{max} = 610 A = 0.8960 Spec abs = 480 a = 48.0 0.04 M AmAc λ_{max} = 610 A = 0.8710 Spec abs = 4780 a = 47.8	
Indigotine Aluminium Lake	52.2 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 610 A = 0.8496 Spec abs = 466 a = 46.6 Lake (red) 0.1 M KHP λ_{max} = 610 A = 0.9568	

Patent Blue V	52.3 mg		Water, pH 5 λ_{max} = 638 A = 0.9421 Spec abs = 2000 a = 200 Water λ_{max} = 638 A = 0.9450 Spec abs = 2006 a = 200.6 0.04 M AmAc λ_{max} = 638 A = 0.9193 Spec abs = 1952 a = 195.2	
Patent Blue V Aluminium Lake	21.6 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 637 A = 0.9175 Spec abs = 1950 a = 195 Lake solution (red) 0.1 M KHP λ_{max} = 637 A = 1.0960	
Ponceau 4R	232.5 mg		Water λ_{max} = 507 A = 0.8345 Spec abs = 430 a = 43.0 0.04 M AmAc λ_{max} = 507 A = 0.8325 Spec abs = 429 a = 42.9	
Ponceau 4R Aluminium Lake	46.6 mg		Straight colour 0.1 M KHP λ_{max} = 507 A = 0.8306 Spec abs = 428 a = 42.8 Lake 0.1 M KHP λ_{max} = 507 A = 1.0250	

Quinoline Yellow Mostly disulfonated positional isomers	116.2 mg		0.1 M KHP λ_{max} = 413 A = 0.6810 Spec abs = 865 a = 86.5 Water λ_{max} = 414 A = 0.6921 Spec abs = 879 a = 87.9 0.04 M AmAc λ_{max} = 414 A = 0.6883 Spec abs = 874 a = 87.4	
Quinoline Yellow Aluminium Lake	24.8 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 413 A = 0.6810 Lake (red) 0.1 M KHP λ_{max} = 413 A = 0.9511	
Red 2G	162.4 mg		Water λ_{max} = 531 A = 0.5784 Spec abs = 620 a = 62,0 0.04 M AmAc λ_{max} = 531 A = 0.5745 Spec abs =616 a = 61.6	
Red 2G Aluminium Lake	33.8 mg		Straight colour 0.1 M KHP λ_{max} = 531 A = 0.5733 Spec abs = 615 a = 61.5 Lake 0.1 M KHP λ_{max} = 529 A = 0.3673	

Sunset Yellow FCF	187.5 mg		Water λ_{max} = 482 A = 0.9208 Spec abs = 555 a = 55.5 0.04 M AmAc λ_{max} = 482 A = 0.9222 Spec abs = 540 a = 54.0	
Sunset Yellow FCF Aluminium Lake	47.4 mg		Straight colour (red) 0.1 M KHP λ_{max} = 481 A = 0.9095 Spec abs = 532 a = 53.2 Lake (blue) 0.1 M KHP λ_{max} = 481 A = 1.028	
Tartrazine	188.6 mg		Water λ_{max} = 427 A = 0.8820 Spec abs = 530 a = 53.0 0.04 M AmAc λ_{max} = 427 A = 0.8974 Spec abs = 530 a = 53.0	
Tartrazine Aluminium Lake	45.6 mg		Straight colour (blue) 0.1 M KHP λ_{max} = 427 A = 0.8853 Spec abs = 522 a = 52.2 Lake (red) 0.1 M KHP λ_{max} = 426 A = 1.012	

PURITY TESTS FOR MODIFIED STARCHES

Carboxyl groups

Principle

The carboxyl containing starch is equilibrated with mineral acid to convert carboxyl salts to the acid form. Cations and excess acid are removed by washing with water. The washed sample is gelatinized in water and titrated with standard alkali.

NOTE: Native phosphate groups present in potato starch increase the titre found in this method (See NOTE 6).

Reagents

Hydrochloric Acid Solution, 0.10 N: Standardization unnecessary
Sodium Hydroxide Solution, 0.10 N : Standardized
Phenolphthalein Indicator, 1%

Procedure

If necessary, grind sample completely through a laboratory cutting mill to 20 mesh or finer, taking precautions to prevent any significant change in moisture, and mix thoroughly.

Weigh accurately a sample containing not more than 0.25 milliequivalents of carboxyl (Note 1), and transfer quantitatively to a 150-ml beaker. Add 25 ml of 0.1 N hydrochloric acid and stir occasionally over a period of 30 min. Vacuum filter the slurry through a medium porosity fritted-glass crucible or small funnel, using a fine stream of water from a wash bottle to aid quantitative transfer of the sample. Wash the sample with distilled water (300 ml usually sufficient) until the filtrate is free from chloride determined by silver nitrate test (NOTE 2).

Transfer the demineralized sample quantitatively to a 600-ml beaker with the aid of distilled water, and slurry the sample in 300 ml of distilled water. Heat sample dispersion in a steam bath or boiling water bath (NOTE 3), stirring continuously until the starch gelatinizes, and continue heating for 15 min to ensure complete gelatinization (NOTE 4).

Remove sample from bath and titrate while hot with standard 0.10 N sodium hydroxide solution to a phenolphthalein end-point. The end-point may be detected electrometrically at pH 8.3. A blank determination is run on the original sample to correct for native acid substances (Note 5). Weigh the same quantity of starch as taken for carboxyl titration, and slurry in 10 ml of distilled water. Stir at about 5-min intervals for 30 min.
Vacuum filter the slurry quantitatively through a medium porosity fritted-glass crucible or small funnel, and wash sample with 200 ml of distilled water. Transfer, gelatinize, and titrate the sample with standard 0.10 N sodium hydroxide in the same manner as the demineralized sample.

Calculation:

$$\text{Carboxyl groups (\%)} = \frac{(\text{ml 0.10N NaOH - Blank}) \times 0.0045 \times 100}{\text{Sample weight (g)}}$$

Notes and Precautions

1. Sample size should not exceed 5.0 g for a mildly oxidized or less than 0.15 g for a highly oxidized commercial starch.

2. Add 1 ml of 1% aqueous silver nitrate solution to 5 ml of filtrate. Turbidity or precipitation occurs within 1 min if chloride is present.

3. Heating on a hot plate or over a Bunsen burner is not recommended. Over-heating or scorching in amounts too small to be visible will cause sample decomposition and apparent high carboxyl results.

4. Thorough gelatinization facilitates rapid titration and accurate end-point detection.

5. A blank titration is run on a water-washed sample to correct for acidic components which are not introduced by oxidation or derivatization. Free fatty acids complexed with amylose in common corn starch are the principal contributors to the blank titre.

6. A correction for phosphate content in potato starch (deduction) should be made after determining the phosphorus content of the sample being examined.

The deduction is calculated:

$$\frac{2 \times 45.02 \times P}{30.97} = 2.907 \times P$$

where

P is the phosphorus content (%).

Phosphorus

Reagents

- Ammonium Molybdate Solution (5%): Dissolve 50 g of ammonium molybdatetetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, in 900 ml of warm water, cool to room temperature, dilute to 1000 ml with water, and mix.

- Ammonium Vanadate Solution (0.25%): Dissolve 2.5 g of ammonium metavanadate, NH4VO3, in 600 ml of boiling water, cool to 60 - 70o, and add 20 ml of nitric acid. Cool to room temperature, dilute to 1000 ml with water, and mix.

- Zinc Acetate Solution (10%): Dissolve 120 g of zinc acetate dihydrate, Zn(C2H3O2)2·2H2O, in 880 ml of water, and filter through Whatman No. 2V or equivalent filter paper before use.

- Nitric Acid Solution (29%): Add 300 ml of nitric acid (sp. gr 1.42) to 600 ml of water, and mix.

- Standard Phosphorus Solution: (100 µg P in 1 ml): Dissolve 438.7 mg of monobasic potassium phosphate, KH2PO4, in water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Standard Curve

Pipet 5.0, 10.0, and 15.0 ml of the Standard Phosphorus Solution into separate 100-ml volumetric flasks. To each of these flasks, and to a fourth blank flask, add in the order stated 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg P per 100 ml.

Sample pre-treatment

Place 20 to 25 g of the starch sample in a 250-ml beaker, add 200 ml of a 7 to 3 methanol-water mixture, disperse the sample, and agitate mechanically for 15 min. Recover the starch by vacuum filtration in a 150 ml medium-porosity fritted-glass or Buchner funnel, and wash the wet cake with 200 ml of the methanol-water mixture. Reslurry the wet cake in the solvent, and wash it a second time in the same manner. Dry the filter cake in an air oven at a temperature below 50°, then grind the sample to 20-mesh or finer, and blend thoroughly. Determine the amount of dry substance by drying a 5 g portion in a vacuum oven, not exceeding 100 mm of Hg, at 120° for 5 h. (NOTE: The treatment outlined above is satisfactory for starch products that are insoluble in cold water.

For pregelatinized starch and other water-soluble starches, prepare a 1% to 2% aqueous paste, place it in a cellophane tube, and dialyze against running distilled water for 30 to 40 h. Precipitate the starch by pouring the solution into 4 volumes of acetone per volume of paste, while stirring. Recover the starch by vacuum filtration in a medium-porosity fritted-glass or Buchner funnel, and wash the filter cake with absolute ethanol. Dry the filter cake, and determine the amount of dry substance as directed for water-insoluble starches).

Sample preparation

Transfer about 10 g of the Treated Sample, calculated on the dry-substance and accurately weighed, into a Vycor dish, and add 10 ml of Zinc Acetate Solution in a fine stream, distributing the solution uniformly in the sample. Carefully evaporate to dryness on a hot plate, then increase the heat, and carbonize the sample on the hot plate or over a gas flame. Ignite in a muffle furnace at 550° until the ash is free from carbon (about 1 to 2 h), and cool. Wet the ash with 15 ml of water and wash slowly down the sides of the dish with 5 ml of Nitric Acid Solution. Heat to boiling, cool, and quantitatively transfer the mixture into a 200-ml volumetric flask, rinsing the dish with three 20-ml portions of water and adding the rinsings to the flask. Dilute to volume with water, and mix. Transfer an accurately measured aliquot (V, in ml) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-ml volumetric flask and add 10 ml of Nitric Acid Solution, 10 ml of Ammonium Vanadate Solution, and 10 ml of Ammonium Molybdate Solution, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min.

Procedure

Determine the absorbance of the Sample Preparation in a 1 cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. From the Standard Curve, determine the mg of phosphorus in the aliquot taken, recording this value as a. Calculate the amount in mg/kg of Phosphorus (P) in the original sample by the formula:

$$\frac{a \times 200 \times 1000}{V \times W}$$

where

W is the weight of the sample taken, in g.

Manganese

Instrumentation
Atomic absorption spectrophotometer with manganese hollow cathode lamp.

Preparation of solutions
Standard solution: Prepare a solution containing 0.5 mg/l of manganese.
Sample solution: Transfer 10.000 g of the sample into a 200-ml Kohlrausch volumetric flask, previously rinsed with 0.5 N hydrochloric acid, add 140 ml of 0.5 N hydrochloric acid, and shake vigorously for 15 min, preferably with a mechanical shaker. Dilute to volume with 0.5 N hydrochloric acid, and shake. Centrifuge approximately 100 ml of the mixture in a heavy-walled centrifuge tube or bottle at 650xg for 5 min, and collect the supernatant liquid. This supernatant comprises the “sample solution”.

Procedure
Follow manufacturer's instructions for operating the atomic absorption spectrophotometer and aspirate distilled water through the air-acetylene burner for 5 min to obtain a base-line reading at 279.5 nm. In the same manner aspirate a portion of the "Standard solution" and note the reading.

Finally, aspirate the "Sample solution" and compare the reading with the reading for the "Standard solution", and multiply this value by 20 to obtain mg per kg of manganese in the original sample taken for analysis.

SPECIFICATIONS FOR CERTAIN FLAVOURING AGENTS

At the 82nd meeting, the Committee prepared specifications of identity and purity of 23 new flavourings in 5 sub-categories for the following numbers: 2211 - 2215, 2216-2221, 2222-2223, 2225 – 2229 and 2230-2234.

