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COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

79th meeting 2014

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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SPECIAL NOTE

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Geneva, 17 – 26 June, 2014

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 79th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Geneva on 17 - 26 June 2014. 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 (flavouring agent) specifications which has been updated and modernized. 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 eleven food additives and these are presented in this publication. The maintained (M) specifications for paprika extract were not revised but are republished as the editorial note reflects that the safety evaluation was concluded.

Benzoe tonkinensis (R)
Carrageenan (R)
Citric acid (R)
Citric and fatty acid esters of glycerol (R)
Gellan gum (R)
Lutein esters from *Tagetes erecta* (N, T)
Modified starches (R)
Octenyl succinic acid modified gum arabic (R, T)
Paprika extract (M)
Polyoxyethylene (20) sorbitan monostearate (R)
Potassium aluminium silicate (R)
Quillaia extract (Type 2) (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 79th meeting of JECFA recommended that the specifications monograph for the modified starches be split into 16 individual specifications monographs. The Committee, as noted at its seventy-sixth meeting, considered that it would also be necessary to revise the specifications for all the modified starches, including test methods, at future meetings.

The FAO Joint Secretariat intends to implement a project to follow up on this recommendation which will be presented for discussion and feedback at the forthcoming session of the Codex Committee on Food Additives (CCFA) in 2015. For this reason the specifications monograph for modified starches is published in print as a future reference for this project.

Editorial changes to specifications

The following specifications monographs were amended editorially and only the online edition of the Joint Compendium is revised:

Specifications monographs	***INS***	***Description of changes***
Potassium aluminium silicate-based pearlescent pigments, Type I, Type II, and Type III	176	Under SYNONYMS the INS no. is added
Potassium acetate	261(i)	Under SYNONYMS the INS no. is corrected
Modified starches	1440	Process description for hydroxypropyl starch: “Esterification” is replaced by “Etherification”
Modified starches	1450	Test for *Octenylsuccinyl groups in starch sodium octenyl succinate*, line 9: “filtrate” is replaced by “filter cake”

BENZOE TONKINENSIS

Prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014) replacing the tentative specifications prepared at the 74th JECFA (2011), published in FAO JECFA Monographs 11 (2011). No safety concern at current estimated dietary exposures.

SYNONYMS Siam Benzoin gum; Siam Benzoin Laos; Styrax tonkinensis

DEFINITION Benzoe tonkinensis is a natural complex balsamic resin obtained from a native tree in Lao PDR: *Styrax tonkinensis* Pierre. It is collected directly from the tree, cleaned and sorted into four grades according to size. All grades have similar chemical composition. The resin is composed mainly of benzoic acid and coniferyl benzoate. The resin also contains minor amounts of, vanilllin and benzyl benzoate. Other compounds namely *p*-coumaryl benzoate, siaresinolic acid 3-oxo-siaresinolic acid, sumaresinolic acid and 3-oxo-sumaresinolic acid are also identified.

These specifications do not cover Sumatra benzoin (resin obtained from *Styrax benzoi*n Dryander and *Styrax paralleloneurum*), which contains cinnamic acid.

C.A.S. number 9000-72-0

DESCRIPTION White-yellow to reddish splits of flattened almond-like grains with a strong vanilla smell.

FUNCTIONAL USES Flavouring agent

CHARACTERISTICS

IDENTIFICATION

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

Benzoic acid, vanillin and benzyl benzoate The sample contains benzoic acid, vanillin and benzyl benzoate identified by their characteristic peaks in the gas-chromatogram.
See description under TESTS

Coniferyl benzoate The sample contains coniferyl benzoate identified by its characteristic peak in the HPLC-chromatogram.
See description under TESTS

Cinnamic acid Not present
See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 5.0% (105°, 4 h). Test 2 g of sample.

Total ash	Not more than 2.0% Evenly distribute 1.0 g of the powdered sample in a crucible. Dry at 100-105° for 1 h and ignite to constant mass in a muffle furnace at 600 ± 25°.
Alcohol-insoluble matter	Not more than 5%. To 2.0 g of the powdered sample add 25 ml of ethanol 90% v/v. Boil until almost completely dissolved. Filter through a previously tared sintered-glass filter and wash with three 5 ml portions of boiling ethanol 90% v/v. Dry the residue at 100-105° for 2 h and weigh after cooling.
Acid value (Vol. 4)	Between 160 and 206
Benzoic acid	Between 15 and 45% See description under TESTS
Coniferyl benzoate	Between 15 and 60% See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Triturate the sample in a centrifugal grinder to a particle size < 200 μm. Determine using an AAS (Electrothermal atomization technique) 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)	*Salmonella spp.:* absent in 25 g *Escherichia coli:* absent in 1 g Yeast and moulds: less than 20 CFU/g

TESTS

IDENTIFICATION AND PURITY

Benzoic acid, vanillin, benzyl benzoate and cinnamic acid

Benzoic acid, vanillin and benzyl benzoate are identified and benzoic acid is quantified by gas chromatography. The absence of cinnamic acid is confirmed.

Reagents

Ethanol 96%
Reference standard of benzoic acid (purity >99%)
Reference standard of vanillin (purity > 99%)
Reference standard of benzyl benzoate (purity > 99%)
Reference standard of cinnamic acid (purity > 99%)

Gas Chromatographic system

Detector: Flame ionization detector
Column: HP-1 (30 m x 0.2 mm I.D., 0.33 μm-film) or equivalent
Carrier gas: helium
Flow rate: 1 ml/min
Temperatures:
-Injector: 250°
-Oven: 5 min at 80°; to 300° at 8°/min; then hold for 40 min
-Detector: 300°
Injection mode: split 1/10
Injection volume: 1 μL

Benzoic acid elutes at 11.7 min. Peaks at 16.1 min, 14.8 min and 20.4 min corresponds to cinnamic acid, vanillin and benzyl benzoate, respectively.

Benzoic acid standard stock solution (20 mg/ml): Weigh accurately 0.4 g (±0.1 mg) of benzoic acid reference standard, transfer to a 20 ml volumetric flask and bring to volume with ethanol.

Benzoic acid standard solutions: Prepare five solutions from the standard stock solutions within the concentration range of 0.25 to 20 mg/ml.

Cinnamic acid, vanillin and benzyl benzoate solution: Prepare a 10 mg/ml solution containing cinnamic acid, vanillin and benzyl benzoate in ethanol.

Sample solution
Accurately weigh about 5.0 g (±0.1 mg) (w_s) of the previously crushed Benzoe tonkinensis and solubilize in 20 ml of ethanol (96%). Sonicate the mixture and filter. Re-extract the residue with a second portion of 20 ml ethanol (96%), sonicate and filter. Combine the ethanol extracts and evaporate the solvent under vacuum. Weigh accurately the extracted resin (w_{ex}). Dissolve the resin with ethanol to a final concentration of 30 mg/ml.

Procedure
Inject 1 µl of each benzoic acid standard solutions and record the peak areas. Inject 1 µl of the 10 mg/ml solution containing cinnamic acid, vanillin and benzyl benzoate.
Plot a standard curve (concentration of benzoic acid (mg/ml) (X-axis) vs. peak area of benzoic acid (Y-axis)) and determine the slope (m) and the linear coefficient (a).
Inject 1 µl of the sample solution and record the peak area.
For identification of cinnamic acid, vanillin and benzyl benzoate, compare the retention times of the corresponding peaks of cinnamic acid, vanillin and benzyl benzoate in the chromatograms obtained with the standard solution and sample solution.

Calculation
Calculate the content of benzoic acid as follows:

$$\text{Benzoic acid (w/w, \%)} = \left(\frac{A_{BzA} - a}{m}\right) \times \frac{1}{30} \times \frac{w_s}{w_{ex}} \times 100$$

where
A_{BzA} is the peak area of benzoic acid in the sample;
a is the linear coefficient of the standard curve;
m is the slope of the standard curve;
w_{ex} is the weight of resin extracted with ethanol (g); and
w_s is the weight of sample Benzoe tonkinensis (g).

Coniferyl benzoate

Coniferyl benzoate is identified and quantified by high performance liquid chromatography.

Reagents
Acetonitrile, HPLC grade
Formic acid
Reference standard of coniferyl benzoate (>95%)

Chromatographic system
HPLC system with a diode array detector (DAD), auto sampler or injector.
Detector wavelength for quantitation: 300 nm
Column: Luna C18 Phenomenex (150 mm x 4.6 mm, 5 μm) or equivalent.
Mobile phase: solvent A: water added of 0.1% formic acid and solvent B: acetonitrile added of 0.1% formic acid
Gradient elution: A:B 65:35 v/v (0 to 5 min) to A:B 0:100 v/v (5 to 25 min)
Column temperature: 25°
Flow rate: 1 ml/min
Injection volume: 10 μl

Coniferyl benzoate elutes at 14.9 min.

Standard stock solution (coniferyl benzoate 8 mg/ml): Weigh accurately 0.04 g (±0.1 mg) of coniferyl benzoate reference standard and transfer to a 5 ml volumetric flask and bring to volume with ethanol.

Standard solutions: Prepare five solutions, by the dilution of the standard stock solution of coniferyl benzoate with ethanol, in the concentration range of 0.05 to 0.8 mg/ml.

Sample preparation
Accurately weigh about 5 g (±0.1 mg) (w_s) of the previously crushed Benzoe tonkinensis sample and solubilize in 20 ml of ethanol (96%). Sonicate the mixture and filter. Re-extract the residue with a second portion of 20 ml ethanol (96%), sonicate and filter. Combine the ethanol extracts and evaporate the solvent under vacuum. Weigh accurately the extracted resin (w_{ex}). Dilute the resin with ethanol to a final concentration (C_{resin}) of 1 mg/ml.

Procedure
Inject 10 µl of each standard solution and record the peak areas. Plot a standard curve (concentration of coniferyl benzoate (mg/ml) (X-axis) vs. peak area of coniferyl benzoate (Y-axis)) and determine the slope (m) and the linear coefficient (a).
Inject 10 µl of the sample and record the peak area.

Calculation
Calculate the content of coniferyl benzoate as follows:

$$\text{Coniferyl benzoate } (w/w\,,\,\%) = \left(\frac{A_{ConBz} - a}{m}\right) \times \frac{1}{C_{resin}} \times \frac{w_s}{w_{ex}} \times 100$$

where
A_{ConBz} is the peak area of coniferyl benzoate in the sample;
a is the linear coefficient of the standard curve;
m is the slope of the standard curve;
C_{resin} is the final concentration of the extracted resin diluted in ethanol;
w_{ex} is the weight of extracted resin with ethanol (g); and
w_s is the weight of sample of Benzoe tonkinensis (g).

CARRAGEENAN

Prepared at the 79th JECFA (2014) and published in FAO JECFA Monographs 16 (2014), superseding specifications prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 4 (2007). A group ADI "not specified" for carrageenan and processed Eucheuma *seaweed was established at the 57th JECFA (2001).*

SYNONYMS Irish moss gelose (from *Chondrus* spp.); Eucheuman (from *Eucheuma* spp.); Iridophycan (from *Iridaea* spp.); Hypnean (from *Hypnea* spp.); Furcellaran or Danish agar (from *Furcellaria fastigiata*); INS No. 407.

DEFINITION A substance with hydrocolloid properties obtained from certain members of the class *Rhodophyceae* (red seaweeds).

The principal commercial sources of carrageenans are the following families and genera of the class of *Rhodophyceae*:

Furcellariacaea such as *Furcellaria*
Gigartinaceae such as *Chondrus*, *Gigartina*, *Iridaea*
Hypnaeceae such as *Hypnea*
Phyllophoraceae such as *Phyllophora*, *Gynmogongrus*, *Ahnfeltia*
Solieriaceae such as *Eucheuma*, *Anatheca*, *Meristotheca*.

Carrageenan is a hydrocolloid consisting mainly of the ammonium, calcium, magnesium, potassium and sodium sulfate esters of galactose and 3,6-anhydrogalactose polysaccharides. These hexoses are alternately linked α-1,3 and β-1,4 in the copolymer. The relative proportions of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.

The prevalent polysaccharides in carrageenan are designated as kappa-, iota-, and lambda-carrageenan. Kappa-carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; iota-carrageenan is similar, except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between kappa-carrageenan and iota-carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In lambda-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked).

Carrageenan is obtained by extraction from seaweed into water or aqueous dilute alkali. Carrageenan may be recovered by alcohol precipitation, by drum drying, or by precipitation in aqueous potassium chloride and subsequent freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol, and isopropanol. Articles of commerce may include sugars for standardization purposes, salts to obtain specific gelling or thickening characteristics, or emulsifiers carried over from drum drying processes.

C.A.S. number 9000-07-1

DESCRIPTION Yellowish or tan to white, coarse to fine powder that is practically odourless.

FUNCTIONAL USES Thickener, gelling agent, stabilizer, glazing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in ethanol; soluble in water at a temperature of about 80°, forming a viscous clear or slightly opalescent solution that flows readily; disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water.
Test for sulfate	Dissolve a 100-mg sample in 20 ml of water (with heating if necessary), and add 3 ml of barium chloride TS and 5 ml of hydrochloric acid, dilute TS; filter if a precipitate forms. Boil the solution or the filtrate for 5 min. A white, crystalline precipitate appears.
Test for galactose and anhydrogalactose (Vol. 4)	Proceed as directed in Vol.4 (under "General Methods, Organic Components, Gum Constituents Identification") using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present.
Identification of hydrocolloid and predominant type of copolymer	Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel. To 50 ml of the solution or gel add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ("brittle") gel indicates a carrageenan of a predominantly kappa type, and a compliant ("elastic") gel indicates a predominantly iota type. If the solution does not gel, the carrageenan is of a predominantly lambda type.
Infrared absorption	Passes test See description under TESTS

PURITY

Loss on drying (Vol. 4)	Not more than 12% (105° to constant weight)
pH (Vol. 4)	Between 8 and 11 (1 in 100 suspension)
Viscosity	Not less than 5 cp at 75° (1.5% solution) See description under TESTS
Sulfate	Not less than 15% and not more than 40% (as SO_4^{2-}) on the dried basis See description under TESTS
Total ash	Not less than 15% and not more than 40% on the dried basis See description under TESTS.
Acid-insoluble ash (Vol. 4)	Not more than 1% Use the ash from the Total ash test

Acid-insoluble matter (Vol. 4)	Not more than 2% Use 2 g of sample obtained from part (a) of the procedure for sulfate determination.
Residual solvents (Vol. 4)	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination See description under TESTS
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 5000 cfu/g *Salmonella* spp.: Negative per test *E. coli*: Negative in 1 g
Arsenic (Vol. 4)	Not more than 3 mg/kg Determine using an AAS (Hydride generation technique) 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").
Lead (Vol. 4)	Not more than 5 mg/kg Determine using an AAS (Electrothermal atomization technique) 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").
Cadmium (Vol. 4)	Not more than 2 mg/kg Determine using an AAS (Electrothermal atomization technique) 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").
Mercury (Vol.4)	Not more than 1 mg/kg Determine using AAS (Cold vapour generation technique). 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

Infrared absorption

Obtain infrared absorption spectra on the gelling and non-gelling fractions of the sample by the following procedure:

Disperse 2 g of the sample in 200 ml of 2.5% potassium chloride solution, and stir for 1 h. Let stand overnight, stir again for 1 h, and transfer into a centrifuge tube. (If the transfer cannot be made because the dispersion is too viscous, dilute with up to 200 ml of the potassium chloride solution.) Centrifuge for 15 min at approximately 1000 x g.