The flavouring agent beta-angelicalactone (No. 2222) in the group Aliphatic, alicyclic-fused and aromatic-fused ring lactones was withdrawn by the sponsor and was removed from agenda.

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. ºC – 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 20º, if not otherwise stated); Specific gravity (S.G) (at 25º, if not otherwise stated). The field called "Other requirements" contains four types of entry:

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

The fields named Session/Status contain the number of the meeting at which the specifications were prepared and the status of the specification. All specifications prepared at the 82nd meeting were assigned full status.

The flavouring agents were evaluated using the Procedure for the Safety Evaluation of Flavouring Agents and a list for conclusions in alphabetical order is given in Annex I.

Group 1. Cinnamyl alcohol and related substances

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / CE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
2211	**Ethyl alpha-acetylcinnamate**	4597	$C_{13}H_{14}O_3$	Practically insoluble or insoluble in water	MS	NA	m.p. = 60-61º
Full	Ethyl (2E)-2-benzylidene-3-oxobutanoate		218.25	Soluble	>95%	NA	
	Ethyl 2-butylidene-3-oxobutanoate; Butanoic acid, 3-oxo-2-(phenylmethylene)-, ethyl ester		White powder; Balsamic aroma with tropical fruit notes	N/A	1		
82		620-80-4					
2212	**3-(3,4-Methylenedioxyphenyl)-2-methylpropanal**	4599	$C_{11}H_{12}O_3$	Insoluble	MS	1.529-1.539	
Full	3-(1,3-benzodioxol-5-yl)-2-methylpropanal		192.21	Soluble	>95%	1.158-1.169	
	alpha-Methyl-3,4-(methylenedioxy) hydrocinnamaldehyde; 2-Methyl-3-(3,4-methyenedioxyphenyl)propanal; 2-Methyl-3-(3,4-methyenedioxyphenyl) propionaldehyde		Pale yellow to yellow clear oily liquid; Floral, fresh, melon, ozone, marine aroma	134-135°	5		
82		1205-17-0					
2213	**Ethyl 2-hydroxy-3-phenylpropionate**	4598	$C_{11}H_{14}O_3$	Insoluble	MS	1.528-1.538	
Full	ethyl 2-hydroxy-3-phenylpropanoate		194.23	Soluble	>95%	1.092-1.102	
	Benzopropanoic acid, alpha-hydroxy-, ethyl ester		Colourless to pale yellow clear liquid; Black pepper like aroma	108 @ 0.3 kPa	1		
82		15399-05-0					
2214	**Cinnamaldehyde propyleneglycol acetal**	4596	$C_{12}H_{14}O_2$	Insoluble	MS	1.542-1.552	Isomeric composition: 56-58% E, 42-44% Z; SC: 4-5% cinnamaldehyde
Full	4-methyl-2-(2-phenylethenyl)-1,3-dioxolane		190.24	Soluble	92% (mixture of isomers)	1.059-1.069	
	4-methyl--2-styryl-1,3-dioxolane		Pale yellow to yellow clear liquid; Spicy Cinnamon aroma	274-275°	1		
82		4353-01-9					

2215	**2-Phenylpropanal propyleneglycol acetal**	4595	$C_{12}H_{16}O_2$	Insoluble	MS, IR, HNMR	1.502-1.508	
Full	4-methyl-2-(1-phenylethyl)-1,3-dioxolane		192.25	Soluble	>95% (racemic mixture of isomers)	1.032-1.038	
			Colourless to pale yellow clear liquid; Green mushroom, chicken coop, newly mown hay aroma	256-257º	1		
82		67634-23-5					

Group 2. Aliphatic secondary alcohols, ketones and related esters

JECFA No.	Name	FEMA	Chemical Formula	Solubility	ID test	R.I. (20°)	Other requirements
Status	Chemical Name	FLAVIS	M.W	Solubility in ethanol	Assay min %	S.G. (25°)	
	Synonyms	CE	Physical form; Odour	B.P. °	Acid value		Information required
Session		CAS					
2216	**9-Decen-2-one**	4706	$C_{10}H_{18}O$	Practically insoluble or insoluble	MS, IR, HNMR, CNMR	1.426-1.446	
Full	dec-9-en-2-one		154.25	Very slightly soluble	>95%	0.834-0.854	
	methyl oct-7-enyl ketone		Colorless clear liquid; Pear, apple green and fatty notes	206-207°			
82		35194-30-0					
2217	**Yuzunone**	4691	$C_{11}H_{18}O$	Slightly soluble	MS, IR, HNMR, CNMR	1.531-1.537	Isomeric composition: 52-55% (6Z,8E); 45-48% (6E,8E).
Full	(8E)-undeca-6,8,10-trien-3-one		164.24	Soluble	>95% (mixture of isomers)	0.889-0.895	
			Colorless Oil; Citrus peel-like, Balsamic Floral Aroma	257-258°			
82		1009814-14-5					
2218	**1,5-Octadien-3-ol**	4732	$C_8H_{14}O$	Slightly soluble	MS, HNMR, CNMR	1.453-1.463	Isomeric composition: 94-96% Z, 4-6% E
Full	(5Z)-octa-1,5-dien-3-ol		126.2	Soluble	>95% (mixture of isomers)	0.854-0.867	
	Octa-1,5-dien-3-ol		Clear colorless Liquid; Earthy, mushroom, geranium, leafy, marine	185-187°			
82		83861-74-9					

2219	**3,5-Undecadien-2-one**	4746	$C_{11}H_{18}O$	Sparingly soluble	HNMR	1.518-1.522	Isomeric composition: 89-94% 3E,5E; 6-11% sum of 3E,5Z; 3Z,5Z; and 3Z,5E
Full	(3E,5E)-undeca-3,5-dien-2-one		166.14	Soluble	>95% (mixture of isomers)	0.794- 0.914	
	3,5-undeca-3,5-dien-2-one		Clear, colourless to slightly yellow liquid; Cucumber, green, tallow, fatty, fruity	253-254°			
82		68973-20-6					
2220	**3-Methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol**	4775	$C_{14}H_{24}O$	Soluble	HNMR, MS	1.481-1.486	Isomeric composition: 50-55% E, 45-50% Z; SC: 4-5% 6-(2,2,3-trimethylcyclopent-3-en-1-yl)hex-5-en-3-ol; 1-2% 3-methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-3-en-2-one;
Full	3-methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol		208.34	Soluble	>90% (mixture of isomers)	0.896-0.906 at 20°C	
	4-Penten-2-ol, 3-methyl-5-(2,2,3-trimethyl-3-cyclopenten-1-yl)		Colourless to pale yellow liquid; Woody aroma with sandalwood-like notes	283-288°			
82		67801-20-1					
2221	**(±)-1-Cyclohexylethanol**	4794	$C_8H_{16}O$	Soluble	HNMR, CNMR	1.465-1.468	Isomeric Composition: Racemic
Full	1-Cyclohexylethanol		128.21	Soluble	95%	0.923-0.928	
	(±)-Methylcyclohexylcarbinol; (±)-cyclohexanemethanol; Cyclohexanemethanol, α-methyl		Clear, colourless liquid; Clean, fresh aroma	189°			
82		1193-81-3					

Group 3. Alicyclic, alicyclic-fused and aromatic-fused ring lactones

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / CE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
2223	**2-(2-Hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone**	4140	$C_{10}H_{14}O_2$	Practically insoluble to insoluble	MS, IR, HNMR	1.490-1.496	Isomeric composition: 81-84% (3aS,7aR), 16-19% (3aR, 7aS)
Full	3,6-dimethyl-3a,4,5,7a-tetrahydro-1-benzofuran-2(3H)-one		166.1	Soluble	>95% (mixture of isomers)	1.065-1.071	
	Wine lactone; 2(3H)-Benzofuranone, 3a,4,5,7a-tetrahydro-3,6-dimethyl-		Colourless to pale yellow liquid; Sweet, spicy, woody aroma	231-232°			
82		57743-63-2					
2224	**2-(2-Hydroxyphenyl)cyclopropanecarboxylic acid delta-lactone**	4270	$C_{10}H_8O_2$	Soluble	HNMR	1.595-1.635	SC: 2-3% dihydrocoumarin
Full	1a,7b-dihydrocyclopropa[c]chromen-2(1H)-one		160.17	Soluble	93%	1.246-1.366	
	Cyclopropylcoumarin		Viscous Oil; Sweet, green, spicy aroma	245-246°			
82		5617-64-1					

Group 4. Aliphatic and aromatic amines and amides

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / CE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
2225	**N1-(2,3-Dimethoxybenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide**	4741	$C_{18}H_{21}N_3O_4$	Practically insoluble to insoluble	MS IR CNMR HNMR	NA	mp: 148.5-149.5º
Full	N-(2,3-dimethoxybenzyl)-N'-[2-(pyridin-2-yl)ethyl]ethanediamide		343.38	Soluble	>95%	NA	
	Ethanediamide, N1-[(2,3-dimethoxyphenyl)methyl]-N2-[2-(2-pyridinyl)ethyl]		White powder / Very slight milky aroma	NA			
82		851670-40-1					

2226	**(R)-N-(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide**	4751	$C_{16}H_{25}NO_2$	Practically insoluble to insoluble	IR CNMR HNMR	NA	mp: 62-64º
Full	N-[(2R)-1-methoxy-4-methylpentan-2-yl]-3,4-dimethylbenzamide		263.38	Soluble	>95%	NA	
	Benzamide, N-[(1R)-1-(methoxymethyl)-3-methylbutyl]-3,4-dimethyl-		White solid / Slightly milky to faint savory aroma	NA			
82		851669-60-8					
2227	**(E)-N-[2-(1,3-benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide**	4773	$C_{20}H_{21}NO_5$	Practically insoluble or insoluble	MS IR HNMR	NA	mp: 153-154º
Full	(2E)-N-[2-(1,3-benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide		355.39	Soluble	>95%		
	Rubescenamine		Pearl white powder / Savoury aroma	NA		NA	
82		125187-30-6					
2228	**(E)-3-Benzo[1,3]dioxol-5-yl-N,N-diphenyl-2-propenamide**	4788	$C_{22}H_{17}NO_3$	Practically insoluble or insoluble	IR HNMR	NA	mp: 144-145º
Full	(2E)-3-(1,3-benzodioxol-5-yl)-N,N-diphenylprop-2-enamide		343.38	Soluble	>95%		
	N-[2-(methoxymethyl)-4-methylpentyl]-3,4-dimethylbenzamide		Solid white powder / Savoury aroma	NA		NA	
82		1309389-73-8					
2229	**N-Ethyl-5-methyl-2-(methylethenyl)cyclohexanecarboxamide**	4808	$C_{13}H_{23}NO$	Very slightly soluble in water	HNMR	NA	Isomeric composition: 72-73% (1R,2R,5R)-isomers and 27-28% (1S,2S,5R)- isomers; mp: 83-84º
Full	N-ethyl-5-methyl-2-(prop-1-en-2-yl)cyclohexane-1-carboxamide		209.33	Soluble	>95% (mixture of isomers)	NA	
			White powder / minty aroma	NA			
82		1582789-90-9					

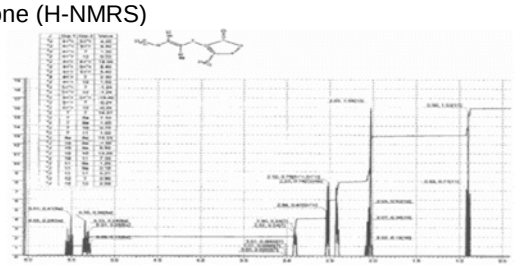
Group 5. Tetrahydrofuran and furanone derivatives