Remove the clear supernatant, resuspend the residue in 200 ml of 2.5% potassium chloride solution, and centrifuge again. Coagulate the

combined supernatants by adding 2 volumes of 85% ethanol or isopropanol (NOTE: Retain the sediment for use as directed below). Recover the coagulum, and wash it with 250 ml of the alcohol. Press the excess liquid from the coagulum, and dry it at 60° for 2 h. The product obtained is the non-gelling fraction (lambda-carrageenan).

Disperse the sediment (retained above) in 250 ml of cold water, heat at 90° for 10 min, and cool to 60°. Coagulate the mixture, and then recover, wash, and dry the coagulum as described above. The product obtained is the gelling fraction (kappa- and iota-carrageenan).

Prepare a 0.2% aqueous solution of each fraction, cast films 0.5 mm thick (when dry) on a suitable non-sticking surface such as Teflon, and obtain the infrared absorption spectrum of each film. (Alternatively, the spectra may be obtained using films cast on potassium bromide plates, if care is taken to avoid moisture).

Carrageenan has strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. Absorption maxima are 1065 and 1020 cm^{-1} for gelling and non-gelling types, respectively. Other characteristic absorption bands and their intensities relative to the absorbance at 1050 cm^{-1} are as follows:

Wave number (cm^{-1})	**Molecular Assignment**	**Absorbance relative to 1050 (cm^{-1})**		
		Kappa	**Iota**	**Lambda**
1220-1260	ester sulfate	0.3-1.4	1.2-1.7	1.4-2.0
928-933	3,6-anhydrogalactose	0.2-0.7	0.2-0.4	0-0.2
840-850	galactose-4-sulfate	0.2-0.5	0.2-0.4	-
825-830	galactose-2-sulfate	-	-	0.2-0.4
810-820	galactose-6-sulfate	-	-	0.1-0.3
800-805	3,6-anhydrogalactose-2-sulfate	0-0.2	0.2-0.4	-

PURITY TESTS

Sulfate

Principle

Hydrolysed sulfate groups are precipitated as barium sulfate.

Procedure

(a) Disperse an accurately weighed 15 g sample of commercial product into 500 ml of 60% w/w isopropanol/water at room temperature. Stir gently for 4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 15-ml portions of 60% isopropanol/water. Dry the material at 105° to constant weight. Approximately 1 g of the dried matter is to be used for part (b). The remainder should be retained for determination of Total ash, Acid-insoluble matter, and viscosity.

(b) Accurately weigh a 1 g sample (W_1) obtained from part (a). Transfer the sample to a 100-ml long-neck round-bottom flask. Add 50 ml of 0.2 N hydrochloric acid. Fit a condenser, preferably one with at least 5 condensing bulbs, to the flask and reflux for 1 h. Add 25 ml of a 10% (by volume) hydrogen peroxide solution and resume refluxing for about 5 h or until the solution becomes completely clear.

Transfer the solution to a 600-ml beaker, bring to a boil, and add dropwise 10 ml of a 10% barium chloride solution. Heat the reaction mixture for 2 h on a boiling water bath. Filter the mixture through ash-free slow-filtration filter paper. Wash with boiling distilled water until the filtrate is free from chloride. Dry the filter paper and contents in a drying oven. Gently burn and ash the paper at 800° in a tared porcelain or silica crucible until the ash is white. Cool in a desiccator.

Weigh the crucible containing the ash. Calculate the percentage sulfate from the weight in g (W_2) of the ash (barium sulfate) using the formula:

$$(W_2/W_1) \times 100 \times 0.4116$$

Total ash

Accurately weigh 2 g of the dried sample (W_1) obtained from part (a) under the procedure for sulfate determination above. Transfer to a previously ignited, tared silica or platinum crucible. Heat the sample with a suitable infrared lamp, increasing the intensity gradually, until the sample is completely charred; continue heating for an additional 30 min. Transfer the crucible with the charred sample into a muffle furnace and ignite at about 550º for 1 h. Cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight (W_2) is obtained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spot with a 1-in-10 solution of ammonium nitrate and dry under an infrared lamp. Repeat the ignition step.

Calculate the percentage of total ash of the sample:

$$(W_2/W_1) \times 100$$

Retain the ash for the Acid-insoluble ash test.

Viscosity

Transfer 7.5 g of the dried sample obtained from part (a) under the procedure for sulfate determination into a tared, 600-ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g, and heat in a water bath with continuous agitation, until a temperature of 80º is reached (20 - 30 min). Add water to adjust for loss by evaporation, cool to 76-77º, and heat in a constant temperature bath at 75º.

Pre-heat the bob and guard of a Brookfield LVF or LVT viscometer to approximately 75º in water. Dry the bob and guard, and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0-100 scale.

If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter or equivalent. (Note. Samples of some types of carrageenan may be too viscous to read when a No. 1 spindle is used. Such samples obviously pass the specification, but if a viscosity reading is desired for other reasons, use a No. 2 spindle and take the reading on the 0-100 scale or on the 0-500 scale.)

Record the results in centipoises, obtained by multiplying the reading on the scale by the factor given by the Brookfield manufacturer.

Residual solvents (Vol.4) See Method 1 under Vol. 4. General Methods, Organic Components, Residual Solvents.

Prepare standard, blank, and calibration solutions as directed under Method 1.

Sample Preparation

Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column and distil about 100 ml, adjusting the heat so that the foam does not enter the column. Quantitatively transfer the distillate to a 200-ml volumetric flask, fill to the mark with water and shake the flask to mix. Weigh accurately 8.0 g of this solution into an injection vial. Add 1.0 ml of the internal standard solution. Heat at 60° for 10 min and shake vigorously for 10 sec.

CITRIC ACID

Prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014), superseding specifications prepared at the 53rd JECFA (1999), published in FNP 52 Add 7 (1999). Group ADI "Not limited" for citric acid and its calcium, potassium, sodium and ammonium salts established at the 17th JECFA in 1973.

SYNONYMS INS No. 330

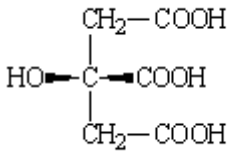
DEFINITION Citric acid may be produced by recovery from sources such as lemon or pineapple juice or fermentation of carbohydrate solutions or other suitable media using *Candida* spp. or non-toxicogenic strains of *Aspergillus niger*

Chemical names 2-hydroxy-1,2,3-propanetricarboxylic acid

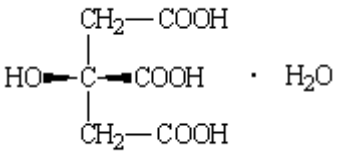
C.A.S. number 77-92-9 (anhydrous)
5949-29-1 (monohydrate)

Chemical formula $C_6H_8O_7$ (anhydrous)
$C_6H_8O_7 \cdot H_2O$ (monohydrate)

Structural formula



Anhydrous



Monohydrate

Formula weight 192.13 (anhydrous)
210.14 (monohydrate)

Assay Not less than 99.5% and not more than 100.5% on the anhydrous basis

DESCRIPTION White or colourless, odourless, crystalline solid; the monohydrate form effloresces in dry air

FUNCTIONAL USES Acidifier; sequestrant; antioxidant synergist; flavouring agent (see "Flavouring agents" monograph)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4) Very soluble in water; freely soluble in ethanol; slightly soluble in ether

Test for citrate (Vol. 4) Passes test

PURITY

Water (Vol. 4)	Anhydrous: Not more than 0.5% (Karl Fischer Method) Monohydrate: Not less than 7.5% and not more than 8.8% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.05% (Method I, use 20 g sample)
Oxalate (Vol. 4)	Not more than 100 mg/kg Dissolve 1.0 g of sample in 4 ml of deionized water, and proceed according to the Oxalate Limit Test (Volume 4). The absorbance of the solution, read at 520 nm, is not more than that of a standard solution. Prepare the standard solution by dissolving 100 mg of oxalic acid (140 mg oxalic acid dehydrate) in 1000 ml of deionized water and dilute 1 ml with 3 ml of deionized water.
Sulfates (Vol. 4)	Not more than 150 mg/kg Test 20 g of the sample by the Sulfates Limit Test (Volume 4) using 6.0 ml of 0.01N sulfuric acid in the standard
Readily carbonizable substances	Heat 1.0 g of sample with 10 ml of 98% sulfuric acid in a water bath at 90 ± 1^{o} for 60 min. No colour darker than *Matching Fluid K* (25^{o}) should be produced (not more than 0.5 absorbance units at 470 nm in a 10 mm cell).
Lead (Vol. 4)	Not more than 0.5 mg/kg Determine using an AAS (Electrothermal atomization technique) 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”).
METHOD OF ASSAY	Weigh, to the nearest mg, 2.5 g of the sample and place in a tared flask. Dissolve in 40 ml of water and titrate with 1 N sodium hydroxide, using phenolphthalein TS as the indicator. Each ml of 1 N sodium hydroxide is equivalent to 64.04 mg of $C_6H_8O_7$.

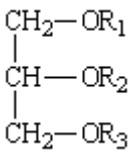
CITRIC AND FATTY ACID ESTERS OF GLYCEROL

Prepared at the 79th JECFA (2014) and published in FAO JECFA Monographs 16 (2014), superseding specifications prepared at the 35th JECFA (1989), published in FNP 49 (1990) and in FNP 52 (1992). Metals and arsenic specifications revised at the 61st JECFA (2003). 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/k Determine using an AAS (electrothermal atomization technique) 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.”

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 not longer than 1.5 h. Add 20 ml of 15% potassium iodide solution, shake gently to mix, and allow to stand at least 1 min. but not more than 5 min. Do not allow to stand in bright or direct sunlight. Add 200 ml of water and titrate with 0.1 N sodium thiosulfate. Use a variable speed electric stirrer to keep the solution thoroughly mixed. Continue the titration to the disappearance of the brown iodine colour from the aqueous layer. Add 2 ml of starch TS and continue the titration to the disappearance of iodine from the tiny chloroform layer separated during titration and the disappearance of the blue iodine-starch complex colour from the aqueous layer. Read the 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 1 h.

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.

$$\% \text{ Total citric acid} = R_s \times 100 \times R_o \times 100 \times (W_o/W_s)Rs$$

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 for standard solution
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:

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

GELLAN GUM

Prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014), superseding specifications prepared at the 49th JECFA (1997), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established at the 37th JECFA (1990)

SYNONYM INS No. 418

DEFINITION Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by *Pseudomonas elodea*, purified by recovery with ethanol or 2-propanol, dried, and milled. The high molecular weight polysaccharide is principally composed of a tetrasaccharide repeating unit of one rhamnose, one glucuronic acid, and two glucose units, and is substituted with acyl (glyceryl and acetyl) groups as the O-glycosidically-linked esters. The glucuronic acid is neutralized to a mixed potassium, sodium, calcium, and magnesium salt. It usually contains a small amount of nitrogen containing compounds resulting from the fermentation procedures.

C.A.S. number 71010-52-1

Formula weight Approximately 500,000

Assay Yields, on the dried basis, not less than 3.3% and not more than 6.8% of carbon dioxide (CO_2).

DESCRIPTION Off-white powder

FUNCTIONAL USES Thickener, gelling agent, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, forming a viscous solution; insoluble in ethanol

Gel test with calcium ion Add 1.0 g of the sample to 99 ml of water, and stir for about 2 h, using a motorized stirrer having a propeller-type stirring blade. Draw a small amount of this solution into a wide bore pipet and transfer into a 10% solution of calcium chloride. A tough worm-like gel will be formed immediately.

Gel test with sodium ion Add 1.0 g of the sample to 99 ml of water, and stir for about 2 h, using a motorized stirrer having a propeller-type stirring blade. Add 0.50 g of sodium chloride, heat to 80° with stirring, and hold at 80° for 1 min. Allow the solution to cool to room temperature. A firm gel is formed.

PURITY

Loss on drying (Vol. 4) Not more than 15% (105°, 2½ h)

Nitrogen (Vol. 4)	Not more than 3%
Residual solvents	Not more than 50 mg/kg of ethanol; not more than 750 mg/kg of 2-propanol See description under TESTS
Microbiological criteria	Total plate count: Not more than 10,000 cfu/g *E. coli:* Negative by test *Salmonella*: Negative by test Yeasts and moulds: Not more than 400 cfu/g See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS (Electrothermal atomization technique) 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

Residual solvents

Standard solutions

Transfer 100.0 mg of chromatographic quality ethanol into a 200-ml volumetric flask and 150.0 mg of 2-propanol into a 20-ml volumetric flask, then dilute to volume with water. Pipet each 1 ml of solutions into a 100-ml volumetric flask and dilute to volume with water as standard solution A. Pipet 10 and 5 ml of standard A into two separate 20-ml volumetric flasks and dilute to volume with water as standard solution B and standard solution C.

Chromatography conditions

Column: 25% diphenyl-75% dimethylpolysiloxane (60 m x 0.25 mm i.d. with 1.4 µm-film) [Aquatic-2 (GL-Sciences Inc.) or equivalent]
Carrier gas: Helium
Flow rate: 1.8 ml/min
Detector: Flame ionization detector (FID)
Temperatures:
- Injection port: 250°
- Oven: Hold for 5 min at 40°, then 40° to 92° at 4°/min
- Detector: 260°

The retention times of ethanol and 2-propanol are about 6.5 and 7.5 min, respectively.

Samples

Weigh accurately 0.10 g of the sample into each of four 20 ml head-space vials. Add a magnetic stirring bar and 10 ml of either water, standard solution A, B or C into each vial and seal. After standing vials overnight at room temperature, stir the solution in the vials for 1 min.

Procedure

Place the sample vial in the sample tray on head-space gas chromatograph. Heat vials at 60° for 40 min with continuous agitation. Inject 1.0 ml of the head space gas (Syringe temperature: 100°,

Transfer temperature: 120°) in the vial into the chromatograph and measure the peak area for ethanol and 2-propanol. Plot the relationship between the added amount against the peak area for ethanol or 2-propanol. Extrapolate the x-intercept for ethanol and 2-propanol (w_e and w_p). Calculate the concentration of ethanol and 2-propanol from;

$$\text{Ethanol (mg/kg)} = w_E / W$$
$$\text{2-Propanol (mg/kg)} = w_P / W$$

Where
W is weight of sample (g).

Microbiological criteria

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.

Continue as per method on page 104 in Volume 4 (under “General methods, *Salmonella*). Identification can be more conveniently done using API or Micro ID systems or equivalent.

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 Proceed as directed in the test for Alginates Assay (Carbon Dioxide Determination by Decarboxylation) in Volume 4 (under ”Assay Methods”), using 1.2 g of the sample.