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / CE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °	ID test / Assay min % / Acid value	R.I. (20°) / S.G. (25°)	Other requirements / Information required
2230	**2,5-Dimethyl-3(2H)-furanone**	4101	$C_6H_8O_2$	Practically insoluble or insoluble	IR MS HNMR	1.470-1.480	
Full	2,5-dimethylfuran-3(2H)-one		112.13	Soluble	>95%	1.041-1.057	
	3(2H)-Furanone, 2,5-dimethyl-		Colourless liquid / sweet herbaceous aroma	259-261°			
82		14400-67-0					
2231	**2,5-Dimethyl-4-ethoxy-3(2H)-furanone**	4104	$C_8H_{12}O_3$	Practically insoluble or insoluble	HNMR	NA	mp: 60-61º
Full	4-ethoxy-2,5-dimethylfuran-3(2H)-one		156.18	Soluble	95%	NA	
	2,3-Dihydro-2,5-dimethyl-3-furanone; 2,5-Dimethyl-2,3-dihydrofuran-3-one; 2,5-Dimethyl-2H-furan-3-one; 2,5-Dimethyl-3(2H)furanone		Almost colourless solid / strawberry aroma	NA			
82		65330-49-6					
2232	**5-Methyl-3(2H)-furanone**	4176	$C_5H_6O_2$	Practically insoluble or insoluble	HNMR	1.492-1.498	
Full	3(2H)-Furanone, 5-methyl		98.10	Soluble	95%	NA	
	5-Methylfuran-3(2H)-one		Colorless liquid / herbaceous sweet odour	162-163°			
82		3511-32-8					

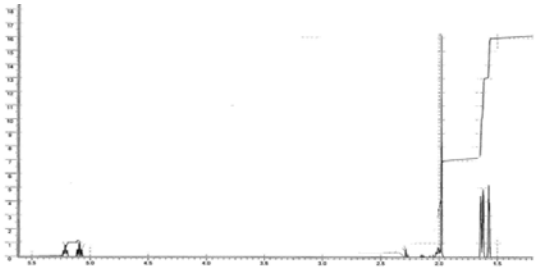
2233	**Ethyl 2,5-dimethyl-3-oxo-4(2H)-furyl carbonate**	4546	$C_9H_{12}O_5$	Practically insoluble or insoluble	MS	1.465-1.475	**SC: 5-6% 2,5-dimethylfuran-3,4-diyl diethyl bis(carbonate)**
Full	2,5-dimethyl-4-oxo-4,5-dihydrofuran-3-yl ethyl carbonate		200.19	Soluble	90%	1.163-1.173	
			Colourless liquid / Cotton candy carmelized sugar aroma	284-285°			
82		39156-54-2					
2234	**4-Acetyl-2,5-dimethyl-3(2H)-furanone**	4070	$C_8H_{10}O_3$	Practically insoluble or insoluble	HNMR	NA	mp: 34-35º
Full	4-acetyl-2,5-dimethylfuran-3(2H)-one		154.17	Soluble	95%	NA	
	4-Acetyl-2,5-dimethyl-3(2H)-furanone		Almost colourless solid / fruity rasberry odour	NA			
82		36871-78-0					

Spectra Of Certain Flavouring Agents

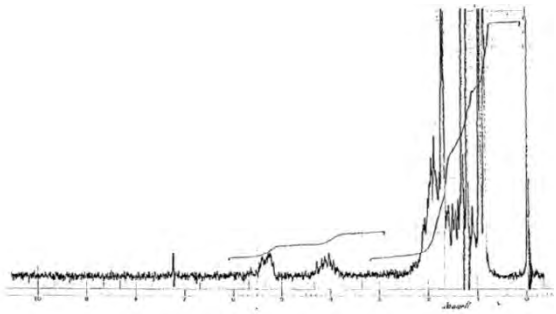
1114 3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one (H-NMRS)



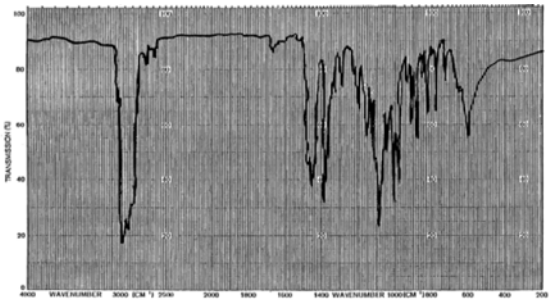
1122 6,10-Dimethyl-5,9-undecadien-2-one (H-NMRS)



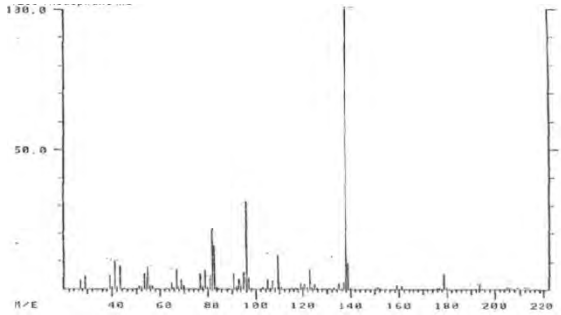
1238 Theaspirane (H-NMRS)



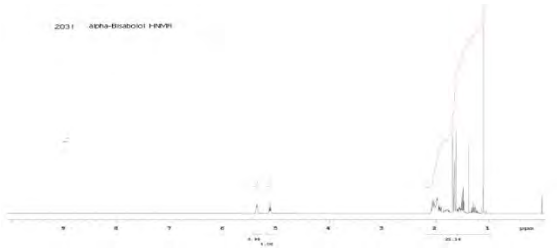
1238 Theaspirane (IR)



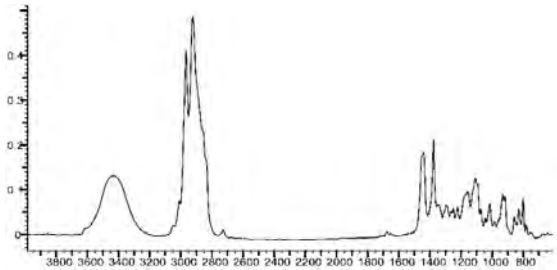
1238 Theaspirane (MS)



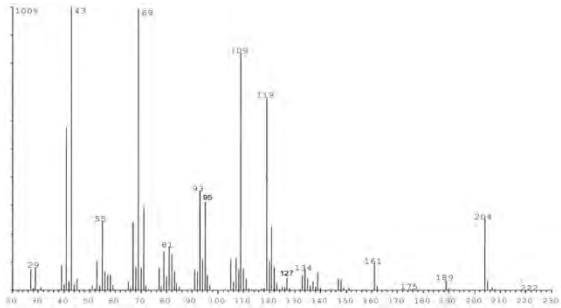
2031 alpha-Bisabolol (H-NMRS)



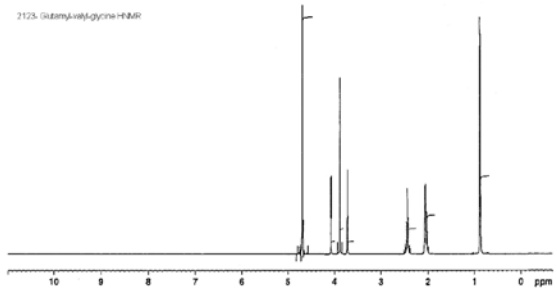
2031 alpha-Bisabolol (IR)



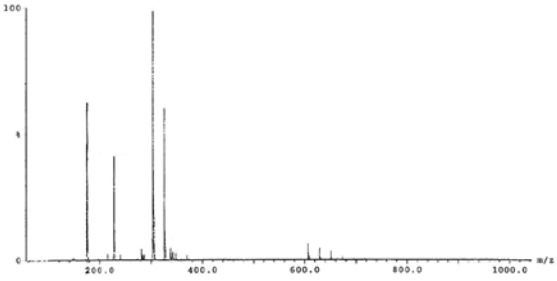
2031 alpha-Bisabolol (MS)



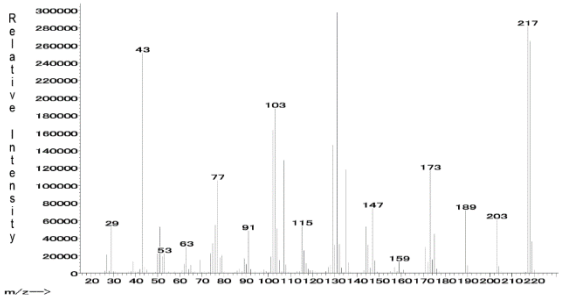
2123 Glutamyl-valyl-glycine (H-NMRS)



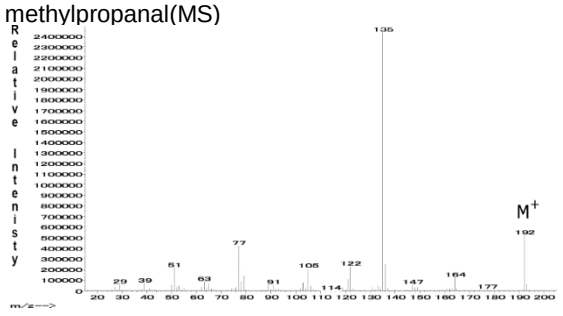
2123 Glutamyl-valyl-glycine (MS)



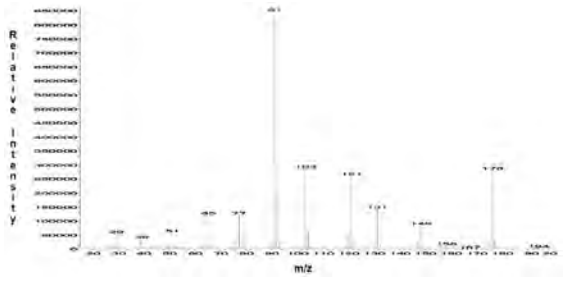
2211 Ethyl alpha-acetylcinnamate (MS)



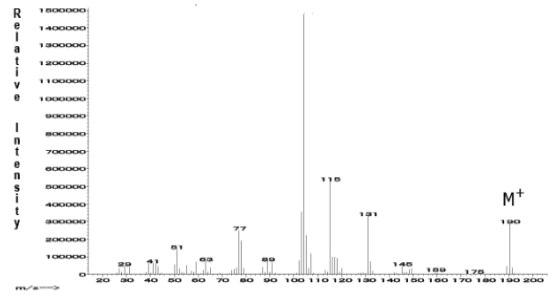
2212 3-(3,4-Methylenedioxyphenyl)-2-methylpropanal(MS)



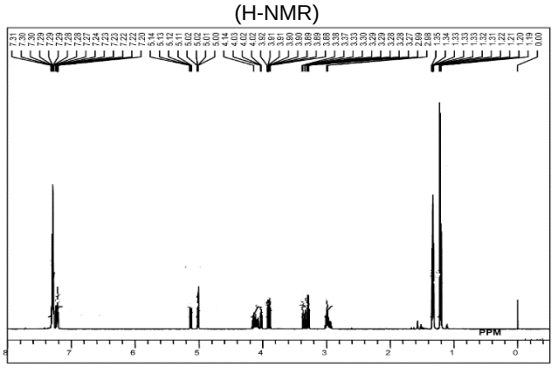
2213 Ethyl 2-hydroxy-3-phenylpropionate (MS)



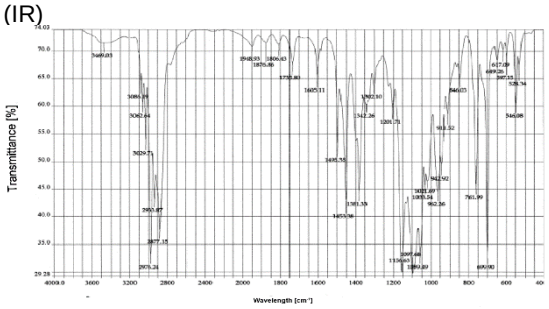
2214 Cinnamaldehyde propyleneglycol acetal (MS)



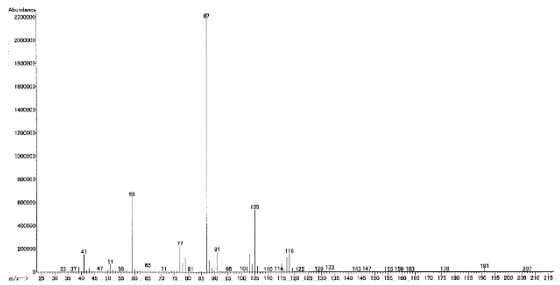
2215 2-Phenylpropanal propyleneglycol acetal (H-NMR)



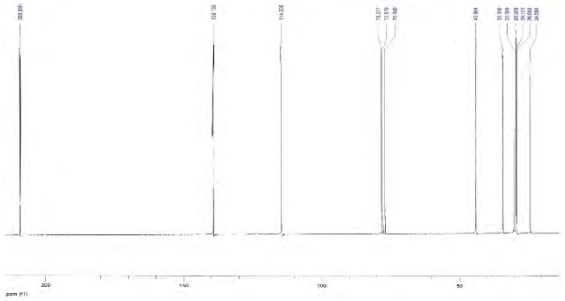
2215 2-Phenylpropanal propyleneglycol acetal (IR)



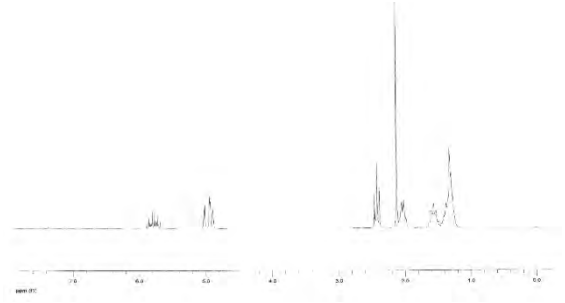
2215 2-Phenylpropanal propyleneglycol acetal (MS)



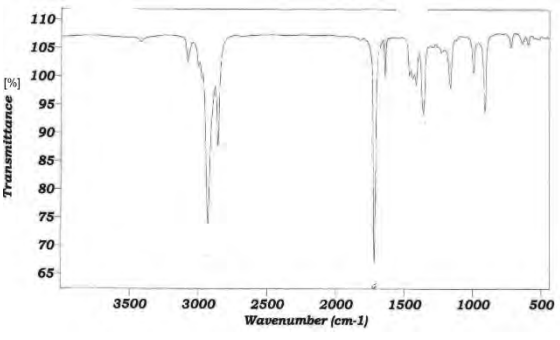
2216 9-Decen-2-one (C-NMRS)



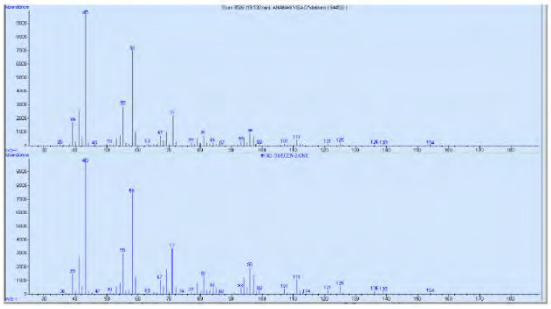
2216 9-Decen-2-one (Proton-NMRS)



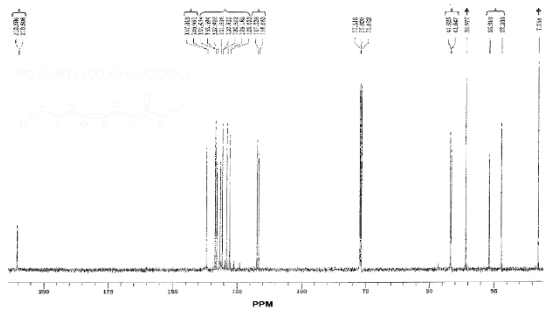
2216 9-Decen-2-one (IR)



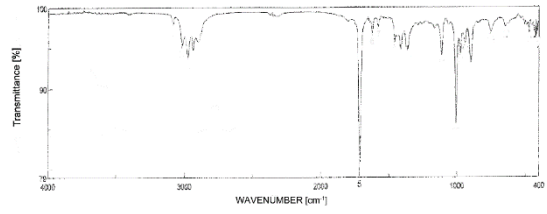
2216 9-Decen-2-one (MS)



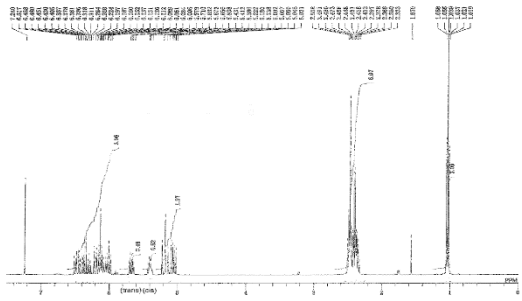
2217 Yuzunone (Carbon-13 NMRS)



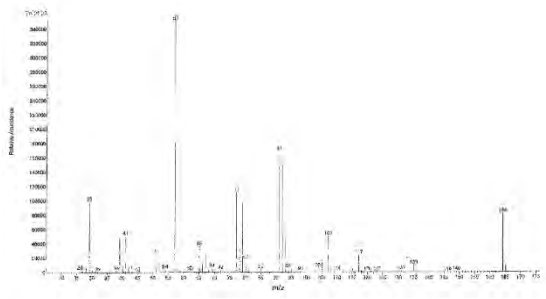
2217 Yuzunone (IR)



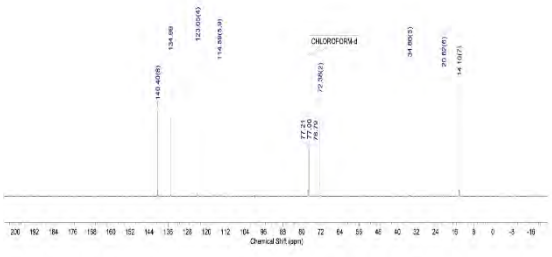
2217 Yuzunone (H-NMR)



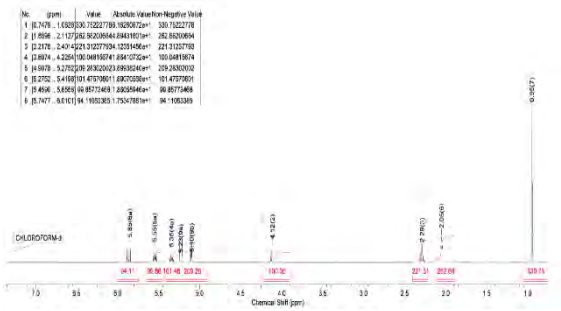
2217 Yuzunone (MS)



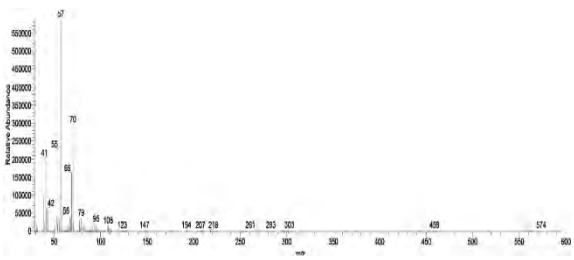
2218 1,5-Octadien-3-ol (CNMR 150MHz)



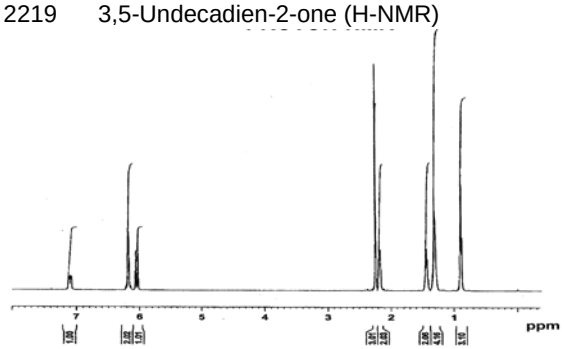
2218 1,5-Octadien-3-ol (H-NMR, 600 MHz)



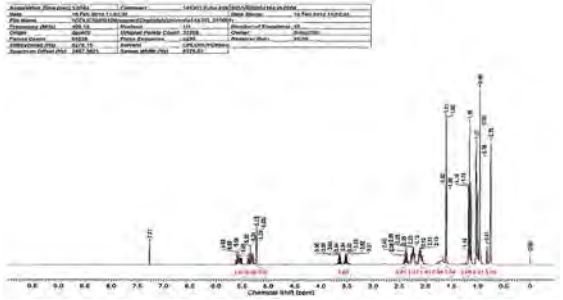
2218 1,5-Octadien-3-ol (MS)



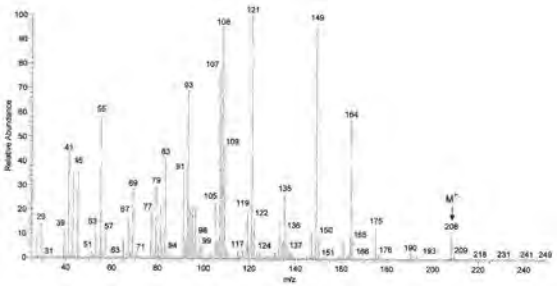
2219 3,5-Undecadien-2-one (H-NMR)



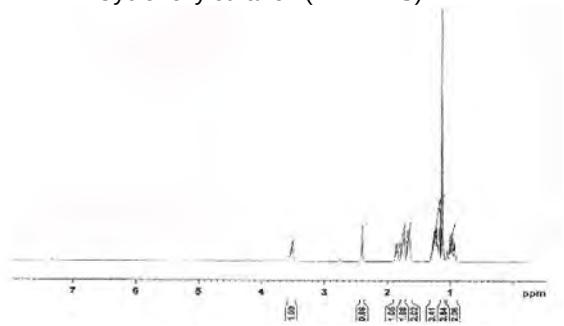
2220 3-Methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol (H-NMRS)



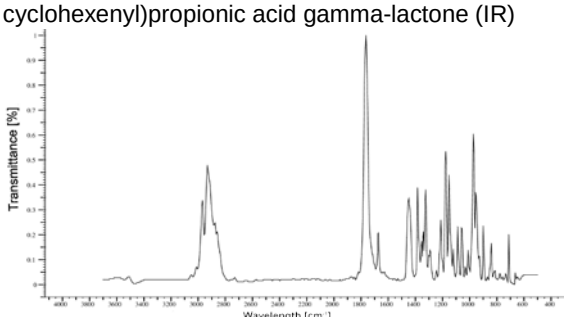
2220 3-Methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol (MS)



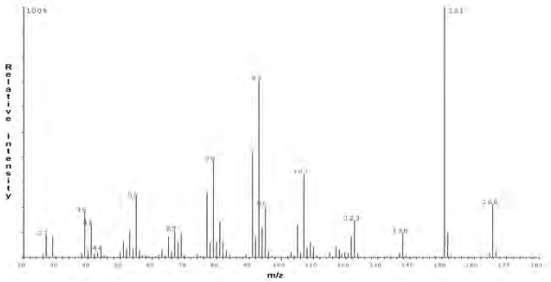
2221 Cyclohexylethanol (H-NMRS)



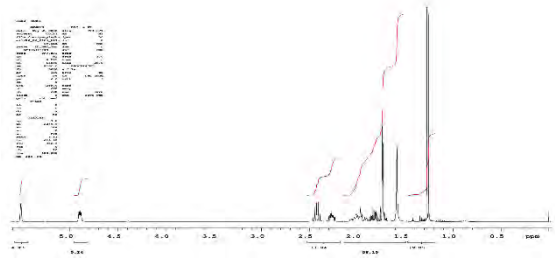
2223 2-(2-Hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (IR)



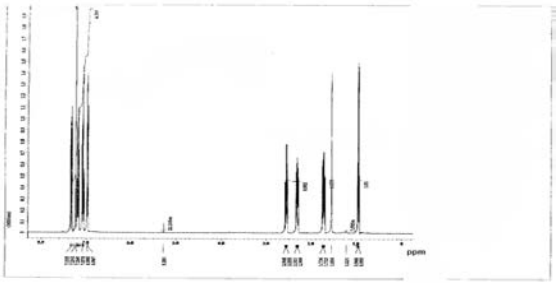
2223 2-(2-Hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (MS)



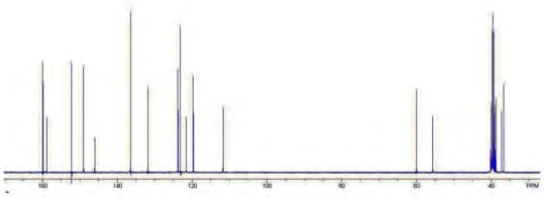
2223 2-(2-Hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone (MS)



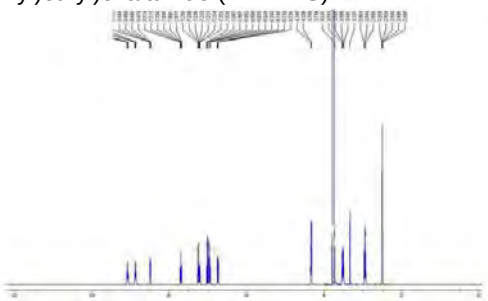
2224 2-(2-Hydroxyphenyl) cyclopropanecarboxylic acid delta-lactone