LUTEIN ESTERS FROM *TAGETES ERECTA*
(TENTATIVE)

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

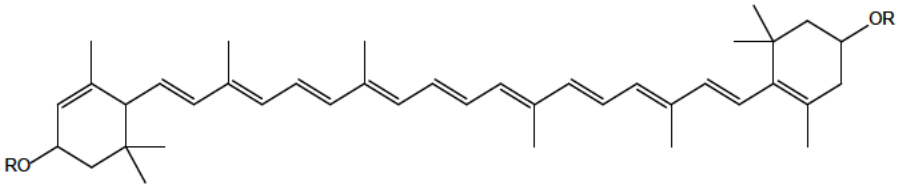
Information required:

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

SYNONYMS Xanthophylls

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

Structural formula



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

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

DESCRIPTION Dark yellow-brown solid

FUNCTIONAL USES Colour, nutrient

CHARACTERISTICS

IDENTIFICATION

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

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

Melting range (Vol. 4)	53 - 55°
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	Not more than 10% of total carotenoids. See description under TESTS.
Residual solvent (Vol. 4)	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.
Glycerides and free fatty acids	Information required
Lead (Vol. 4)	Not more than 2 mg/kg. Determine using an AAS (electrothermal atomization) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Zeaxanthin	Information required
Waxes	Determine by gas chromatography using the following conditions:

Apparatus

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

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

Internal standard pentacosane (C25)
Standard curves are prepared through the addition of absolute hydrocarbon standards to methylene chloride to provide hydrocarbon concentrations of 2.0, 10, 25, 50, 75, and 100 mg/kg.

Sample Preparation
Accurately weigh 200 mg of sample into a centrifuge tube and dissolve in exactly 20 ml of methylene chloride. Sonication or vortex mixing may be required to completely dissolve the product.
Centrifuge sample at 2500 rpm for 5 min if the sample appears turbid. Transfer 40 µl into 2 ml autosampler vial that contains 1.6 ml of methylene chloride and 20 µl of (5000 mg/kg) pentacosane for a final concentration of 50 mg/kg.

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

Results
The approximate retention according to GC/FID times of C29, C30, C31, C32, C33, C34, C35, and the internal standard pentacosane (C25) are 18.6, 19.1, 19.6, 20.0, 20.5, 20.9, 21.4, and 16.3 minutes, respectively.
Construct standard curve using the peak areas from analysis of the standard solutions and use it to calculate total and individual wax content in the sample.

METHOD OF ASSAY

Determine the total content of carotenoid esters as follows:

Apparatus:
UV/VIS spectrophotometer
1-cm cuvettes

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

Calculation:

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

Where:
Abs = measured absorbance
d = dilution factor
$A^{1\%}_{\text{isobestic}}$ (specific absorbance of lutein ester at the wavelength of the isobestic point) = 898
W = weight of sample (g)

MODIFIED STARCHES

Prepared at the 79th JECFA and published in FAO JECFA Monographs 16 (2014), superseding the specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI "not specified" was established at the 26th JECFA (1982) for all modified starches listed below except for acetylated oxidized starch for which an ADI "not specified" was established at the 57th JECFA (2001).

Modified starches comprise the following:

Dextrin roasted starch: INS No. 1400
Acid treated starch: INS No. 1401
Alkaline treated starch: INS No. 1402
Bleached starch: INS No. 1403
Oxidized starch: INS No. 1404
Enzyme-treated starch: INS No. 1405
Monostarch phosphate: INS No. 1410
Distarch phosphate: INS No. 1412
Phosphated distarch phosphate: INS No. 1413
Acetylated distarch phosphate: INS No. 1414
Starch acetate: INS No. 1420
Acetylated distarch adipate: INS No. 1422
Hydroxypropyl starch: INS No. 1440
Hydroxypropyl distarch phosphate: INS No. 1442
Starch sodium octenylsuccinate: INS No. 1450
Acetylated oxidized starch: INS No. 1451

DEFINITION

Food starches which have one or more of their original characteristics altered by treatment in accordance with good manufacturing practice by one of the procedures listed in Table 1. In the case of starches treated with heat in the presence of acid or with alkali, the alteration is a minor fragmentation. When the starch is bleached, the change is essentially in the colour only. Oxidation involves the deliberate production of carboxyl groups. Acetylation results in substitution of hydroxyl groups with acetyl esters. Treatment with reagents such as orthophosphoric acid results in partial substitution in the 2, 3- or 6- position of the anhydroglucose unit unless the 6-position is occupied for branching. 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. The article of commerce can be specified by the parameter specific for the particular type of modification as indicated in Column 3 of Table 1, and may also be further specified as to the loss on drying, sulfated ash, protein and fat.

C.A.S. numbers

Starch acetate:	9045-28-7
Acetylated distarch adipate:	68130-14-3
Hydroxypropyl starch:	9049-76-7
Hydroxypropyl distarch phosphate:	53124-00-8
Acetylated oxidized starch:	68187-08-6
Starch sodium octenylsuccinate:	66829-29-6

DESCRIPTION Most modified starches are white or off-white, odourless powders. According to the drying method these powders can consist of whole granules having the appearance of the original native starch, or aggregates consisting of a number of granules (pearl starch, starch-grits) or, if pre-gelatinized, of flakes, 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
Differentiation test	Passes test for type of starch See description under TESTS for: 1. Hypochlorite oxidized starch 2. Specific reaction for acetyl groups 3. Positive test for ester groups

PURITY

Sulfur dioxide	Not more than 50 mg/kg for modified cereal starches Not more than 10 mg/kg for other modified starches unless otherwise specified in Table I See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS (electrothermal atomization technique) 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”).
Additional purity specifications for individual chemically modified starches	See column 3 of Table I See description under TESTS

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

Differentiation test

To differentiate between various treated starches perform the following tests:

1. Test for hypochlorite-oxidized starch (not for slightly oxidized potato 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.

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.

2. Specific reaction of 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 N 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 N 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.

3. Positive test for 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, adipyl or succinyl groups in the product.

PURITY TESTS

Sulfur dioxide

Scope
The method is applicable, with minor modifications, to liquid or solid samples even in the presence of other volatile sulfur compounds.

Principle
The sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in dilute hydrogen peroxide where it is oxidized to sulfuric acid and titrated with standard alkali. Alternatively, the sulfuric acid may be determined gravimetrically as barium sulfate.

Apparatus
“Monier-Williams" apparatus for the determination of sulfurous acid, constructed with standard-taper glass connections, can be obtained from any reliable scientific glass apparatus store. It is customary, however, to construct the apparatus with regular laboratory glassware using stopper connections (see Figure 1).

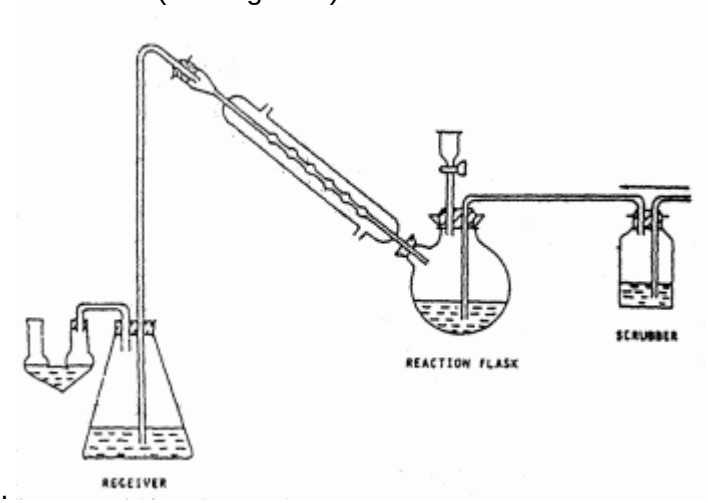


Figure 1

The assembly consists of a 1000-ml two-neck round-bottom boiling flask to which a gas-inlet tube, a 60-ml dropping funnel having a 2-mm bore stopcock, and a sloping Allihn reflux condenser are attached. A delivery tube connects the upper end of the condenser to the bottom of a 250-ml conical receiving flask, which is followed by a Peligot tube.
In operation, carbon dioxide is passed through the scrubber and bubbled through the heated reaction mixture, sweeping sulfur dioxide through the condenser and into the receivers where it is absorbed quantitatively.

Preparation of solutions
Sodium carbonate solution: Dissolve approximately 15 g of Na_2CO_3 or 40 g of $Na_2CO_3 \cdot 10H_2O$ in distilled water, and dilute to 100 ml.
Hydrogen peroxide, 3%: Dilute 10 ml of C.P. (Chemical Purity) neutral 30% hydrogen peroxide (H_2O_2) with distilled water to 100 ml.

Procedure

Pass carbon dioxide from a generator or cylinder through the sodium carbonate scrubber solution to remove chlorine, thence into the gas-inlet tube of the boiling flask. Place 15 ml of the 3% hydrogen peroxide in the receiving flask and 5 ml in the Peligot tube. Connect the apparatus and introduce into the boiling flask, by means of the dropping funnel, 300 ml of distilled water and 20 ml of concentrated hydrochloric acid. Boil the contents approximately 10 min in a current of carbon dioxide. Weigh, to the nearest g, 100 g of the sample and disperse in approximately 300 ml of recently-boiled distilled water. Transfer the slurry to the boiling flask by means of dropping funnel, regulating the sample addition rate and the gas flow rate through the apparatus to prevent drawback of hydrogen peroxide, inclusion of air, or burning of sample. Boil the mixture gently for 1 h in a slow current of carbon dioxide. Stop the flow of water in the condenser just before the end of the distillation. When the delivery tube just above the receiving flask becomes hot, remove the tube from the condenser immediately. Wash the delivery tube and the Peligot tube contents into the receiving flask, and titrate with 0.1 N sodium hydroxide, using bromphenol blue indicator (see NOTE).

Perform a blank determination on the reagents, and correct results accordingly.

$$\% \text{ sulfur dioxide} = \frac{(S - B) \times 0.0032 \times 100}{W}$$

where

S is ml of 0.1 N sodium hydroxide used for the sample;
B is ml of 0.1 N sodium hydroxide used for the blank; and
W is the weight (in grams) of the sample.

NOTE: A gravimetric determination may be made after titration. Acidify with HCl, precipitate with $BaCl_2$, settle, filter, wash, ignite, and weigh as $BaSO_4$.

Table 1. Additional purity specifications for individual chemically modified starches
(All percentages calculated on dry substance)

Modification	Process limitations	End-product specifications
Dextrin roasted starch	Dry heat treatment with hydrochloric acid or ortho-phosphoric acid	Final pH 2.5-7.0
Acid treated starch	Treatment with hydrochloric acid or ortho-phosphoric acid or sulfuric acid	Final pH 4.8-7.0
Alkaline treated starch	Treatment with sodium hydroxide or potassium hydroxide	Final pH 5.0-7.5

Modification	Process limitations	End-product specifications
Bleached starch	Treatment 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	Added carbonyl group not more than 0.1% No residual reagent Residual sulfur dioxide not more than 50 mg/kg Residual manganese not more than 50 mg/kg
Enzyme-treated starch	Treatment in an aqueous solution at a temperature below the gelatinezation point with one or more food-grade amyolytic enzymes	Residual sulfur dioxide not more than 50 mg/kg
Oxidized starch	Treatment with sodium hypochlorite	Carboxyl groups not more than 1.1% Residual sulfur dioxide not more than 50 mg/kg
Monostarch phosphate	Esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate	Phosphate calculated as phosphorus not more than 0.5% for potato or wheat, and not more than 0.4% for other starches
Distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride	Phosphate calculated as phosphorus not more than 0.5% for potato and wheat, and not more than 0.4% for other starches
Phosphated distarch phosphate	Combination of treatments for Monostarch phosphate and Distarch phosphate	Phosphate calculated as phosphorus not more than 0.5% for potato and wheat, and not more than 0.4% for other starches
Acetylated distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate	Acetyl groups not more than 2.5%; phosphate calculated as phosphorus not more than 0.14% for potato and wheat, and 0.04% for other starches; and vinyl acetate not more than 0.1 mg/kg
Starch acetate	Esterification with acetic anhydride or vinyl acetate	Acetyl groups not more than 2.5%
Acetylated distarch adipate	Esterification with acetic anhydride and adipic anhydride	Acetyl groups not more than 2.5% and adipate groups not more than 0.135%
Hydroxypropyl starch	Etherification with propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg

Modification	Process limitations	End-product specifications
Hydroxypropyl distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg; and residual phosphate calculated as phosphorus not more than 0.14% for potato and wheat, and not more than 0.04% for other starches
Starch sodium octenylsuccinate	Esterification with octenylsuccinic anhydride	Octenylsuccinyl groups not more than 3%; and residual octenylsuccinic acid not more than 0.3%
Acetylated oxidized starch	Treatment with sodium hypochlorite followed by esterification with acetic anhydride	Acetyl groups not more than 2.5 % and carboxyl groups not more than 1.3 %

METHODS FOR ADDITIONAL PURITY SPECIFICATIONS

pH (Vol. 4)

As specified in Column 3 of Table 1
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).

Carboxyl groups

As specified in Column 3 of Table 1.

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:

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

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 (%).

Manganese (Vol. 4) As specified in Column 3 of Table 1.

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.

Phosphorus (Vol. 4) As specified in the Column 3 of Table 1.
Reagents

- Ammonium Molybdate Solution (5%): Dissolve 50 g of ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 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.

Acetyl groups

As specified in Column 3 of Table 1.

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 N sodium hydroxide to a permanent pink end-point. Add 25.0 ml of 0.45 N sodium hydroxide, stopper the flask, and shake vigorously for 30 min, preferably with a mechanical shaker. (NOTE: the temperature should not exceed 30° as some starches may 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 N hydrochloric acid to the disappearance of the pink colour. Record the volume, in ml of 0.2 N hydrochloric acid required as S.(S). Perform a blank titration on 25.0 ml of 0.45 N sodium hydroxide, and record the volume, in ml, of 0.2 N hydrochloric acid required as B.—(B).

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

where

N is the normality 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.

Adipate groups As specified in Column 3 of Table 1.

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 (%).

Hydroxypropyl groups — As specified in Column 3 of Table 1

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

As specified in Column 3 of Table 1.
Determine by gas chromatography

Gas Chromatographic system
Use a Hewlett Packard model 5750 or equivalent.. A dual-column and a flame-ionization detector is recommended.. An integrator should be part of the recording system

Gas Chromatography column: Use a stainless steel column, 3 m x 3.2 mm (o.d.), packed with 10% Carbowax 20 M on 80/100-mesh Gas Chrom 2, or equivalent. After packing and prior to use, condition the column overnight at 200°, using a helium flow of 25 ml per min.
Concentrator: Use a Kuderna-Danish concentrator having a 500-ml flask, available from Kontes Glass Co., Vineland, N.J., USA, (Catalogue No. K-57000), or equivalent.

Pressure Bottles: Use 200-ml pressure bottles, with a Neoprene washer, glass stopper, and attached wire clamp, available from Fisher Scientific Co., Pittsburgh, PA, USA (Vitro 400, Catalogue No. 3-100), or equivalent.

Reagents
Diethyl ether: Use anhydrous, analytical reagent-grade, diethyl ether.
Florisil: Use60/100 mesh material,, available from Floridin Co., 3 Penn Center, Pittsburgh, PA 15235, USA, or an equivalent product.
Propylene chlorohydrins: Use Eastman No. P 13251-Chloro-2-propanol Practial containing 25% 2-chloro-1-propanol available from Eastman Kodak Co., Rochester, N.Y. 14650, USA or equivalent).

Standard preparation

Draw 25 µl of mixed propylene chlorohydrin isomers containing 75% of 1-chloro-2-propanol and 25% of 2-chloro-propanol into a 50-µl syringe. Accurately weigh the syringe and discharge the contents into a 500-ml volumetric flask partially filled with water. Reweigh the syringe, and record the weight of the chlorohydrins taken. Dilute to the volume with water, and mix. This solution contains about 27.5 mg of mixed chlorohydrins, or about 55 µg per ml. Prepare this solution fresh on the day of use.

Sample preparation

Transfer a blended representative 50.0 g sample into a Pressure Bottle, and add 125 ml of 2 N sulfuric acid. Clamp the top in place, and swirl the contents until the sample is completely dispersed. Place the bottle in a boiling water bath, heat for 10 min, then swirl the bottle to mix the contents, and heat in the bath for an additional 15 min. Cool in air to room temperature, then neutralize the hydrolyzed sample to pH 7 with 25% sodium hydroxide solution, and filter through Whatman No. 1 paper, or equivalent, in a Buchner funnel, using suction. Wash the bottle and filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, and stir with a magnetic stirring bar for 5 to 10 min, or until the sodium sulfate is completely dissolved. Transfer the solution into a 500-ml separator equipped with a teflon plug, rinse the flask with 25 ml of water, and combine the washings with the sample solution. Extract with five 50 ml portions of diethyl ether, allowing at least 5 min in each extraction for adequate phase separation. Transfer the combined ether extracts in a Concentrator, place the graduated receiver of the concentrator in a water bath maintained at 50 - 55°, and concentrate the extract to a volume of 4 ml.
(NOTE: Ether extracts of samples may contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. These residues are believed to be degradation products arising during the hydrolysis treatment. Analytical problems created by their presence can be avoided through application of a clean-up treatment performed as follows: Concentrate the ether extract to about 8 ml, instead of 4 ml specified above. Add 10 g of Florisil, previously heated to 130° for 16 h just before use, to a chromatographic tube of suitable size, then tap gently, and add 1 g of anhydrous sodium sulfate to the top of the column. Wet the column with 25 ml of diethyl ether, and quantitatively transfer the concentrated extract to the column with the aid of small portions of the ether. Elute with three 25-ml portions of the ether, collect all of the eluate, transfer it to a concentrator, and concentrate to a volume of 4 ml).
Cool the extract to room temperature; transfer it quantitatively to a 5.0 ml volumetric flask with the aid of small portions of diethyl ether, dilute to volume with the ether, and mix.

Control preparation

Transfer 50.0 g portions of unmodified (underivatized) waxy corn starch into five separate pressure bottles, and add 125 ml of 2 N sulfuric acid to each bottle. Add 0.0, 0.5, 1.0, 2.0, and 5.0 ml of the Standard Preparation to the bottles, respectively, giving propylene chlorohydrin concentrations, on the starch basis, of 0, 0.5, 1.0, 2.0, and 5.0 mg/kg, respectively. Calculate the exact concentration in each bottle from the weight of propylene chlorohydrins used in making the Standard Preparation. Clamp the tops in place, swirl until the contents of each bottle are completely dissolved, and proceed with the hydrolysis, neutralization, filtration, extraction, extract concentration, and final dilution as directed under Sample Preparation.

Procedure

The operating conditions may be varied, depending upon the particular instrument used, but a suitable chromatogram is obtained with the Hewlett-Packard Model 5750 using a column oven temperature of 110°, isothermal; injection port temperature of 210°; detector temperature of 240°; and hydrogen (30 ml per min), helium (25 ml per min), or air (350 ml per min) as the carrier gas. A 1.0 mV full-scale recorder is recommended; range, attenuation, and chart speed should be selected to optimize signal characteristics. Inject 2.0 µl aliquots of each of the concentrated extracts, prepared as directed under Control preparation, allowing sufficient time between injections for signal peaks corresponding to the two chlorohydrin isomers to be recorded (and integrated) and for the column to be purged. Record and sum the signal areas (integrator outputs) from the two chlorohydrin isomers for each of the controls. Using identical operating conditions, inject a 2.0 µl aliquot of the concentrated extract prepared as directed under Sample preparation, and record and sum the signal areas (integrator outputs) from the sample.

Calculation

Prepare a calibration plot on linear coordinate graph paper by plotting the summed signal areas for each of the controls against the calculated propylene chlorohydrin concentrations, in mg/kg, derived from the actual weight of chlorohydrin isomers used. Using the summed signal areas corresponding to the 1-chloro-2-propanol and 2-chloro-1-propanol from the sample, determine the concentration of mixed propylene chlorohydrins, in mg/kg, in the sample by reference to the calibration plot derived from the control samples. After gaining experience with the procedure and demonstrating that the calibration plot derived from the control samples is linear and reproducible, the number of controls can be reduced to one containing about 5 mg/kg of mixed propylene chlorohydrin isomers. The propylene chlorohydrin level in the sample can then be calculated as follows:

$$\text{Propylene chlorohydrins (mg/kg)} = \frac{C \times a}{A}$$

where

C is the concentration, in mg/kg, of propylene chlorohydrins (sum of isomers) in the control;

a is the sum of signal areas produced by the propylene chlorohydrin isomers in the sample; and

A is the sum of the signal areas produced by the propylene chlorohydrin isomers in the control.

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 N 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 N 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

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

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
N is the normality 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 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 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 octenyl succinic 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 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 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.

OCTENYL SUCCINIC ACID MODIFIED GUM ARABIC
(TENTATIVE)

Tentative specifications prepared at the 79th JECFA (2014) and published in the FAO Monographs 16 (2014), superseding tentative specifications prepared at the 77th JECFA (2013) and published in the FAO Monographs 14 (2013). A temporary ADI "not specified" was established at the 71st JECFA (2009).

Information required:

- *Details of the manufacturing process including purification steps.*
- *Chemical characterization of the product in commerce.*
- *Updated analytical methods for the determination of esterified (bound) and residual (free) OSA and results of at least five different batches of commercially available product.*
- *Applicability of the HPLC-method for the determination of residual octenyl succinic acid.*

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 not more than 3% of octenyl succinic acid anhydride. It 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

Precipitate formation Add 0.2 ml of dilute lead subacetate TS to 10 ml of a cold 1:50 aqueous solution. A white, flocculent precipitate forms immediately.

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 Information required

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

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	Information required
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 an AAS (Electrothermal atomization technique) 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	Information required
Residual octenyl succinic acid	Information on the applicability of this method is requested 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 octenyl succinic 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 height 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 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.

PAPRIKA EXTRACT

Prepared at the 77th JECFA, published in FAO JECFA Monographs 14 (2013), superseding tentative specifications prepared at the 69th JECFA (2008). An ADI of 0 - 1.5 mg/kg bw was allocated at the 79th JECFA (2014).

SYNONYMS INS No. 160c(ii), Capsanthin

DEFINITION Paprika extract is obtained by solvent extraction of the dried ground fruit pods of *Capsicum annuum.* The major colouring compound is capsanthin. Other coloured compounds, such as capsorubin, canthaxanthin, cryptoxanthin, zeaxanthin and lutein, as well as other carotenoids are also present. The balance of the extracted material is lipidic in nature and varies depending on the primary extraction solvent. Commercial preparations may be diluted and standardised with respect to colour content using refined vegetable oil.

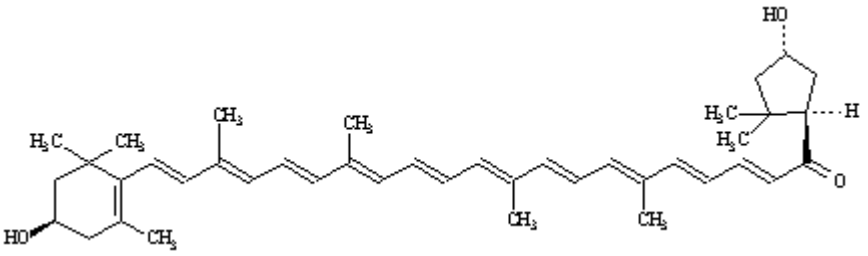
Only methanol, ethanol, isopropanol, acetone, hexane, ethyl acetate and supercritical carbon dioxide may be used as solvents in the extraction.

Chemical names Capsanthin: (3R, 3'S, 5'R)-3,3'-dihydroxy-β,κ-carotene-6-one

C.A.S number Capsanthin: 465-42-9

Chemical formula Capsanthin: $C_{40}H_{56}O_3$

Structural formula



Capsanthin

Formula weight Capsanthin: 584.85

Assay Total carotenoids: not less than 7%
Capsanthin: Not less than 30% of total carotenoids.

DESCRIPTION Dark-red viscous liquid

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility Practically insoluble in water, soluble in acetone

Spectrophotometry	Maximum absorption in acetone at about 462 nm and in hexane at about 470 nm.
Colour reaction	To one drop of sample add 2-3 drops of chloroform and one drop of sulfuric acid. A deep blue colour is produced.
High performance liquid chromatography (HPLC)	Passes test. See Method of assay, Capsanthin

PURITY

Residual solvents	Acetone Ethanol Ethyl acetate Hexane Isopropanol Methanol	Not more than 50 mg/kg, singly or in combination
	See description under TESTS	
Capsaicinoids	Not more than 200 mg/kg See description under TESTS	
Arsenic (Vol. 4)	Not more than 1 mg/kg Determine using an AAS (Hydride generation technique) 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”).	
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS (Electrothermal atomization technique) 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

PURITY TESTS

Capsaicinoids

Capsaicinoids are determined by reversed-phase HPLC (Volume 4 under “Chromatography”) using a standard to allow quantification.

Preparation of standard

Prepare all standard solutions in ethanol and keep out of direct sunlight.
Standard solution A,150 µg/ml: Accurately weigh and transfer 75 mg of N-vanillyl-n-nonenamide, >99 % (CAS Registry Number 2444-46-4) into a 500 ml volumetric flask, dissolve and dilute to volume. Mix thoroughly.
Standard solution B, 15 µg/ml: Pipet 10 ml standard solution A into a 100 ml volumetric flask, dilute to volume, and mix well.
Standard solution C, 0.75 µg/ml: Pipet 5 ml of standard solution B into 100 ml volumetric flask, dilute to volume, and mix well.

Preparation of sample

Accurately weigh up to 5 g extract into a 50 ml volumetric flask, do not allow the extract to coat the sides of the flask. Add 5 ml acetone (ACS Grade) to the flask and swirl until the sample is completely dispersed. Ensure the extract has not coated the bottom of flask when neck is at a 45° angle. Slowly add ethanol (95% or denatured) with mixing until the

solution becomes cloudy. Dilute to volume and mix well. Pipet 5 ml sample mixture into a 10 ml syringe attached to a 6 ml preconditioned C-18 SEP-PAK cartridge. Take care to avoid coating of sample on the sides of syringe. Allow the aliquot to pass through the SEP-PAK and collect the eluent in a 25 ml volumetric flask. Rinse the SEP-PAK with three 5 ml portions of ethanol, and collect in the flask. Dilute to volume with ethanol and mix. Filter through a 0.45 µm syringe filter and collect in a glass vial.

Apparatus

Liquid chromatograph equipped with a 20 µl sample loop injector, a fluorescence detector and/or ultraviolet detector and integrator.

Column: LC-18 (150 mm x 4.6 mm id, 5 µm)
Detector: Fluorescence - Excitation 280 nm and emission 325 nm
UV Detector - 280 nm
Mobile phase: 40% acetonitrile and 60% deionised water containing 1% Acetic acid (v/v).
Flow rate: 1.5 ml/min

Procedure

Inject 20 µl of the sample solution in duplicate. Inject the appropriate standard solution (Standard solution C is appropriate for samples expected to contain low levels of capsaicins) prior to the first sample injection and after every 6 sample injections. Purge the column with 100% acetonitrile for 30 min at 1.5 ml/min after no more than 30 sample injections. Equilibrate with mobile phase prior to further determinations.

Calculations

Calculate individual capsaicinoids (µg/ml) as follows:

Nordihydrocapsaicin: $C_N = (N/a) \times (Cs/RN)$
Capsaicin: $C_C = (C/a) \times (Cs/RC)$
Dihydrocapsaicin: $C_D = (D/a) \times (Cs/RD)$

Total capsaicins (µg/ml) = nordihydrocapsaicin + capsaicin + dihydrocapsaicin

where

a is the average peak area of standard;
N, C, and D are average peak areas for respective capsaicinoids (nordihydrocapsaicin, capsaicin and dihydrocapsaicin) from duplicate injections;
Cs is the concentration of std in µg/ml;
$C_{N,C,D}$ is the concentration of compound in extract expressed as µg/ml;
RN, RC, and RD are response factors of respective capsaicinoids relative to standard.

Response factors:

Nordihydrocapsaicin (N) UV: RN = 0.98; FLU: RN = 0.92
Capsaicin (C) UV: RC = 0.89; FLU: RC = 0.88
Dihydrocapsaicin (D) UV: RD = 0.93; FLU: RD = 0.93
N-vanillyl-n-nonenamide UV: R = 1.00; FLU: R = 1.00

Relative retention times: Nordihydrocapsaicin 0.90; N-vanillyl-n-nonenamide 1.00, Capsaicin 1.00; Dihydrocapsaicin 1.58

Residual solvents

Water and methanol are not suitable for the head-space gas chromatographic analysis of solvent-extracted paprika extracts as given in Method I and Method II for the method for residual solvent determination by head-space gas chromatography in Vol.4. A refined vegetable oil (e.g. soybean oil) is the preferred solvent for sample dissolution. Weigh accurately 1.0 g sample into a headspace vial and add 10 ml soybean oil. Cap and seal immediately. Prepare blanks, standard solutions and calibration samples in a similar fashion. Use the same soybean oil to determine residual solvents in the blank. Determine residual solvents following the Procedure given in Vol. 4.

METHOD OF ASSAY

Total carotenoids

Determine by spectrophotometry. Accurately weigh 300 to 500 mg of sample, and transfer quantitatively to a 100 ml volumetric flask. Dilute with acetone to volume, dissolve by shaking and leave to stand for 2 min. Pipet 1 ml of this extract into another 100 ml volumetric flask, dilute to volume with acetone, and shake well. Transfer a portion to the spectrophotometer cell, and read the absorbance A at 462 nm. Adjust the sample concentration to obtain an absorbance between 0.3 and 0.7.

$$\text{Total carotenoids (\%)} = \frac{A}{2100} x \frac{10000}{W}$$

where

A is the absorbance of sample

2100 is $A^{1\ \%}_{1\ cm}$ for capsanthin in acetone at 462 nm

W is the weight of sample (g)

Capsanthin

Determine the identity of the sample and the content of capsanthin by reversed-phase HPLC. See Volume 4 under “Chromatography”.
The sample is saponified to release the parent hydroxy-carotenoids from the extracts prior HPLC analysis.