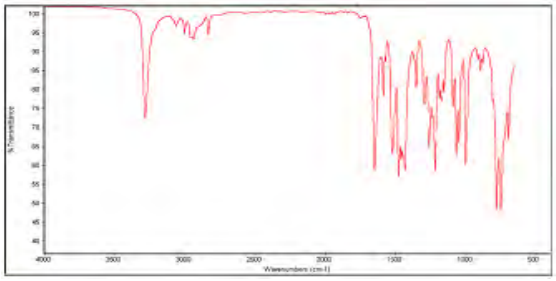
2225 N1-(2,3-Dimethoxybenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide (CNMRS)



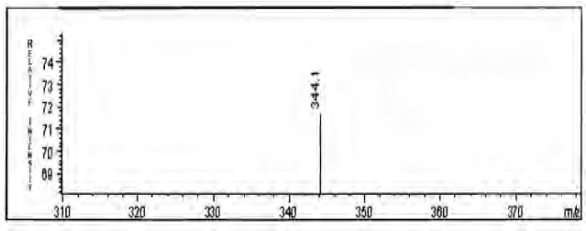
2225 N1-(2,3-Dimethoxybenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide (H-NMRS)



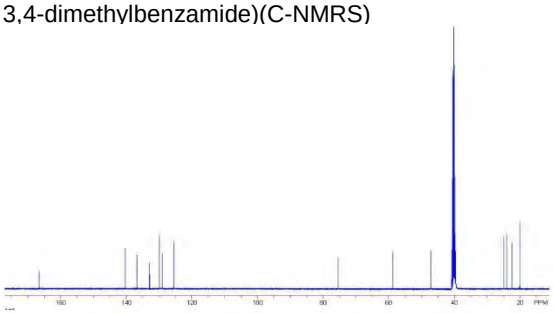
2225 N1-(2,3-Dimethoxybenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide (IR)



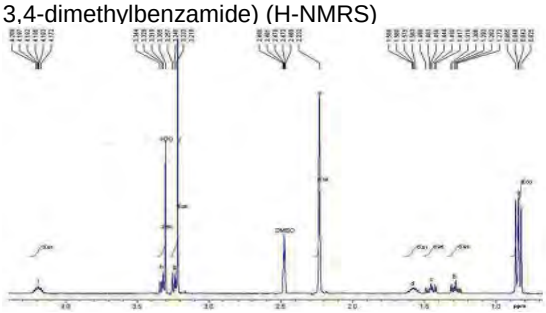
2225 N1-(2,3-Dimethoxybenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide (MS)



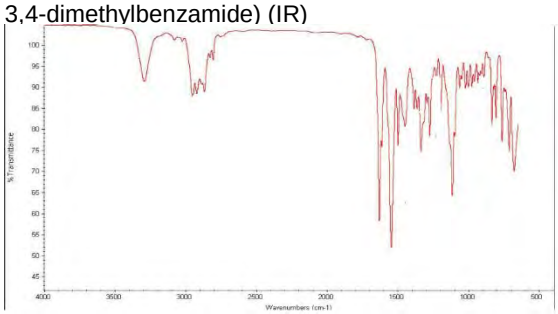
2226 (R)-N-(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide)(C-NMRS)



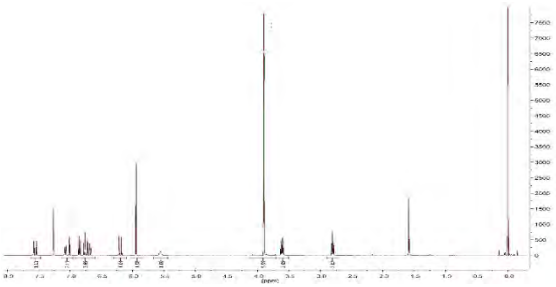
2226 (R)-N-(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide) (H-NMRS)



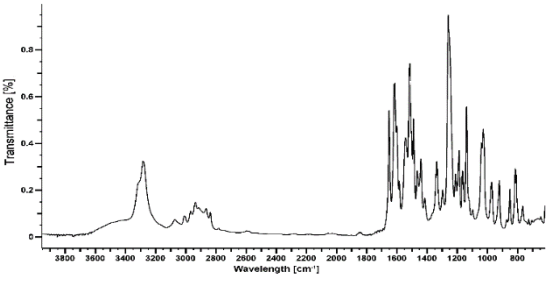
2226 (R)-N-(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide) (IR)



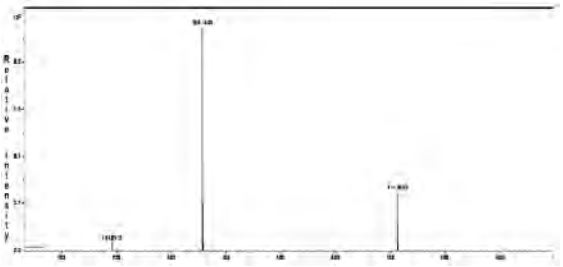
2227 (E)-N-[2-(1,3-benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide (H-NMRS)



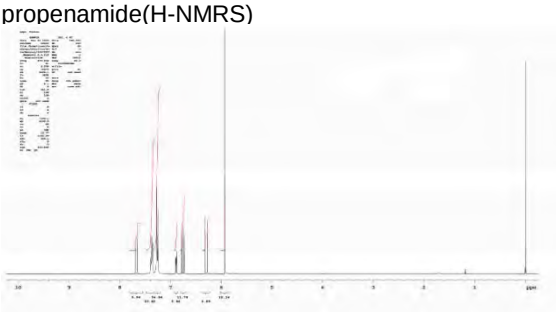
2227 (E)-N-[2-(1,3-benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide (H-NMRS)



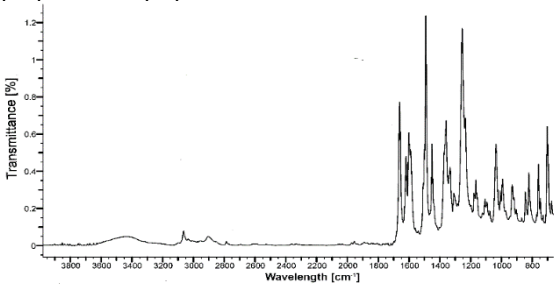
2227 (E)-N-[2-(1,3-benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide (H-NMRS)



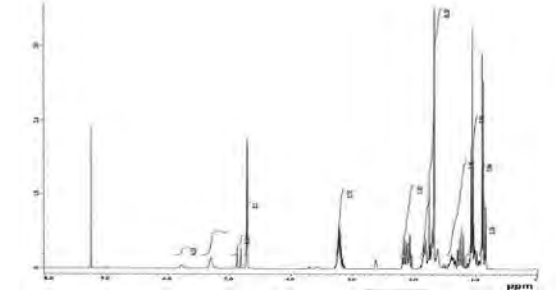
2228 (E)-3-Benzo[1,3]dioxol-5-yl-N,N-diphenyl-2-propenamide(H-NMRS)



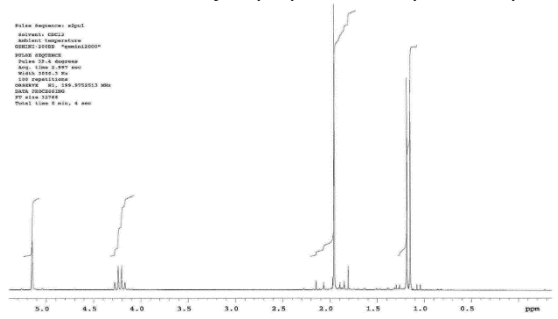
2228 (E)-3-Benzo[1,3]dioxol-5-yl-N,N-diphenyl-2-propenamide(IR)



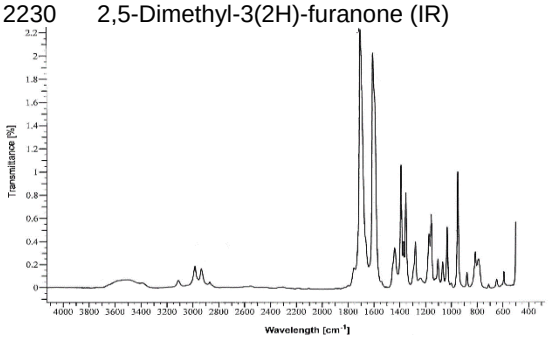
2229 N-Ethyl-5-methyl-2-(methylethenyl) cyclohexanecarboxamide (H-NMRS)



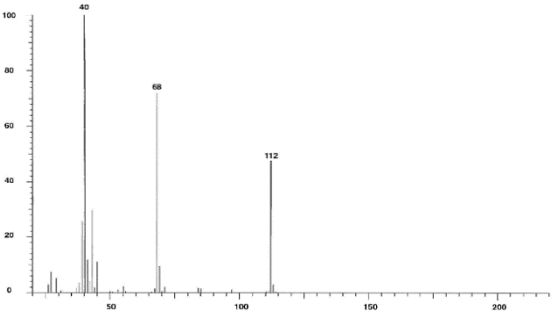
2230 2,5-Dimethyl-3(2H)-furanone (H-NMRS)



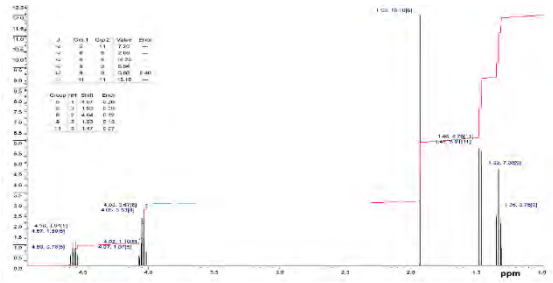
2230 2,5-Dimethyl-3(2H)-furanone (IR)



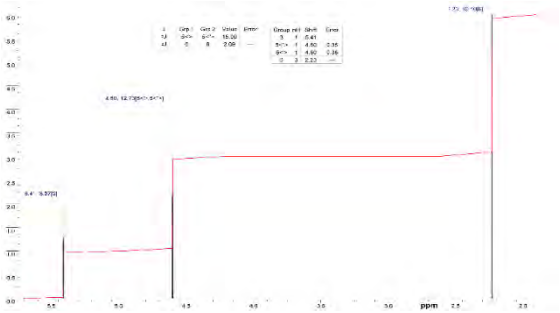
2230 2,5-Dimethyl-3(2H)-furanone (MS)



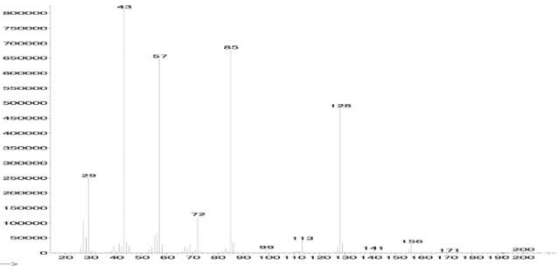
2231 2,5-Dimethyl-4-ethoxy-3(2H)-furanone (H-NMRS)



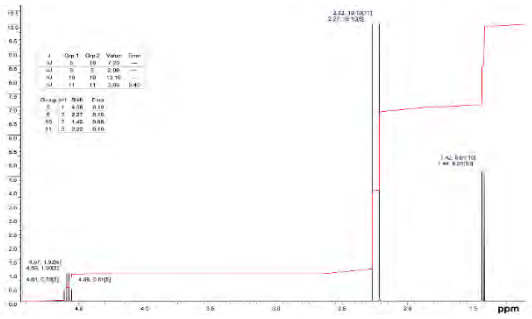
2232 5-Methyl-3(2H)-furanone (H-NMRS)



2233 5-Methyl-3(2H)-furanone (MS)



2234 4-Acetyl-2,5-dimethyl-3(2H)-furanone (MS)