Sample preparation

Dissolve 0.2 g of the sample in acetone, quantitatively transfer into a 500 ml separatory funnel and add enough acetone to make up to 100 ml. Add 100 ml diethyl ether and mix well. Remove any insoluble particles by filtration. Add 100 ml of KOH-methanol (20%) and leave the solution for one hour. Shake periodically. Remove the aqueous phase and wash the organic phase several times with distilled water until the washings are neutral. Filter through a bed of anhydrous Na_2SO_4 and evaporate to dryness in a rotary evaporator at a temperature below 35°. Dissolve the pigments in acetone and make up to 25 ml in a volumetric flask. Keep the samples refrigerated until analysis by HPLC. Thoroughly disperse the samples, e.g. by sonication, and filter through a 0.45 µm filter before analysis.

Chromatography

Filter acetone (HPLC grade) and deionised water and de-gas before use.

Column:	Reversed-phase C-18 (250 x 4 mm i.d.)
Precolumn:	Reversed-phase C-18 (50 x 4 mm i.d.)
Mobile phase:	Program a gradient acetone/water as follows:

Time (min)	Acetone (%)	Water (%)
-10 (pre-injection)	75	25
0	75	25
5	75	25
10	95	5
17	95	5
22	100	0
27	75	25

Flow rate: 1.5 ml/min
Detector: Diode array detector, store spectra in the range of 350-600 nm.
Detection wavelength: 450 nm
Injection volume: 5 µl
Identify peaks by comparing the peaks obtained with known standards and quantify the individual carotenoids. Saponified carotenoids will elute in the same order, with capsorubin and some minor carotenoids eluting first and β-carotene in last place. The order of elution is:
- Neoxanthin
- Capsorubin
- Violaxanthin
- Capsanthin
- Antheraxanthin
- Mutatoxanthin
- Cucurbitaxanthin A (Capsolutein)
- Zeaxanthin
- Cryptocapsin
- β-Cryptoxanthin
- β-Carotene

$$\text{Total capsanthin (\% of total carotenoids)} = \frac{a}{a_{total}} x100$$

Where
a is the area of capsanthin peak
a_{total} is the total area of the peaks in the chromatogram
.

POLYOXYETHYLENE (20) SORBITAN MONOSTEARATE

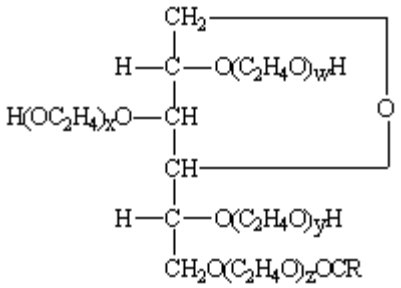
Revised specifications prepared at the 79th JECFA (2014) published in FAO JECFA Monographs 16 (2014) superseding specifications prepared at the 25th JECFA (1981), published in FNP 19 (1981) and in FNP 52 (1992). Metals and arsenic specifications revised at the 55th JECFA (2000). An ADI 'not specified' was established at the 51st JECFA (1998)

SYNONYMS Polysorbate 60; INS No. 435

DEFINITION Polyoxyethylene (20) sorbitan monostearate consists of a mixture of the partial esters of sorbitol and its mono- and dianhydrides (which have an acid value below 10 and a water content below 0.2%) with the food-grade stearic acid and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides.

C.A.S. number 9005-07-6

Structural formula Nominal formula and approximate composition:



where w + x + y + z = approx. 20 and RCO- is the fatty acid moiety

Assay Not less than 65.0 and not more than 69.5% of oxyethylene groups, equivalent to not less than 97.0 and not more than 103.0% of polyoxyethylene (20) sorbitan monostearate, on the anhydrous basis

DESCRIPTION Yellow to orange coloured oily liquid or semi-gel at 25°, with a faint characteristic odour

FUNCTIONAL USES Emulsifier, dispersing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, ethyl acetate and toluene; insoluble in mineral oil and vegetable oils

Infrared absorption The infrared spectrum of the sample is characteristic of a partial fatty acid ester of a polyoxyethylated polyol

Colour reaction To 5 ml of a 5% (w/v) aqueous solution of the sample add 10 ml of ammonium cobaltothiocyanate solution and 5 ml of chloroform, shake well

	and allow to separate; a blue colour is produced in the chloroform layer. (Ammonium cobaltothiocyanate solution: 37.5 g of cobalt nitrate and 150 g of ammonium thiocyanate made up to 100 ml with water - freshly prepared).
Test for fatty acids	To 5 ml of a 5% (w/v) aqueous solution of the sample add 5 ml sodium hydroxide TS. Boil for a few min, cool, and acidify with dilute hydrochloric acid. The solution is strongly opalescent, owing to the fatty acids liberated.
Gelatinization	A mixture of 60 parts by volume of the sample and 40 parts of water yields a gelatinous mass at or below room temperature
Saponification (Vol. 4)	100 g of the sample yields approximately 25 g of fatty acids and 77 g of polyols
PURITY	
Water (Vol. 4)	Not more than 3% (Karl Fischer Method)
Sulfated ash (Vol. 4)	Not more than 0.25% Test 5 g of the sample
Acid value (Vol. 4)	Not more than 2
Saponification value (Vol. 4)	Not less than 45 and not more than 55
Hydroxyl value (Vol. 4)	Not less than 81 and not more than 96
1,4-Dioxane (Vol. 4)	Not more than 10 mg/kg
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS (Electrothermal atomization technique) 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”)
METHOD OF ASSAY	Determine the content of *Oxyethylene groups* (Vol. 4)

POTASSIUM ALUMINIUM SILICATE

Prepared at the 79th JECFA (2014), published in FAO Monographs 16 (2014), superseding the specifications prepared at the 77th JECFA (2013), published in FAO Monographs 14 (2013). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives.

SYNONYMS Mica, Muscovite, INS No. 555

DEFINITION Potassium aluminium silicate is mined from natural sources and then further purified.

Chemical name Potassium aluminium silicate

C.A.S. number 12001-26-2

Chemical formula $KAl_2[AlSi_3O_{10}](OH)_2$ (Idealized)

Formula weight 398.31

Assay Not less than 98%

DESCRIPTION Light grey to white crystalline platelets or powder.

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble or insoluble in water, dilute acids and alkali and organic solvents

Test for aluminium and silicon Passes test
See description under TESTS

PURITY

Loss on Drying (Vol. 4) Not more than 0.5% (105°, 2 h)

Impurities soluble in 0.5 M hydrochloric acid

Antimony:	Not more than 20 mg/kg
Arsenic:	Not more than 3 mg/kg
Barium:	Not more than 25 mg/kg
Cadmium:	Not more than 2 mg/kg
Chromium:	Not more than 100 mg/kg
Copper:	Not more than 25 mg/kg
Lead:	Not more than 5 mg/kg
Mercury:	Not more than 1 mg/kg
Nickel:	Not more than 50 mg/kg
Zinc:	Not more than 25 mg/kg

See description under TESTS

TESTS

IDENTIFICATION TESTS

Test for aluminium and silicon

Use the test solution as shown under method of assay. Analyze aluminium and silica in the test solution by ICP-AES technique (Volume 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical lines for Al (396.15 nm) and Si (251.611 nm).

PURITY TESTS

Impurities soluble in 0.5 M hydrochloric acid

Extract 20 g of finely ground sample under reflux conditions (to prevent loss of mercury) with 100 ml of 0.5 M hydrochloric acid (spectroscopic grade) for 30 min. Let solution cool, then filter through a 0.1 µm membrane filter. Wash the filter twice with hot 0.5 M hydrochloric acid. Combine the filtrate and wash solution in a 200 ml volumetric flask and make up to volume with 0.5 M hydrochloric acid.
Determine arsenic using an AAS (Hydride generation) technique; antimony, barium, chromium, copper, nickel and zinc by an ICP-AES technique; lead and cadmium using an AAS (Electrothermal atomization) technique; and mercury using an AAS (Cold vapour generation) technique. See “Metallic impurities” in the Combined Compendium of Food Additive Specifications (Volume 4).

METHOD OF ASSAY

Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, mix and melt completely using a torch burner (alkali fusion) and allow to stand at room temperature. Place the reaction product along with crucible in a 250-ml PTFE beaker, add 150 ml hot deionized water and dissolve residue by agitation. Wash the crucible with a small amount of hot water and add the washings to the beaker. Add 50 ml hydrochloric acid and transfer the contents into a 250-ml volumetric flask. Wash the beaker three times with hot water, transfer the washings to the volumetric flask and make up to volume (Solution A). Prepare the test solution by diluting Solution A with 2% hydrochloric acid solution to get the solution within the linear dynamic range of the analyzer. Analyze aluminium in the test solution using ICP-AES (Vol. 4). Set instrument parameters as specified by the instrument manufacturer and use the analytical line for aluminium (396.152 nm). Determine the concentration (as µg/ml) of aluminium from the respective standard curve. Calculate the percentage of potassium aluminium silicate in the sample from aluminium using the formula below.

$$\text{Potassium Aluminium Silicate (\%)} = \frac{4.92 \times C_{Al} \times 250 \times DF}{W \times 10^6}$$

Where:
C = Concentration of Al in the test solution, µg/ml
DF = Dilution factor (dilution of Solution A to get test solution)
W = Weight of sample, g

QUILLAIA EXTRACT (TYPE 2)

Revised specifications prepared at the 79th JECFA (2014) and published in FAO JECFA Monographs 16 (2014) superseding specifications prepared at the 65th JECFA, published in FNP 52 Add 13 (2005). A group ADI of 0-1 mg quillaia saponins/kg bw for Quillaia ExtractsTypes 1 & 2 was established at 65th JECFA (2005)

SYNONYMS Quillaja extract, soapbark extract, quillay bark extract, bois de Panama, Panama bark extract, quillai extract; INS No. 999(ii)

DEFINITION Quillaia extract (Type 2) is obtained either by chromatographic separation or ultrafiltration of the aqueous extraction of the milled inner bark or of the wood of pruned stems and branches of *Quillaja saponaria* Molina (family *Rosaceae*). It contains triterpenoid saponins (quillaia saponins, QS) consisting predominantly of glycosides of quillaic acid. Polyphenols and tannins are minor components. Some sugars and calcium oxalate will also be present.

Quillaia extract (Type 2) is available commercially as a liquid product or as a spray-dried powder that may contain carriers such as lactose, maltitol or maltodextrin. The liquid product is usually preserved with sodium benzoate or ethanol.

C.A.S. number 68990-67-0

Formula weight Monomeric saponins range from ca. 1800 to ca. 2300, consistent with a triterpene with 8-10 monosaccharide residues

Assay Saponin content:
not less than 65 % and not more than 90 % on the dried basis

DESCRIPTION Light red-brownish liquid or powder

FUNCTIONAL USES Emulsifier, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very soluble in water, insoluble in ethanol, acetone, methanol, and butanol

Foam Dissolve 0.5 g of the powder form in 9.5 ml of water or 1 ml of the liquid form in 9 ml of water. Add 1 ml of this solution to 350 ml of water in a 1000-ml graduated cylinder. Cover the cylinder, vigorously shake it 30 times, and allow settling. Record the foam volume (ml) after 30 min. Typical volumes are about 260 ml.

Chromatography Determine as in METHOD OF ASSAY. The retention time of the major sample peak corresponds to the major saponin peak (QS-18) of the standard.

Colour and turbidity Powder form only: Dissolve 0.5 g in 9.5 ml of water. The solution shall not be turbid. Determine the absorbance of the solution against water at 520 nm. The absorbance shall be less than 0.7.

PURITY

Water (Vol. 4)	Powder form: not more than 6% (Karl Fischer Method)
Loss on drying (Vol. 4)	Liquid form: 50 to 90% (2 g, 105°, 5 h)
pH (Vol. 4)	3.7 -5.5 (4 % solution)
Ash (Vol. 4)	Not more than 5% on a dried basis (use 1.0 g for powder samples; for liquid samples, use the residue from Loss on drying)
Tannins	Not more than 8% on a dried basis See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg. Determine using an AAS (Electrothermal atomization technique) 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

Tannins

Weigh either 3.0 g of the powder form or an equivalent amount of liquid sample, accounting for solids content determined from loss on drying. Dissolve in 250 ml of water. Adjust the pH to 3.5 with acetic acid. Dry 25 ml of this solution at 105° for 5 h and determine the weight of the dried solid, in g (W_i). Mix 50 ml of the solution with 360 mg of polyvinyl polypyrrolidone. Stir the solution for 30 min at room temperature; then centrifuge at 800 × g. Recover the supernatant and dry this solution at 105^o (5 h).Weigh the recovered solid (W_f, in g). The percentage of tannins in the sample is:

$$\% \text{ tannins (dried basis)} = 100 \times (W_i - W_f/2) / W_i$$

METHOD OF ASSAY

Principle:
The saponins QS-7, QS-17, QS-18 and QS-21 are separated by reversed phase HPLC and their quantitation is used as an indicator for total saponins levels in Quillaia extract (Type 2).

Sample preparation:
Powders: Weigh 0.5 g of sample and dissolve in 9.5 ml of water. Filter through a 0.2 µm filter.
Aqueous extracts (~ 550 mg solids/ml): Weigh 1 g of sample and dilute with 9 ml of water. Filter through a 0.2 µm filter.
In each case, the sample volume is ca. 10 ml.

Standard preparation:
Weigh 1.5 g of purified saponins (SuperSap, Natural Response, Chile; Quil-A, Superfos, Denmark or similar, containing a known saponin content) and dissolve in 100 ml of water. Filter through a 0.2 µm filter.

High performance liquid chromatography (HPLC):
HPLC conditions:
Column: Vydac 214TP54 (4.6 x 250 mm length, 5 µm particle size) or equivalent
Column temperature: Room temperature
Pump: Gradient
Solvent A: 0.15% trifluoroacetic acid in HPLC-grade water.
Solvent B: 0.15% trifluoroacetic acid in HPLC-grade acetonitrile.

Gradient:

Time(min)	% solvent A	% solvent B
0	70	30
40	55	45
45	70	30

Flow rate: 1 ml/min
Detection wavelength: 220 nm
Injection volume: 20 µl

Calculation:
The concentration of saponins, C_{sap}, in mg/ml, in the solution prepared as directed under sample preparation is:

$$C_{sap} = (A_{sample}/A_{standard})C_{Standard}$$

Where
$C_{Standard}$ (mg/ml) is the saponins concentration of the standard injected (e.g., $C_{Standard}$ = 13.5 mg/ml if the saponin content of 1.5 g of standard sample is 90 %) and A_{sample} and $A_{standard}$ are the sums of the peak areas attributed to the four principle saponins in the sample preparation and in the standard preparation, respectively, as noted in the figure. (Tannins and polyphenols will elute before the saponins. The peaks corresponding to the saponins will appear after the major peak corresponding to the polyphenols)

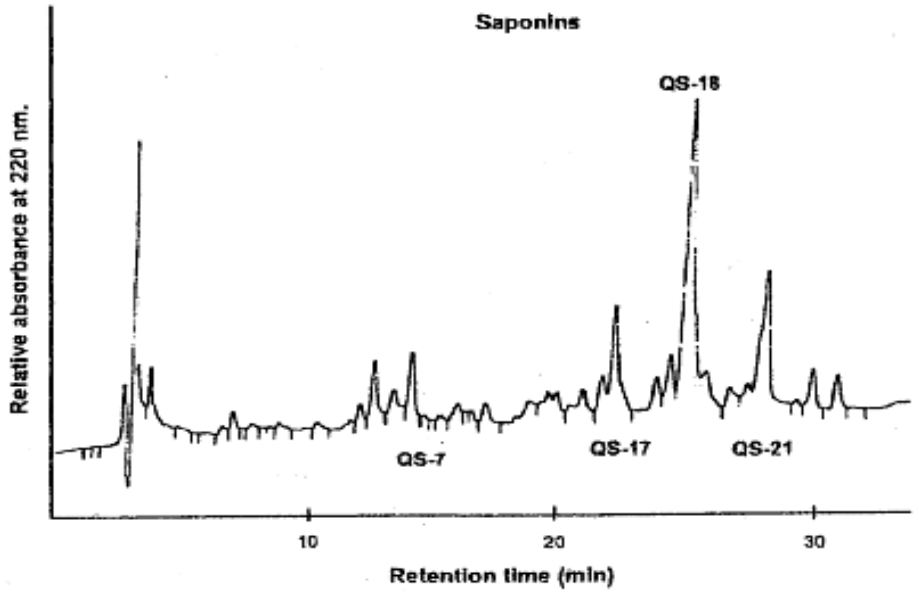
The percentage of saponins in the test sample is:

$$\% \text{ Saponins} = 100 \times C_{sap}/(0.1W_{sample})$$

Where
W_{sample} is the weight of the sample (mg) taken for the sample preparation and 0.1 is the inverse of the sample volume, 10 ml.

Appendix

Chromatogram of Standard (15 mg solids/ml equivalent to 13.5 mg saponins/ml).



ANALYTICAL METHODS

The following analytical methods were prepared by the Committee at the 79th meeting.

While reviewing the specifications for citric acid, the Committee recognized that the oxalic acid standard used for comparison in the oxalate limit test, as described in Volume 4 of the Combined Compendium of Food Additive Specifications, does not represent the limit specified in the individual specifications monographs for different food additives. The method was revised to use an appropriate standard solution that represents the limit specified.

While reviewing the specifications for gellan gum, the Committee noted that the method of assay referred to the alginates assay method in Volume 4 of the Combined Compendium of Food Additive Specifications, which uses mercury as one of its reagents. The Committee replaced the alginates assay method with a new method, based on the United States Pharmacopeia, without the use of mercury.

The on-line version of the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1, Volume 4, will be revised correspondingly.

Oxalate Limit Test

Dissolve a known quantity of sample as specified in the specifications monograph in 4 ml of water; add 3 ml concentrated hydrochloric acid and then 1g of granulated zinc. Heat for 1 min in a boiling water bath. Let stand for 2 min at room temperature; decant the supernatant solution into a test tube containing 0.25 ml of a 1% solution of phenylhydrazine hydrochloride. Mix, heat to boiling and cool immediately. Transfer the solution into a glass cylinder with a ground glass stopper and add an equal volume of concentrated hydrochloric acid. Add 0.25 ml of a 5% solution of potassium hexacyanoferrate (III), mix well and let stand for 30 min. Measure the absorbance at 520 nm in 10 mm cell. The absorbance is not more than that of a standard solution, prepared in the same manner, using 1 ml of oxalic acid standard solution (prepared by dissolving calculated amount, in mg, equivalent to the limit specified for oxalic acid or oxalic acid dihydrate in 1000 ml of deionized water) diluted with 3 ml of deionized water.

Alginates Assay (Carbon Dioxide Determination by Decarboxylation) [1]

This method can also be used for gellan gum.

Apparatus

The apparatus required is shown in Figure 1. It consists of a capillary metering valve, A, followed by a flow meter, B, to control and monitor the flow of nitrogen through the system. Halogenated vinyl plastic tubing and a rubber fitting, C, are used to connect the flow meter to a sidearm of a reaction flask, D. Flask D is a 250-ml round-bottom, boiling flask, resting in a suitable heating mantle, E. Flask D is provided with a 225-mm Hopkins coil reflux condenser, F. The condenser terminates in a U-shaped trap, G, which contain two 25-g bands of 20-mesh zinc, the bands being bounded and separated by three 3-inch plugs of glass wool. The trap terminates in an adapter, H, that by means of a halogenated vinyl plastic tubing and a twist cock connector, I, connects with a 250-ml gas washing bottle, J. The inlet (bubbling) tube extends almost to the bottom of the gas washing bottle, and it terminated in a fritted disk having a coarse porosity. The size of all glass joints is 24/40, except for the 45/50 joint of the gas washing bottle.

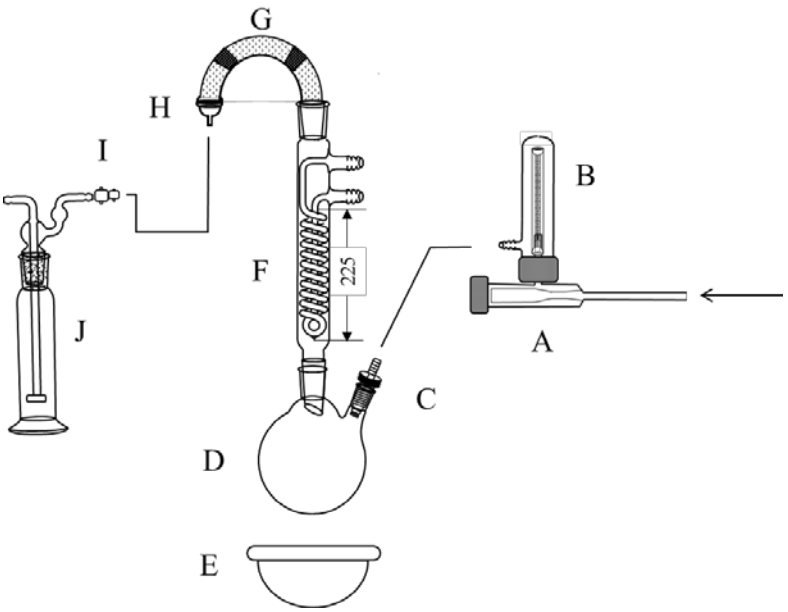


Figure 1. Apparatus

System suitability

Using D-glucuronolactone as the standard, proceed as directed for procedure, but do not perform the preboiling steps. Calculate the value for system suitability using the formula. The value for system suitability should be between 0.02 and 0.06.

$$\text{Value for system suitability} = C_{NaOH} \times V_{NaOH} - C_{HCl} \times V_{blank}$$

Where

C_{NaOH} is the concentration of sodium hydroxide solution added, mol/l
V_{NaOH} is the volume of sodium hydroxide solution pipetted, ml
C_{HCl} is the concentration of hydrochloric acid, mol/l
V_{blank} is the volume of hydrochloric acid used for the titration of the blank, ml

Procedure

Weigh accurately about 0.25 g of the sample into the reaction flask, D. Add 50 ml of 0.1 mol/l hydrochloric acid, insert several boiling chips and connect the flask to the reflux condenser, F, using syrupy phosphoric acid as a lubricant. (Note: Stopcock grease may be used for the other connections)

[1] Adapted from USP 37-NF32 with permission. Copyright 2013. The United States Pharmacopeia Convention

Connect the nitrogen line to the sidearm of the flask, and adjust the flow of cooling water to about 2 l/min. Maintain the flow of nitrogen through the apparatus at 90 to 100 ml/min. Raise the heating mantle, E, to the flask, heat the sample to boiling and boil gently for 2 min. Turn the heat off, lower the mantle, E, and allow to cool for about 10 min. Connect the empty gas washing bottle assembly, J, and sweep the system with nitrogen at a rate of 90 to 100 ml/min for 5 min. Reduce the nitrogen flow to 60 to 65 ml/min, add 10 drops of 1-butanol, pipette 25.0 ml of 0.25 mol/l sodium hydroxide solution and add 50 ml of distilled water into the bottle, rinsing down the inside of the gas washing bottle, and replace the cap. Detach the rubber fitting, C, from sidearm, and add 46 ml of hydrochloric acid through the sidearm of the boiling flask. Reattach the nitrogen line, raise the heating mantle and heat the reaction mixture to boiling.

After 3 hours of boiling, increase the nitrogen flow to 90 to 100 ml/min, discontinue the heating and lower the mantle. Allow to cool for 10 min. Disconnect and disassemble the gas washing bottle. Using a directed stream of distilled water, thoroughly rinse all parts of the bubbling tube and cap, collecting the washings in the gas washing bottle. Use nitrogen to gently force all water out of the bubbling tube. To the bottle immediately add 10 ml of 10% barium chloride solution and a magnetic stirring bar. Insert a tight stopper and stir gently for 1 min. Allow to stand for at least 5 min. Add three drops of phenolphthalein TS and titrate with 0.1 mol/l hydrochloric acid. Perform a blank determination.
Calculate the percentage of carbon dioxide from;

$$\text{Carbon dioxide (\%)} = \frac{22\,[(C_{NaOH}\,xV_{NaOH} - C_{HCl}\,xV_{sample}) - (C_{NaOH}\,xV_{NaOH} - C_{HCl}\,xV_{blank})]}{1000\,W\,(1 - 0.01\,LD)} \times 100$$

$$= \frac{22\,C_{HCl}\,(V_{blank} - V_{sample})}{1000\,W\,(1 - 0.01LD)} \times 100$$

Where

C_{NaOH} is the concentration of sodium hydroxide solution, mol/l
V_{NaOH} is the volume of sodium hydroxide solution pipetted, ml
C_{HCl} is the concentration of hydrochloric acid, mol/l
V_{sample} is the volume of hydrochloric acid used for the titration of the sample, ml
V_{blank} is the volume of hydrochloric acid used for the titration of the blank, ml
LD is the loss on drying obtained, %
W is the weight of the sample, g

Each ml of 1 mol/l sodium hydroxide is equivalent to 22 mg of carbon dioxide.

SPECIFICATIONS FOR CERTAIN FLAVOURING AGENTS

At the 79th meeting of the Committee prepared specifications of identity and purity of 25 new flavourings for the following numbers: 2186 - 2192, 2194 2204.1, 2205 - 2210. The specifications for number 2137 (nerolidol oxide) were maintained (see FAO JECFA Monographs 13), the specifications for number 1051 (2,5-dimethyl-3-acetylthiophene) were withdrawn.

The flavouring agent number 2193 (α-ionene) was not evaluated at the 79th meeting as it was determined not to fit into the proposed group of aliphatic and alicyclic hydrocarbons.

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 79th meeting were assigned full status.

The spectra used for identification tests are provided from page 75 onwards. A list of the new flavourings evaluated in alphabetical order is added on page 81.

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / COE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °C	ID test / Assay min % / Acid value	R.I. / S.G.	Other requirements / Information required
2137	**Nerolidol oxide**	4536	$C_{15}H_{26}O_2$	Practically insoluble to insoluble in water	MS	1.468-1.472	
	2-(5-Ethenyl-5-methyltetrahydrofuran-2-yl)-6-methylhept-5-en-2-ol	-	238.37	Soluble	95	0.928-0.933 (20º)	
Full	Ethenyl tetrahydro-alpha-5-dimethyl-alpha-(4-methyl-3-pentenyl)-2-furan methanol	-	Colourless liquid; Faint floral aroma	324-325 (decomposes)			
76		1424-83-5					
2186	**beta-Isomethylionone**	4151	$C_{14}H_{22}O$	Practically insoluble to insoluble	HNMR	NA	m.p. 62°
	(3E)-3-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	07.041	206.32	Soluble	95	NA	
Full	3-Buten-2-one,3-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl);3-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	650	Colourless solid; Warm floral aroma	NA			
79		79-89-0					
2187	**Pseudoionone**	4299	$C_{13}H_{20}O$	Practically insoluble to insoluble	IR, MS	1.529-1.535	3E,5E isomer: 80-95%;3E,5Z isomer: 5-20%
	(3E,5E)-6,10-Dimethylundeca-3,5,9-trien-2-one	07.198	192.30	Soluble	95	0.894-0.903	
Full	3,5,9-Undecatrien-2-one, 6,10-dimethyl-; phi-Ionone; psi-Ionone; 2,6-Dimethyl-2,6,8-undecatrien-10-one; 2,6-Dimethylhendeca-2,6,8-trien-10-one; 2-Pseudoionone; 2-Pseudojonon; 6,10-Dimethyl-3,5,9-undecatrien-2-one; Citrylideneacetone	11191	Pale yellow liquid; Warm floral aroma	143-145			
79		141-10-6					
2188	**trans-alpha-Damascone**	4088	$C_{13}H_{20}O$	Practically insoluble to insoluble	IR,MS	1.493-1.499	
	(2E)-1-(2,6,6-trimethylcyclohex-2-en-1-yl)but-2-en-1-one	07.226	192.30	Soluble	95	0.937-0.943	
Full	2-Buten-1-one, 1-(2,6,6-trimethyl-2-cyclohexen-1-yl)-,(2E)-; trans-1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)but-2-en-1-one	11053	Colourless to pale yellow liquid; Warm balsamic aroma	54 (1 mm Hg)			
79		24720-09-0					