List of new flavourings evaluated in numerical order

3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	1114
6,10-Dimethyl-5,9-undecadien-2-one	1122
Theaspirane	1238
alpha-Bisabolol	2031
Glutamyl-valyl-glycine	2123
Ethyl alpha-acetylcinnamate	2211
3-(3,4-Methylenedioxyphenyl)-2-methylpropanal	2212
Ethyl 2-hydroxy-3-phenylpropionate	2213
Cinnamaldehyde propyleneglycol acetal	2214
2-Phenylpropanal propyleneglycol acetal	2215
9-Decen-2-one	2216
Yuzunone	2217
1,5-Octadien-3-ol	2218
3,5-Undecadien-2-one	2219
3-Methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol	2220
(±)-1-Cyclohexylethanol	2221
2-(2-Hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone	2223
2-(2-Hydroxyphenyl)cyclopropanecarboxylic acid delta-lactone	2224
N1-(2,3-Dimethoxybenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide	2225
(R)-N-(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide	2226
(E)-N-[2-(1,3-benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide	2227
E)-3-Benzo[1,3]dioxol-5-yl-N,N-diphenyl-2-propenamide	2228
N-Ethyl-5-methyl-2-(methylethenyl)cyclohexanecarboxamide	2229
2,5-Dimethyl-3(2H)-furanone	2230
2,5-Dimethyl-4-ethoxy-3(2H)-furanone	2231
5-Methyl-3(2H)-furanone	2232
Ethyl 2,5-dimethyl-3-oxo-4(2H)-furyl carbonate	2233
4-Acetyl-2,5-dimethyl-3(2H)-furanone	2234

Annex I: Summary of recommendations from the 82nd JECFA

Food additives considered for specifications only

Food additive	Specifications
Acetylated distarch adipate	R, T[a,b]
Acetylated distarch phosphate	R, T[a,b]
Acetylated oxidized starch	R[b]
Acid treated starch	R, T[a,b]
Alkaline treated starch	R, T[a,b]
Aspartame	R[c]
Bleached starch	R, T[a,b]
Cassia gum	R, T[d]
Citric and fatty acid esters of glycerol	R[e]
Dextrin roasted starch	R, T[a,b]
Distarch phosphate	R, T[a,b]
Enzyme-treated starch	R, T[a,b]
Hydroxypropyl distarch phosphate	R, T[a,b]
Hydroxypropyl starch	R, T[a,b]
Monostarch phosphate	R, T[a,b]
Octanoic acid	R[f]
Oxidized starch	R[b]
Phosphated distarch phosphate	R, T[a,b]
Starch acetate	R[b]
Starch sodium octenyl succinate	R, T[a,b,g]
Total colouring matters	R[h]

R: existing specifications revised; T: tentative specifications

[a] Additional information is required for the removal of the tentative status (see Future work and recommendations below).

[b] The Committee noted that all the modified starches may additionally be subjected to bleaching and therefore included the appropriate purity tests in the revised specifications.

[c] The purity tests for 5-benzyl-3,6-dioxo-2-piperazineacetic acid and other optical isomers were replaced by new published and validated high-performance liquid chromatography (HPLC) tests. The identification characteristic for solubility in ethanol was changed from “slightly soluble” to “practically insoluble or insoluble”.

[d] The Committee decided to remove the current method for anthraquinones from the specifications and make the specifications tentative. The additional information required for the removal of the tentative status is noted under Future work and recommendations below.

[e] A limit for lead of 0.5 mg/kg for use in infant formula was introduced.

[f] The infrared spectrum identity test conditions and the reference spectrum were included.

[g] The limit for lead (2 mg/kg) was maintained, as no data were received in response to the call for data.

[h] Procedure 1 (water-soluble colouring matters) and Procedure 3 (lakes) were revised. Table 1 was revised to give spectrophotometric data for 17 synthetic colours, their aluminium lakes, cochineal extract and carmine dissolved in water and buffers. Reagents, solution preparations and sample preparation information were added. Equations shown in Procedures 1, 2 and 3 were edited. The tentative status of the method was removed. Where available, information on the wavelength of maximum absorbance, absorptivity and/or specific absorbance (including information on the solvent used) for the 17 synthetic colours and cochineal extract used to form a lake was included in Table 1 of the revised method. The Committee noted that chloroform is listed as a reagent in Procedure 2 (organic solvent–soluble colouring matters) and decided that efforts should be made to replace it.

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Allura Red AC	R[a]	The Committee concluded that the new data do not give reason to revise the ADI and **confirmed the ADI of 0–7 mg/kg body weight** (bw). The Committee noted that the range of estimated dietary exposures to Allura Red AC for children based on reported or industry use data were below the upper bound of the ADI and concluded that dietary exposure to Allura Red AC for children and all other age groups does not present a health concern.
Carob bean gum	R[b]	**The Committee concluded that the available studies are not sufficient for the evaluation of carob bean gum for use in infant formula at the proposed use level**.[c] The Committee requests toxicological data from studies in neonatal animals, adequate to evaluate the safety for use in infant formula, to complete the evaluation.
Lutein esters from *Tagetes erecta*	R[d]	**The Committee removed the temporary designation[e]** (because the tentative status of the specifications was removed) **and established an ADI "not specified"** for lutein esters from *Tagetes erecta*.
Octenyl succinic acid (OSA)–modified gum arabic	R[f]	**The Committee removed the temporary designation[e] and established an ADI "not specified"** for OSA-modified gum arabic. The Committee confirmed the validity of the dietary exposure estimate for risk assessment purposes set at a previous meeting.
Pectin	R[g]	The no-observed-adverse-effect level (NOAEL) in a previously evaluated neonatal pig study was recalculated to be 1049 mg/kg bw per day using measured concentrations of pectin in milk replacer rather than target concentrations. At the new maximum proposed use level of 0.2%, the estimated exposure of infants 0–12 weeks of age would be up to 360 and 440 mg/kg bw per day at mean and high consumption. The margins of exposure for average and high consumers are 2.9 and 2.4, respectively, when compared with the NOAEL of 1049 mg/kg bw per day. On the basis of a number of considerations, **the Committee concluded that the margins of exposure calculated for the use of pectin at 0.2% in infant formula indicate low risk for the health of infants and are not of concern**.
Quinoline Yellow	R[h]	The Committee concluded that it was reasonable to use toxicology data on D&C Yellow No. 10 to support the database for Quinoline Yellow. **The Committee established an ADI of 0–3 mg/kg bw** (rounded value) for Quinoline Yellow on the basis of a NOAEL of 250 mg/kg bw per day for effects on body weight and organ weights in two long-term studies in rats on D&C Yellow No. 10. An uncertainty factor of 100 was applied to account for interspecies and intraspecies variability. The Committee concluded that dietary exposure to Quinoline Yellow for children and all other age groups does not present a health concern.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Rosemary extract	T[i]	**The Committee established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid and carnosol,** on the basis of a NOAEL of 64 mg carnosic acid + carnosol/kg bw per day, the highest dose tested in a short-term toxicity study in rats, with application of a 200-fold uncertainty factor. This uncertainty factor incorporates a factor of 2 to account for the temporary designation of the ADI. The Committee made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. An additional uncertainty factor to account for the lack of a chronic toxicity study was not considered necessary based on the absence of adverse effects in the short-term toxicity studies at doses up to and including the highest dose tested. **The temporary ADI applies to rosemary extract that meets the specifications prepared at the present meeting. It will be withdrawn if the required data are not provided by the end of 2018.** The Committee noted that the dietary exposure estimates for rosemary extract for high consumers, 0.09–0.81 mg/kg bw per day (as carnosic acid plus carnosol), may exceed the upper bound of the temporary ADI by up to 2.7-fold (for young children at the top end of the range of estimated dietary exposures). Based on the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained rosemary extracts at the maximum use level, the Committee concluded that this exceedance of the temporary ADI does not necessarily represent a safety concern.
Steviol glycosides	N[j] N,T[k]	**The Committee confirmed the ADI of 0–4 mg/kg bw, expressed as steviol. The Committee also confirmed that rebaudioside A from multiple gene donors expressed in *Yarrowia lipolytica* is included in the ADI.** The Committee concluded that it was not necessary to make the ADI temporary because the requested information to complete the specifications refers only to an update of the method and has no safety implication. The Committee noted that the predicted maximum dietary exposure to steviol glycosides of 4.0–4.4 mg/kg bw per day for young children who were high consumers exceeded the upper bound of the ADI (up to 110%), but the ADI was not exceeded for other age groups. Considering the conservative nature of the dietary exposure estimate, based on maximum use levels applied to all food consumed from categories with permissions for use in the countries assessed, steviol glycosides are not likely to present a health concern for any age group.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological and dietary exposure conclusions
Tartrazine	R[l]	**The Committee established an ADI of 0–10 mg/kg bw**, on the basis of a NOAEL of 984 mg/kg bw per day for reductions in body weight in a chronic rat study, with application of a 100-fold uncertainty factor to account for interspecies and intraspecies variability. **The Committee withdrew the previous ADI of 0–7.5 mg/kg bw per day.** The Committee noted that the dietary exposure estimate for children aged 1–10 years was below the upper bound of the ADI and concluded that dietary exposure to tartrazine for the general population, including children, does not present a health concern.
Xanthan gum	R[m]	A NOAEL of 750 mg/kg bw per day was established for xanthan gum in neonatal pigs, which are an appropriate animal model for the assessment of the safety of the additive for infants. The margin of exposure based on this NOAEL and the conservative estimate of xanthan gum intake of 220 mg/kg bw per day by infants (high energy requirements for fully formula-fed infants) is 3.4. On the basis of a number of considerations, the Committee concluded that the consumption of xanthan gum in infant formula or formula for special medical purposes intended for infants is of no safety concern at the maximum proposed use level of 1000 mg/L.

N: new specifications; R: existing specifications revised; T: tentative specifications

[a] The method for the determination of lead was changed from atomic absorption to any method appropriate to the specified level. Updated HPLC conditions were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

[b] For carob bean gum and carob bean gum (clarified). A limit for lead of 0.5 mg/kg for use in infant formula was introduced. There were insufficient data to set a limit for arsenic. The method descriptions for the determination of lead and sample preparation for residual solvents were updated.

[c] The Committee noted that the current use level of carob bean gum for infant formula or for formula for special medical purposes intended for infants in CODEX STAN 72-1981 (1000 mg/L) is much lower than the proposed use level (10 000 mg/L).

[d] The tentative status was removed. The assay value was increased from 60% to 75% for total carotenoids, a method for the determination of the proportion of zeaxanthin in total carotenoids (<10%) was included and amendments were made to the method for the determination of waxes.

[e] ADI “not specified” is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

[f] The tentative status was removed.

[g] The limit for lead for general use was lowered from 5 to 2 mg/kg, a limit for lead of 0.5 mg/kg for use in infant formula was introduced and the method descriptions for the determination of lead and sample preparation for residual solvents were updated.

[h] The tentative status was removed. Methods for determining lead and zinc were revised, the titanium trichloride assay was replaced with assay by spectrophotometry, the maximum wavelength of absorbance and absorptivity value for the colour dissolved in water were added, and HPLC conditions for determining the subsidiary colouring matters and organic compounds other than colouring matter and for assaying the colouring components were added.

[i] The published gas chromatography–mass spectrometry method for the determination of key volatiles of rosemary extract was included. Additional information is required to finalize the specifications (see Future work and recommendations below).

[j] A new specifications monograph (Rebaudioside A from Multiple Gene Donors Expressed in *Yarrowia lipolytica*) was prepared for the yeast-derived product.

[k] New tentative specifications for steviol glycosides were established, including a new title name (Steviol Glycosides from *Stevia rebaudiana* Bertoni) to reflect the separation of specifications by source material. The Definition and Assay specification was expanded from nine named leaf-derived steviol glycosides to include any mixture of steviol glycoside compounds derived from *Stevia rebaudiana* Bertoni, provided that the total percentage of steviol glycosides is not less than 95%. Additional information is required to finalize the specifications (see Future work and recommendations below).

[l] The method for the determination of lead was changed from atomic absorption to any method appropriate to the specified level. Updated HPLC conditions were added for determining subsidiary colouring matters and organic compounds other than colouring matters. The method of assay was changed to visible spectrophotometry, and spectrophotometric data were provided for the colour dissolved in water.

[m] The limit for lead in xanthan gum was maintained at 2 mg/kg for general use, and a limit for lead of 0.5 mg/kg for use in infant formula was introduced. The test method for the determination of residual solvents that employs a gas chromatographic method using a packed column was replaced with a method using a capillary column.