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / COE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °C	ID test / Assay min % / Acid value	R.I. / S.G.	Other requirements / Information required
2189	**Cassyrane**	4731	$C_{12}H_{22}O$	Practically insoluble to insoluble	MS, IR, CNMR	1.4480-1.4520	Racemate
Full	2-tert-butyl-5-methyl-2-propyl-2,5-dihydrofuran		182.30	Soluble	95	0.8680-0.8720	
	2-tert-Butyl-5-methyl-2-propyl-2,5-dihydrofuran; (±)-cis and trans-2-(1,1-Dimethylethyl)-2,5-dihydro-5-methyl-2-propylfuran		Colourless to pale yellow clear liquid; Fruity reminiscent of black currant	202-203			
79		871465-49-5					
2190	**1-Cyclopropanemethyl-4-methoxybenzene**	4759	$C_{11}H_{14}O$	Practically insoluble to insoluble	HNMR	1.5228-1.5260	
Full	1-(cyclopropylmethyl)-4-methoxybenzene		162.23	Soluble	95	0.9849-0.9900	
	Anisole, p-(cyclopropylmethyl)		Clear colourless liquid	238-240			
79	Toscanol, sassafras acetate	16510-27-3	Anise-like sweet; Spicy aroma				
2191	**1-Octene**	4293	C_8H_{16}	Practically insoluble to insoluble	MS		
Full	Oct-1-ene	01.007	112.21	Slightly soluble	95	1.413-1.416 0.718-0.722	
	alpha-octene; alpha-octylene; Octylene; Caprylene; n-1-octene; n-octene-1		Colourless liquid; petroleum-like aroma	121 -122	1		
79		111-66-0					
2192	**2,4-Nonadiene**	4292	C_9H_{16}	Practically insoluble to insoluble	CNMR, IR	1.443-1.449	1,3-Nonadiene: 11%; 1-2,7
Full	(2E,4E)-nona-2,4-diene		124.22		79-80	0.745-0.763	nonadiene: 1-6%;
		1.078		Slightly soluble			2,6 nonadiene: 3-4%
79	(2E,4E)-2,4-nonadiene; (2E,4E)-nona-2,4-diene; nona-2,4-diene; (E,E)-2,4-nonadiene; trans-2,trans-4-nonadiene	56700-78-8	Colourless liquid; petroleum-like aroma	153-155	1		
2194	**4-Methyl-cis-2-pentene**	4650	C_6H_{12}	Practically insoluble to insoluble	IR,HNMR	1.3860-1.3900	Z-isomer: 90% and E isomer: 5-7%
Full	(2Z)-4-methylpent-2-ene		84.16	Slightly soluble	95	0.6690-0.6730	
	2-Pentene, 4-methyl-, (2Z)-; cis-2-methyl-3-penten		Clear colourless liquid; Fruity green aroma upon dilution	57-58			
79		691-38-3					

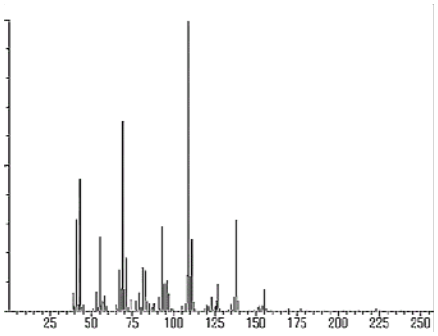
JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / COE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °C	ID test / Assay min % / Acid value	R.I. / S.G.	Other requirements / Information required
2195	**1-Nonene**	4651	C_9H_{18}	Practically insoluble to insoluble	MS	1.4160-1.4180	
Full	Non-1-ene		126.24		95		
			Clear colourless liquid;	Slightly soluble		0.7310-0.7330	
79	N-heptyl ethylene	124-11-8	Strong green aroma reminiscent of cucumber	146-148			
2196	**1,3,5,7-Undecatetraene**	4652	$C_{11}H_{16}$	Practically insoluble to insoluble	MS	1.581-1.591 (20 °)	
Full	(3E,5E,7E)-undeca-1,3,5,7-tetraene		148.24		95	0.809-0.819 (25 °)	
			Clear colourless liquid;	Slightly soluble			
79		116963-97-4	Woody, earthy, leafy galbanum aroma	116 (2.6 hPa)			
2197	**Mixture of methyl cyclohexadiene and methylene cyclohexene**	4311	C_7H_{10}	Practically insoluble to insoluble	HNMR	1.474-1.484	2-methyl-1,3-cyclohexadiene:
Full	2-methylcyclohexa-1,3-diene and 4-methylidenecyclohexene	01.025	94.15		98	0.830-0.837	45%; 3-methylene-1-cyclohexene:
	Cyclohexadiene, methyl-/		Colourless liquid;	Slightly soluble			39%; 1-methyl-1,3-cyclohexadiene:
79		30640-46-1; 1888-90-0	Strong lemon-lime top note	105			15%
2198	**2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol**	4521	$C_{13}H_{20}O$	Slightly soluble	MS	1.447-1.453	
Full	1,4,4,7a-Tetramethyl-2,4,5,7a-tetrahydro-1*H*-inden-2-ol		193.20	Soluble	98.9	0.936-0.943	
	(3aR)-3,3a,7,7-tetramethyl-4,5,6,7-tetrahydro-3aH-inden-4-ol		Clear colourless to yellow liquid; Fruity aroma	281-282	1		
79		97866-86-9					
2199	**dl-Camphor**	4513	$C_{10}H_{16}O$	Slightly soluble	MS	NA	m.p 175-180
Full	(4S,R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	07.006 / 07.209	152.23				
	dl-1,7,7-Trimethylbicyclo[2.2.1]hepta-2-one;dl-2-camphanone;dl-2-keto-1,7,7-trimethylnorcamphane		White to pale yellow crystalline solid; Camphoraceous aroma	Soluble	96	NA	
79	;	21368-68-3					

JECFA No. Status Session	Name Chemical Name Synonyms 	FEMA FLAVIS COE CAS	Chemical Formula M.W Physical form; Odour	Solubility Solubility in ethanol B.P. °C	ID test Assay min % Acid value	R.I. S.G.	Other requirements Information required
2200	**l-Fenchone**	4519	$C_{10}H_{16}O$	Pratically insolube to insoluble	MS	1.461-1.465	Optical rotation: ([α]20D= -55 to -47.5)
Full	(1R,4S)-1,3,3-trimethylbicyclo[2.2.1]heptan-2-one		152.23				
	Alpha-fenchone		Clear colourless to pale yellow liquid;	Soluble	98	0.941-0.946	
79		7787-20-4	Camphor herbal earthy woody aroma	192-194			
2201	**2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one**	4522	$C_{13}H_{18}O$	Slightly soluble	MS	1.444-1.449	90-93% cis-isomer; 6-7% trans-isomer
Full	1,4,4,7a-tetramethyl-1,4,5,7a-tetrahydro-2H-inden-2-one		190.28	Soluble	95 mixture of isomers	0.918-0.923	
	2,2,6,7-tetramethylbicyclo(4.3.0)nona-4,9(1)-dien-8-one;		Clear colourless to yellow liquid;				
79		97844-16-1	Fruity aroma	102-105 (4 mmHg)	1		
2202	**Ethyl 3-(2-hydroxyphenyl)propanoate**	4758	$C_{11}H_{14}O_3$	Slightly soluble	IR, MS, CNMR	NA	m.p. = 39-42°
Full	ethyl 3-(2-hydroxyphenyl)propanoate		194.23	Soluble	95	NA	
	Hydrocinnamic acid, o-hydroxy-, ethyl ester; Ethyl 3-(2-hydroxyphenyl)propionate; Ethyl melilotate; Ethyl o-hydroxyhydrocinnamate; Benzenepropanoic acid, 2-hydroxy-, ethyl ester		White solid; Slight spicy aroma	281-283			
79		20921-04-4					
2203	**3-[3-(2-Isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester**	4766	$C_{17}H_{32}N_2O_3$	Practically insoluble to insoluble	MS, HNMR, IR	NA	m.p. = 108-113°; Isomers: 93-98%
Full	ethyl 3-(3-(2-isopropyl-5-methylcyclohexyl)ureido)butanoate		312.45	Soluble	95 (sum of isomers)	NA	(3R(1S, 2S, 5R)), 3S((1S, 2S, 5R)), (3R(1R, 2R, 5S)), (3S((1R, 2R, 5S)); and 0-5% sum of all other isomers
	3-[3-(2-Isopropyl-5-methyl-cyclohexyl)ureido-butyric acid ethyl ester		Pearl-white powder; Bland aroma				
79		1160112-20-8					

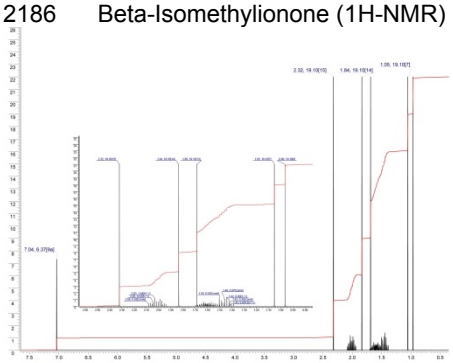
JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / COE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °C	ID test / Assay min % / Acid value	R.I. / S.G.	Other requirements / Information required
2204	**4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid**	4774	$C_{19}H_{25}N_3O_4$	Soluble in 1.9 mM phosphate buffer, pH 7.1	IR	NA	m.p. = 212-215°
Full	4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid		359.42	Soluble	95	NA	
	3-Quinolinecarboxylic acid, 4-amino-5-[2,2-dimethyl-3-[(1-methylethyl)amino]-3-oxopropoxy]-2-methyl-		Off-white solid; Lightly milky aroma				
79		1359963-68-0					
2204.1	**4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid hemisulfate monohydrate salt**	4774	$C_{19}H_{25}N_3O_4 \cdot 1/2\ H_2O_4\ S \cdot H_2O$	Soluble	HNMR	NA	m.p. =135.9-137.9
Full	4-amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid hemisulfate monohydrate salt		426.47	Sparingly soluble	95	NA	
	3-Quinolinecarboxylic acid, 4-amino-5-[2,2-dimethyl-3-[(1-methylethyl)amino]-3-oxo-propoxy]-2-methyl-, sulfate, hydrate (2:1:2)		Off-white to pale yellow powder; Lightly milky aroma				
79		1460210-04-1					
2205	**Triethylthialdine**	4748	$C_9H_{19}NS_2$	Practically insoluble to insoluble	HNMR	1.5306-1.5309	Racemate
Full	2,4,6-triethyl-1,3,5-dithiazinane	15.054	205.38	Soluble	95%	1.0365-1.0375 (20º)	
			Clear to yellow liquid; Alliaceous, onion, fatty scallion aroma	287-288	5.2		
79		54717-17-8					
2206	**2-Isopropyl-4-methyl-3-thiazoline**	4767	$C_7H_{13}NS$	Practically insoluble to insoluble	MS, HNMR	1.4971-1.5470	
Full	4-methyl-2-(propan-2-yl)-2,5-dihydro-1,3-thiazole		143.25	Soluble	95%	0.9936-1.0740 (20º)	
	2,5-Dihydro-2-isopropyl-4-methylthiazole; Thiazole, 2,5-dihydro-4-methyl-2-(1-methylethyl)		Clear liquid; Toasted, sulfury, sweet cocoa aroma	45-46 (0.75 mm Hg)	2.0		
79		67936-13-4					

JECFA No. / Status / Session	Name / Chemical Name / Synonyms	FEMA / FLAVIS / COE / CAS	Chemical Formula / M.W / Physical form; Odour	Solubility / Solubility in ethanol / B.P. °C	ID test / Assay min % / Acid value	R.I. / S.G.	Other requirements / Information required
2207	**Myricitrin**	4491	$C_{21}H_{20}O_{12}$	Practically insoluble to insoluble	MS, IR, HNMR, CNMR	NA	m..p. = 197-199°
Full	3',4',5,5',7-pentahydroxy-3-(α-L-rhamnopyranosyloxy) flavone 3,3',4',5,5',7-Hexahydroxyflavone, 3-rhamnoside; Myricetin 3-O-.alpha.-L-rhamnopyranoside; Myricetin 3-O-.alpha.-L-rhamnoside; Myricetin 3-O-.alpha.-rhamnopyranoside; Myricetin 3-O-rhamnoside; Myricetin 3-rhamnoside; Myricetin-3-O-alpha-rhamnoside; Myricitrine; Myricitroside		464.38 Light yellow powder or mass; Slight bayberry aroma	Slightly soluble	95	NA	
79		17912-87-7					
2208	**Naringin dihydrochalcone**	4495	$C_{27}H_{34}O_{14}$	Soluble	HNMR	NA	m.p. = 169 - 171° (decomposition)
Full	1-[4-[[2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(4-hydroxyphenyl)-1-propanone Glucopyranoside, 3,5-dihydroxy-4-(p-hydroxyhydrocinnamoyl)phenyl 2-O-(6-deoxy-α-L-mannopyranosyl)-, β-D-;	9.265	582.55 White solid; Bland aroma	Soluble	95	NA	
79		18916-17-1					
2209	**1-(2,4-Dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one**	4764	$C_{16}H_{16}O_5$	Practically insoluble to insoluble	MS, IR, HNMR	NA	m.p. = 120-122º
Full	1-(2,4-dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one		288.30 Pearly white powder; Bland aroma	Sparingly soluble	95	NA	
79		50297-39-7					
2210	**(-)-Matairesinol**	4762	C20H22O6	Practically insoluble to insoluble	IR, HNMR	NA	Optical rotation: -46.7º to -46.1º in acetone
Full	(3R,4R)-3,4-bis(4-hydroxy-3-methoxybenzyl)dihydrofuran-2(3H)-one 2(3H)-Furanone, dihydro-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl]-,(3R-trans)-; 2(3H)-Furanone, dihydro-3,4-divanillyl-; Matairesinol; (8R,8'R)-(-)-Matairesinol		358.39 Pearly white powder; Bland aroma	Sparingly soluble	95	NA	
79		580-72-3					

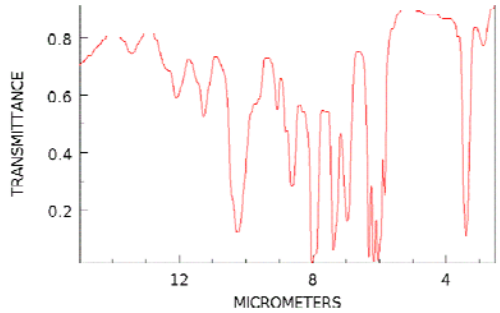
2137 Nerolidol oxide (MS)



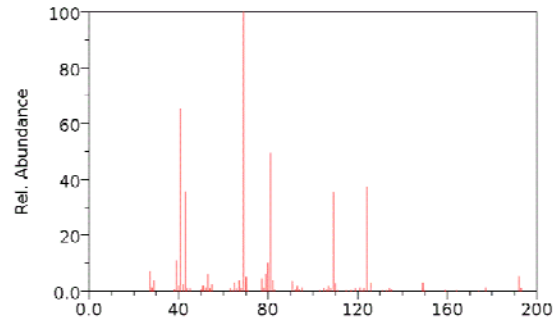
2186 Beta-Isomethylionone (1H-NMR)



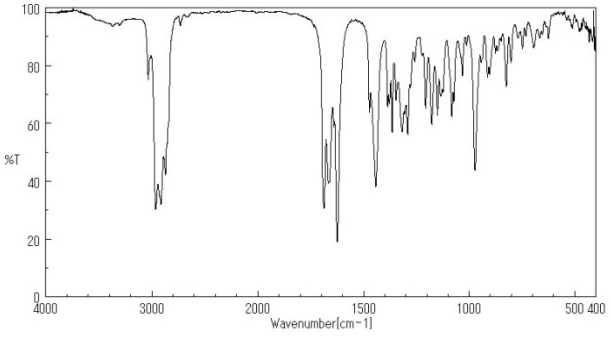
2187 Pseudoionone (IR)



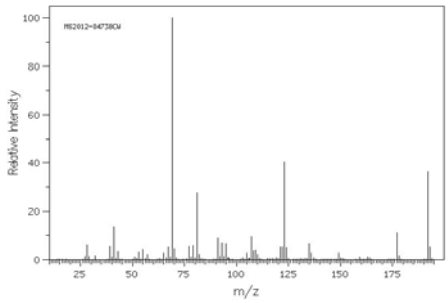
2187 Pseudoionone (MS)