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

A. Alicyclic, alicyclic-fused and aromatic-fused ring lactones

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
2-(2-Hydroxy-4-methyl-3-cyclohexenyl)propionic acid gamma-lactone	2223	N	No safety concern
2-(2-Hydroxyphenyl)-cyclopropanecarboxylic acid delta-lactone	2224	N	No safety concern

N: new specifications

B. Aliphatic and aromatic amines and amides

The Committee concluded that the concerns previously expressed by the Committee at its sixty-ninth meeting as to in vivo genotoxicity and how to address the kidney effects and identify a NOAEL have not been sufficiently addressed and that the Procedure still could not be applied to 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595).[6]

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
N1-(2,3-Dimethoxybenzyl)-*N2*-(2-(pyridin-2-yl)ethyl)oxalamide	2225	N	No safety concern
(*R*)-*N*-(1-Methoxy-4-methylpentan-2-yl)-3,4-dimethylbenzamide	2226	N	No safety concern
(*E*)-*N*-[2-(1,3-Benzodioxol-5-yl)ethyl]-3-(3,4-dimethoxyphenyl)prop-2-enamide	2227	N	No safety concern
(*E*)-3-Benzo[1,3]dioxol-5-yl-*N*,*N*-diphenyl-2-propenamide	2228	N	No safety concern
N-Ethyl-5-methyl-2-(methylethenyl)cyclohexanecarboxamide	2229	N[a]	Additional data required to complete evaluation

[6] The statement currently contained in the specifications indicating that the safety evaluation had not been completed will be maintained.

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
N-Ethyl-2,2-diisopropylbutanamide	2005	M[b]	Additional data required to complete evaluation
N-(2-Hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide	2010	M[b]	Additional data required to complete evaluation
N-(1,1-Dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide	2011	M[b]	Additional data required to complete evaluation

M: existing specifications maintained; N: new specifications

[a] The specifications include a statement that the safety evaluation for the flavouring agent had not been completed.

[b] The statement currently contained in the specifications indicating that the safety evaluation had not been completed will be maintained.

C. Aliphatic secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
9-Decen-2-one	2216	N	No safety concern
Yuzunone	2217	N	No safety concern
1,5-Octadien-3-ol	2218	N	No safety concern
3,5-Undecadien-2-one	2219	N	No safety concern
3-Methyl-5-(2,2,3-trimethylcyclopent-3-en-1-yl)pent-4-en-2-ol	2220	N	No safety concern
(±)-1-Cyclohexylethanol	2221	N	No safety concern

N: new specifications

D. Cinnamyl alcohol and related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Ethyl alpha-acetylcinnamate	2211	N	No safety concern
Ethyl 2-hydroxy-3-phenylpropionate	2213	N	No safety concern
Structural class III			
3-(3,4-Methylenedioxyphenyl)-2-methylpropanal	2212	N[a]	Additional data required to complete evaluation
Cinnamaldehyde propyleneglycol acetal	2214	N	No safety concern
2-Phenylpropanal propyleneglycol acetal	2215	N	No safety concern

N: new specifications

[a] The specifications include a statement that the safety evaluation for the flavouring agent had not been completed.

E. Tetrahydrofuran and furanone derivatives

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
2,5-Dimethyl-3(2*H*)-furanone	2230	N	No safety concern
Structural class III			
2,5-Dimethyl-4-ethoxy-3(2*H*)-furanone	2231	N	No safety concern
5-Methyl-3(2*H*)-furanone	2232	N	No safety concern
Ethyl 2,5-dimethyl-3-oxo-4(2*H*)-furyl carbonate	2233	N	No safety concern
4-Acetyl-2,5-dimethyl-3(2*H*)-furanone	2234	N[a]	Additional data required to complete evaluation

N: new specifications

[a] The specifications include a statement that the safety evaluation for the flavouring agent had not been completed.

Flavouring agents considered for specifications only

Flavouring agent	No.	Specifications
3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	1114	R[a]
6,10-Dimethyl-5,9-undecadien-2-one	1122	R[b]
3-Ammonium isovalerate	1203	R[c]
Theaspirane	1238	R[d]
alpha-Bisabolol	2031	R[e]
Glutamyl-valyl-glycine	2123	R[f]

[a] The Committee changed the assay minimum from greater than 98% as the *cis* isomer to greater than 95% as a sum of isomers, revised the ranges for refractive index and specific gravity, and introduced new information on the isomeric composition of the flavouring agent.

[b] The Committee indicated that the assay minimum was for a sum of isomers, changed the Chemical Abstracts Service (CAS) number, revised the information for solubility in ethanol, revised the ranges for refractive index and specific gravity, and introduced new information on the isomeric composition of the flavouring agent.

[c] The Committee corrected the molecular weight and chemical formula and revised the melting point range for the flavouring agent.

[d] The Committee lowered the assay minimum from greater than 97% (sum of stereoisomers) to greater than 85% (sum of stereoisomers), revised the ranges for refractive index and specific gravity, and introduced new information on the isomeric composition and secondary components of the flavouring agent.

[e] The Committee changed the assay minimum from greater than 93% to greater than 95% as a sum of isomers, added a second CAS number, revised the ranges for refractive index and specific gravity, clarified the range of the secondary component, and introduced new information on the isomeric composition of the flavouring agent.

[f] The Committee lowered the assay minimum from greater than 99% to greater than 95%.

Annex 2. General information

Revisions of the Procedure for the Safety Evaluation of Flavouring Agents

The European Food Safety Authority (EFSA) and WHO recently reviewed the general threshold for toxicological concern (TTC) approach in a joint project, building on existing and ongoing work in this area. An expert workshop was convened in December 2014, primarily to provide recommendations as to how the existing TTC framework may be improved and expanded by updating/revising the Cramer classification scheme and extending the TTC approach. An important aspect was also to develop a globally harmonized decision-tree for a tiered approach on the application of the TTC in the risk assessment of chemicals from oral exposures.[7]

Based on the recommendations from this expert workshop, the Committee discussed the consequences for the existing JECFA Procedure for the Safety Evaluation of Flavouring Agents, which is based on the TTC concept, and proposed a revised Procedure. The main change proposed is to remove question 2 of the existing Procedure ("Can the substance be predicted to be metabolized to innocuous products?") and in consequence combine the A-side and B-side of the existing Procedure, because:

1) metabolism is an inherent part of the Cramer, Ford & Hall scheme[8] and the TTC values for the different classes;
2) models for predicting metabolism can have significant limitations, including lack of information on interspecies extrapolation and alterations in metabolite profiles arising from saturation of metabolic pathways;
3) prediction of the major pathways of metabolism may not reflect the hazard associated with a minor pathway; and
4) the B-side of the existing procedure requires toxicity data on the compound or a structurally related substance even if the dietary exposure was below the TTC value, which is inconsistent with the TTC concept.

Another change is to add an initial question regarding genotoxicity and in consequence to delete step B5 ("Do the conditions of use result in an intake greater than 1.5 µg/day?") from the Procedure. The Committee noted that this is the original United States Food and Drug Administration threshold of regulation value of 1.5 µg/person per day, but that this value is of little practical application in the Procedure. Moreover, the Cramer class thresholds as applied would be adequately protective for a non-genotoxic cancer end-point.

The Committee recommends these points for consideration when deciding on the adequacy of a resulting margin of exposure at step 5 of the revised procedure:

- What is the overall strength of the database?
- Is the margin of exposure based on a NOAEL for the flavouring agent or for a structurally related substance?
- What is the effect on which the NOAEL is based?
- Is the NOAEL the highest dose tested or identified from a single-dose study?
- What is the duration of the study from which the NOAEL is identified?

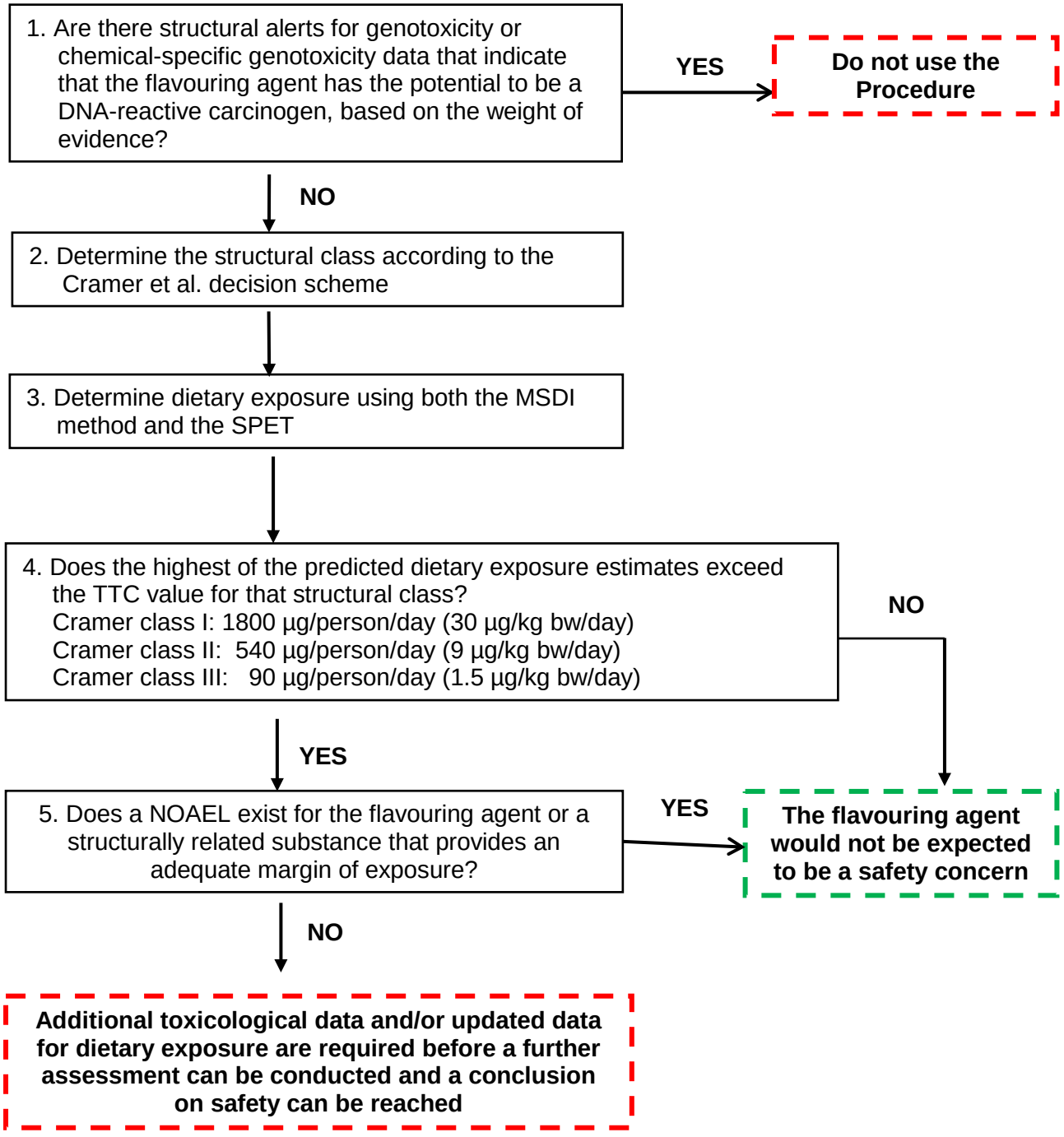
If the overall database is considered, based on expert judgement, to be sufficiently robust, the Committee considered that a margin of exposure that accommodates at least a default safety factor as used in the assessment of food additives may be sufficient to conclude that the flavouring agent would not be expected to be a safety concern at current estimated levels of dietary exposure.

The Committee further concluded that the revised Procedure for the Safety Evaluation of Flavouring Agents (see Fig. 1) should be applied in its future evaluations.

[7] http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/1006e.pdf

[8] Cramer GM, Ford RA, Hall RL. Estimation of toxic hazard – a decision tree approach. Food Cosmet Toxicol. 1978;16:255–76.

Fig. 1. **Revised Procedure for the Safety Evaluation of Flavouring Agents**



The Committee noted that application of the new Procedure would not have an impact on previous evaluations, because genotoxicity is considered in the current Procedure, metabolism is considered in the Cramer decision-tree and, overall, this new procedure is equally robust.

Approach for prioritizing flavouring agents for re-evaluation

The Committee at its seventy-ninth meeting held a preliminary discussion concerning the fact that the submission of additional toxicology data, including genotoxicity data, and/or exposure data for previously evaluated flavouring agents may trigger the need for re-evaluation of previously evaluated flavouring agents. The present Committee reiterated the need for the development of an approach, including a prioritization process, for the re-evaluation of flavouring agents based on all available toxicological data and updated exposure estimates. When developing such an approach, compounds that are used as comparators for structurally related compounds will require specific attention when new data on these become available. The Committee also noted that there is a need to compile data on all flavouring agents that were reported in the monographs of previous meetings and from other sources but not re-evaluated, to assist the prioritization for the re-evaluation.

Moreover, for any flavouring agents for which new toxicological studies are submitted, the sponsor needs to provide updated exposure data.

Limits for lead in specifications of food additives for use in infant formula

The Committee at its seventy-ninth meeting considered four additives for use in infant formula and formula for special medical purposes – namely, carrageenan, pectin, citric and fatty acid esters of glycerol (CITREM) and starch sodium octenyl succinate. At its Eighth Session, the Codex Committee on Contaminants in Foods (CCCF) set a maximum limit (ML) of 0.01 mg/kg for lead in infant formula (as consumed). The Committee at the seventy-ninth meeting noted that three of the four food additives considered for risk assessment at that meeting (pectin, CITREM and starch sodium octenyl succinate) could result in exceedance of the ML for lead in infant formula at proposed use levels if lead were present at the specification limits listed in the individual monographs (i.e. at 5 mg/kg in pectin and at 2 mg/kg in both CITREM and starch sodium octenyl succinate). The seventy-ninth JECFA also noted that the introduction of lower lead limits in the specifications (e.g. 1 mg/kg for pectin, 0.5 mg/kg for CITREM and 0.1 mg/kg for starch sodium octenyl succinate) would result in none of these additives exceeding the ML for lead in the final infant formula (i.e. 0.01 mg/kg) if these additives were included in infant formula at the maximum use level reviewed by JECFA.
For the current meeting, data were requested on the levels of lead present in CITREM, pectin and starch sodium octenyl succinate for use in infant formula, and the Committee received data on levels of lead in CITREM and pectin, but not for starch sodium octenyl succinate.

The Committee evaluated the data presented for levels of lead in 12 non-consecutive lots of CITREM. The levels of lead were below 0.1 mg/kg, the limit of quantification of the method (inductively coupled plasma optical emission spectrometry), demonstrating that the lead level of 0.5 mg/kg proposed by the seventy-ninth JECFA was achievable for CITREM used in infant formula. The current limit of 2 mg/kg for lead in the CITREM specifications monograph was maintained for general use, and a limit of 0.5 mg/kg was included for use in infant formula. The Committee also evaluated data presented for levels of lead in pectin for use in infant formula analysed by two different analytical methods. Levels reported for lead in 12 non-consecutive lots of pectin analysed by inductively coupled plasma atomic emission spectrometry were below the limit of detection of the method (0.4 mg/kg). The mean level of lead reported for five non-consecutive lots of pectin analysed by inductively coupled plasma mass spectrometry was 0.017 mg/kg. Based on the data provided, the Committee noted that the levels of lead in pectin intended for use in infant formula were below the level of 1 mg/kg considered by the Committee at the seventy-ninth meeting. The current limit of 5 mg/kg for pectin in the specifications monograph was reduced to 2 mg/kg for general use, and a limit of 0.5 mg/kg was included for use in infant formula.

The Committee also considered the levels of lead in the specifications monographs of two other additives on the agenda for consideration for use in infant formula – namely, carob bean gum and xanthan gum – in light of this discussion. Based on the data provided, the Committee maintained the lead limits in the specifications monographs for these two additives for general use (2 mg/kg) and reduced them to 0.5 mg/kg for use in infant formula.

Based on the data submitted for CITREM, pectin, carob bean gum and xanthan gum, the Committee was reassured that the overall criterion for lead levels in the ingredients for use in infant formula is achievable. However, the Committee further reaffirmed that it is the responsibility of the infant

formula manufacturers to ensure that the lead levels in the final infant formula (as consumed) comply with the ML for lead as set by the Eighth Session of CCCF.

The Committee recommended that all additives (including starch sodium octenyl succinate) for use in infant formula be reviewed for lead levels in the specifications.

Annex 3. Future work and recommendations

General considerations

Revisions of the Procedure for the Safety Evaluation of Flavouring Agents

The Committee recommended that the revised Procedure for the Safety Evaluation of Flavouring Agents should be applied in its future evaluations.

Approach for prioritizing flavouring agents for re-evaluation

The Committee reiterated the need for the development of an approach, including a prioritization process, for the re-evaluation of flavouring agents based on all available toxicological data and updated exposure estimates.

Replacement of packed column gas chromatographic methods in the specifications monographs

The Committee recommended that the FAO JECFA Secretariat establish a process to identify the food additive specifications monographs containing packed column gas chromatographic methods and request suitable methods (through a call for data), in order for the Committee to replace these methods in the specifications monographs.

Revision of the FAO JECFA Monographs 1, Combined Compendium of Food Additive Specifications, Volume 4

The Committee recommended that the FAO JECFA Secretariat establish a process for the revision of FAO JECFA Monographs 1, Combined Compendium of Food Additive Specifications, Volume 4.

Limits for lead in specifications of food additives for use in infant formula

The Committee recommended that all additives for use in infant formula be reviewed for lead levels in the specifications.

Limits for arsenic in specifications of food additives for use in infant formula

The Committee recommended that all additives for use in infant formula be reviewed for arsenic levels in the specifications.

Use of chloroform as solvent in the test methods associated with specifications monographs for synthetic colours

The Committee recommended the development of analytical methods with suitable replacement solvent(s), in order to replace chloroform, in the future.

General inclusion of infrared spectra

The Committee recommended that all future specifications for new flavouring agents contain a high-quality readable infrared spectrum in the data submission.

Inclusion of chemical structures in the JECFA flavourings database

The Committee recommended that chemical structures be included in the JECFA flavourings database.

Specific food additives (other than flavouring agents)

Carob bean gum

The Committee concluded that the available information is not sufficient for the evaluation of carob bean gum for use in infant formula at the proposed use level and requests toxicological data on neonatal animals, adequate to evaluate the safety for use in infant formula, to complete the evaluation.

The Committee noted that the sponsor also identified a cold-soluble carob bean gum for use in infant formula. However, no information was provided on the manufacturing and composition of the product, and the Committee was unclear which product is used in infant formula and formula for special medical purposes intended for infants.

Cassia gum

The Committee noted that cassia gum can be obtained from a number of companies and requested information on validated methods of analysis currently in use by providers of cassia gum. The methods submitted should contain details of the use of standard (reference) materials, the extraction efficiency of the initial steps, the recovery of the analytes in question, performance data and the results of the analysis of several batches of the material in commerce.

The tentative specifications will be withdrawn unless the requested information is submitted **before 31 December 2017**.

Citric and fatty acid esters of glycerol (CITREM)

The Committee recommended that data be submitted for the replacement of the packed column gas chromatography test method for the determination of total citric acid with a suitable method using a capillary/wide-bore column for consideration at a future meeting.

Lutein esters from Tagetes erecta

The Committee at its seventy-ninth meeting considered establishing a group ADI “not specified” for lutein esters from *Tagetes erecta* that would include lutein from *Tagetes erecta* and synthetic zeaxanthin and related xanthophylls. The current Committee was not able to consider this aspect in detail and recommended that this be taken up at a future meeting.

Modified starches

The Committee prepared tentative specifications for the following 13 modified starches and requires the following information for the removal of the tentative status:

Modified starch	Information required on
Dextrin roasted starch (INS No. 1400)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Acid treated starch (INS No. 1401)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Alkaline treated starch (INS No. 1402)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Bleached starch (INS No. 1403)	• Typical levels of residual reagents or by-products
Enzyme-treated starch (INS No. 1405)	• A suitable method for the Dispersion or Reducing Sugars Distinguishing Test
Monostarch phosphate (INS No. 1410)	• A suitable test for identification of the phosphate groups

Modified starch	Information required on
Distarch phosphate (INS No. 1412)	• A suitable test for identification of the phosphate groups and of crosslinking
Phosphated distarch phosphate (INS No. 1413)	• A suitable test for identification of the phosphate groups and of crosslinking
Acetylated distarch phosphate (INS No. 1414)	• A suitable test for identification of the phosphate groups and of crosslinking
Acetylated distarch adipate (INS No. 1422)	• A suitable test for identification of the adipate groups • Levels of free adipic acid
Hydroxypropyl starch (INS No. 1440)	• A suitable method for the determination of propylene chlorohydrin
Hydroxypropyl distarch phosphate (INS No. 1442)	• A suitable method for the determination of propylene chlorohydrin • A suitable test for identification of the phosphate groups
Starch sodium octenyl succinate (INS No. 1450)	• A suitable test for identification of octenylsuccinate groups

The Committee recommended that the call for data also include method of manufacture for each of the 16 modified starches. The missing data are required **by 31 December 2017**.

Rosemary extract

The Committee made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. The temporary ADI will be withdrawn if the required data are not provided **by the end of 2018**.

The Committee prepared tentative specifications and requested validation information on the method for determination of residual solvents **by the end of 2018**.

The Committee requested that data on typical use levels in foods be provided **by the end of 2018** in order to refine the dietary exposure estimates.

Steviol glycosides

The specifications were made tentative pending submission of following information **by 31 December 2017**:

- method of assay to replace the existing method and including as many steviol glycosides as possible (at least those listed in Appendix 1 of the specifications) in steviol glycoside mixtures, along with supporting validation information and chromatograms;
- analysis results from a minimum of five batches for commercial samples, including supporting chromatograms.

Flavouring agents

Aliphatic and aromatic amines and amides

The Committee concluded that the concerns previously expressed by the Committee at its sixty-ninth meeting as to in vivo genotoxicity and how to address the kidney effects and identify a NOAEL had not been sufficiently addressed and that the Procedure for the Safety Evaluation of Flavouring Agents still could not be applied to 2-isopropyl-*N*,2,3-trimethylbutyramide (No. 1595). Information that would assist in resolving the concerns would include data informing on the difference in response observed in the kidney of male and female rats in the comet assay and on the potential of this compound to form reactive metabolites, as well as additional information on the kidney effects found at relatively low doses.

For *N*-ethyl-2,2-diisopropylbutanamide (No. 2005), *N*-(2-hydroxyethyl)-2,3-dimethyl-2-isopropylbutanamide (No. 2010) and *N*-(1,1-dimethyl-2-hydroxyethyl)-2,2-diethylbutanamide (No. 2011), NOAELs for these flavouring agents or structurally related substances were not available. Although No. 1595 is structurally related, the Committee concluded that No. 1595 could not be evaluated using the Procedure, and therefore this flavouring agent was not suitable to support the evaluation of these three flavouring agents. Therefore, for these three flavouring agents, the Committee concluded that additional data would be necessary to complete the evaluation.

For some previously evaluated flavouring agents in this group, additional toxicity data were available for this meeting. For *N*-isobutyl (*E*,*E*)-2,4-decadienamide (No. 1598) and (2*E*,6*E*/*Z*,8*E*)-*N*-(2-methylpropyl)-2,6,8-decatrienamide (No. 2077), the new studies resulted in lower NOAELs. In light of general considerations on the Procedure for the Safety Evaluation of Flavouring Agents and the need for an approach for re-evaluation in light of new data (see above), the Committee recommends re-evaluation of these two flavouring agents at a future meeting.

Additional data required to complete the evaluation according to the Procedure for the Safety Evaluation of Flavouring Agents

Additional toxicological and/or dietary exposure information is required to complete the toxicological evaluation of six flavouring agents (Nos 2005, 2010, 2011, 2212, 2229 and 2234).

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

82nd Meeting 2016

This document contains food additive specification monographs, analytical methods, and other information prepared at the eighty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, Switzerland, 7 - 16 June 2016. 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.

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