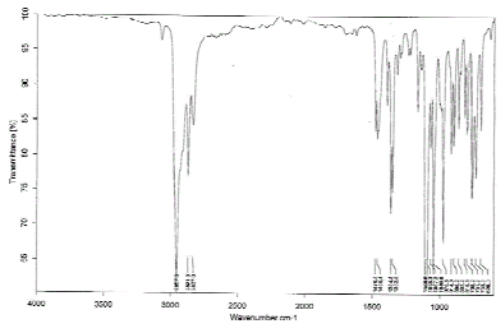
2188 trans-alpha-Damascone (IR)



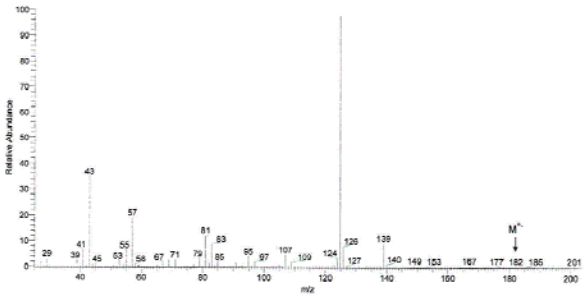
2188 trans-alpha-Damascone (MS)



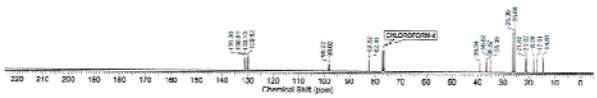
2189 Cassyrane (IR)



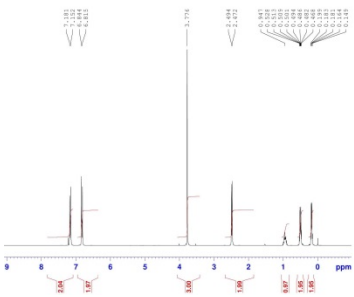
2189 Cassyrane (MS)



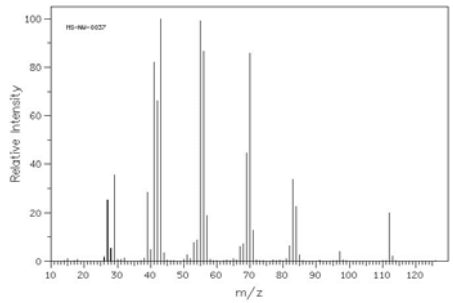
2189 Cassyrane (13C-NMR)



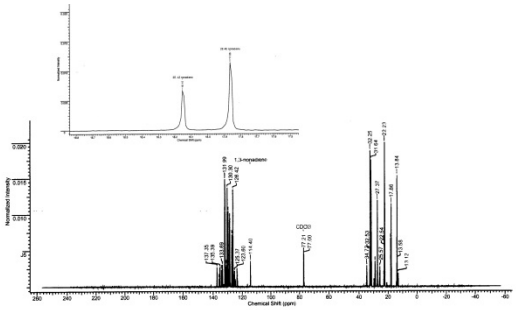
2190 1-Cyclopropanemethyl-4-methoxybenzene (1H-NMR)



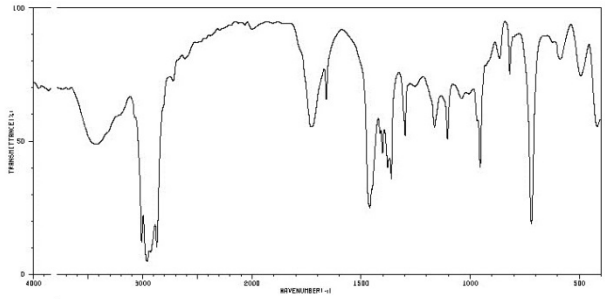
2191 1-Octene (MS)



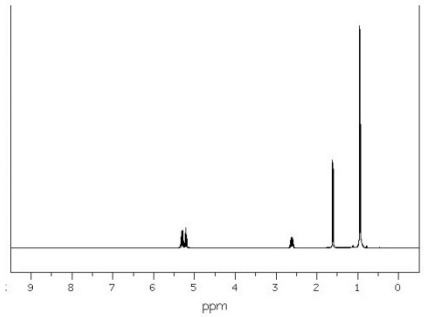
2192 2,4-Nonadiene (13C-NMR)



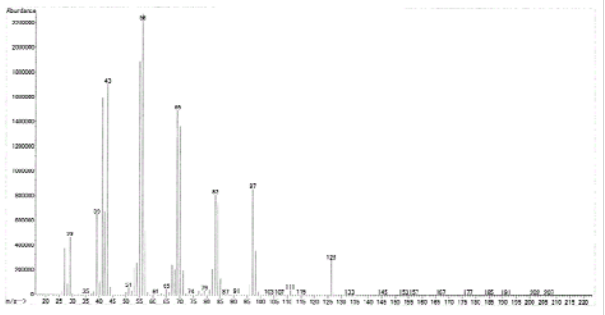
2194 4-Methyl-cis-2-pentene (IR)



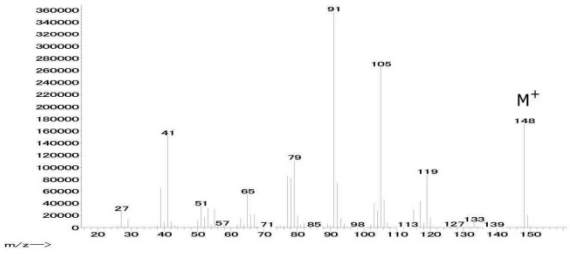
2194 4-Methyl-cis-2-pentene (13C-NMR)



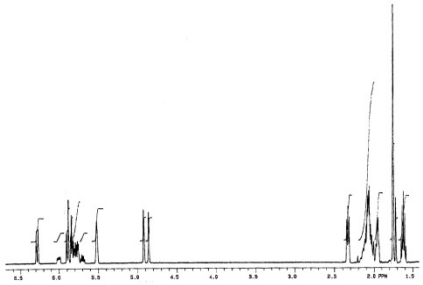
2195 1-Nonene (MS)



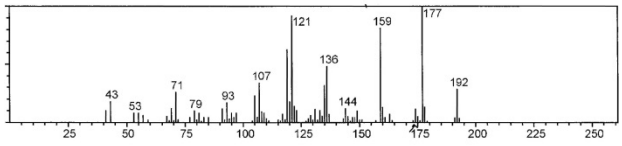
2196 1,3,5,7-Undecatetraene (MS)



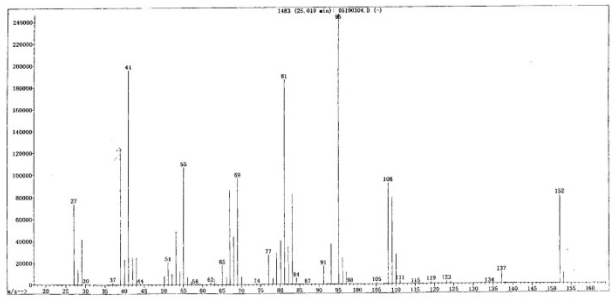
2197 Mixture of methyl cyclohexadiene and methylene cyclohexene (1H-NMR)



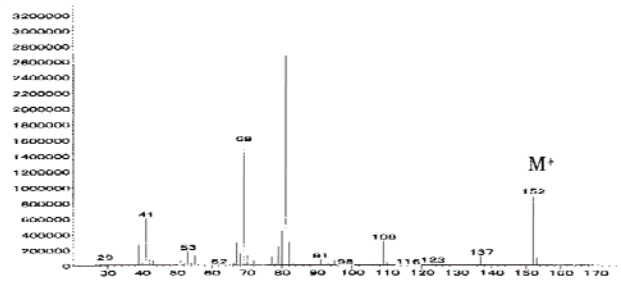
2198 2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol (MS)



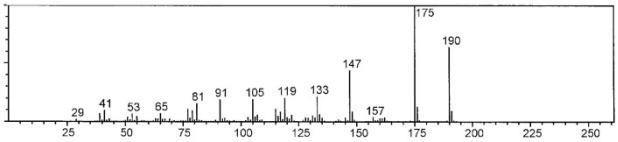
2199 dl-Camphor (MS)



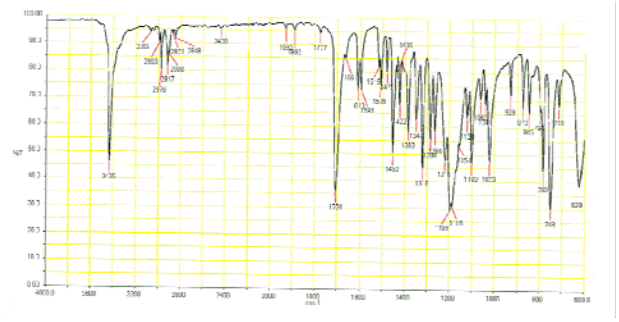
2200 l-Fenchone (MS)



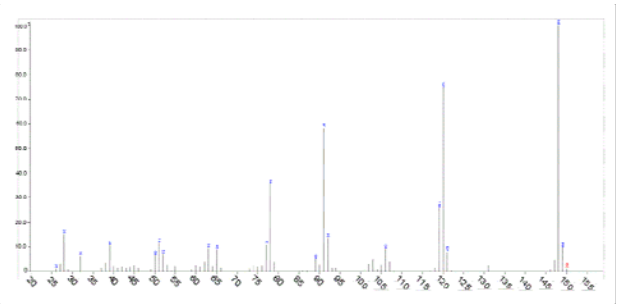
2201 2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one (MS)



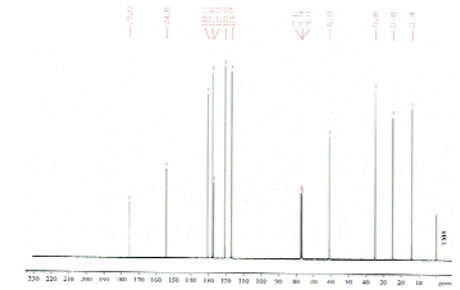
2202 Ethyl 3-(2-hydroxyphenyl)propanoate (IR)



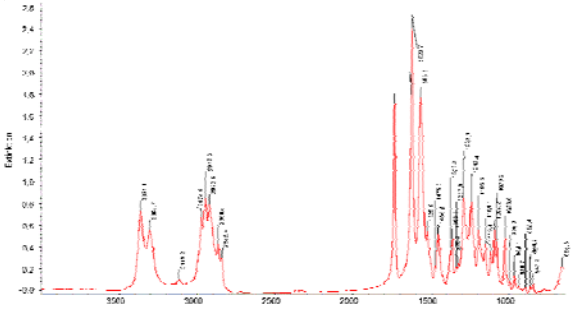
2202 Ethyl 3-(2-hydroxyphenyl)propanoate (MS)



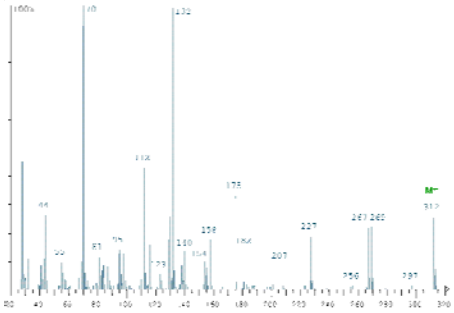
2202 Ethyl 3-(2-hydroxyphenyl)propanoate (13C-NMR)



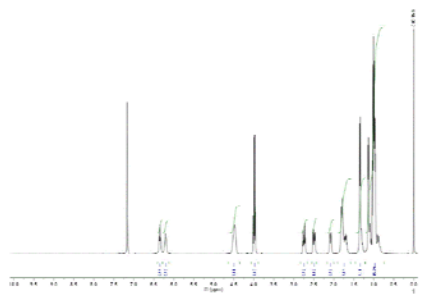
2203 3-[3-(2-Isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester (IR)



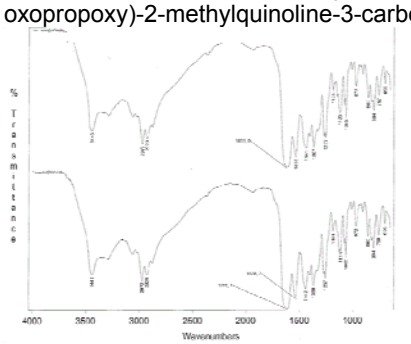
2203 3-[3-(2-Isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester (MS)



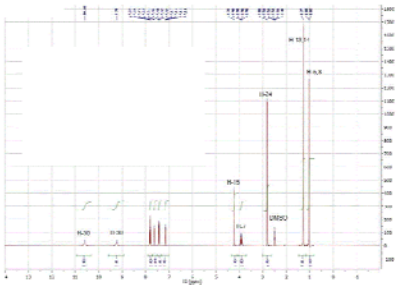
2203 3-[3-(2-Isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester (1H-NMR)



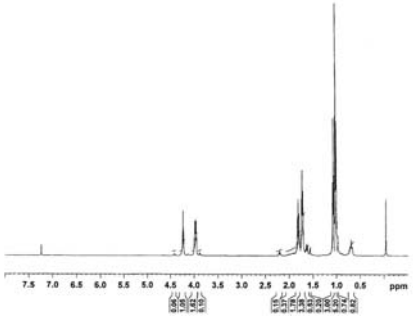
2204 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (IR)



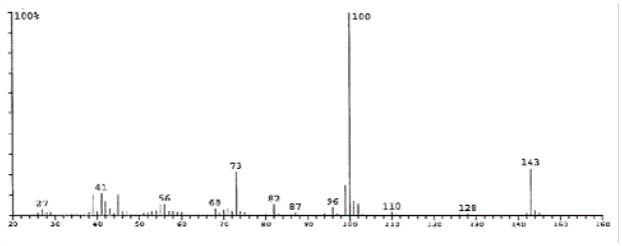
2204.1 4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid hemisulfate monohydrate salt (1H-NMR)



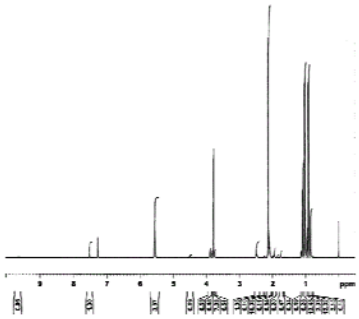
2205 Triethylthialdine (1H-NMR)



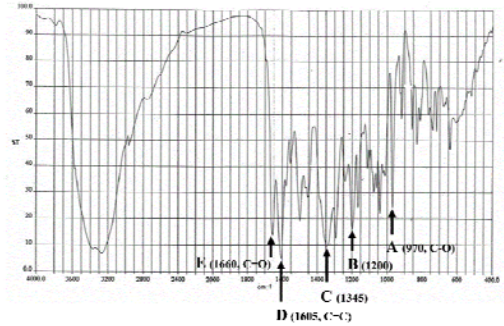
2206 2-Isopropyl-4-methyl-3-thiazoline (MS)



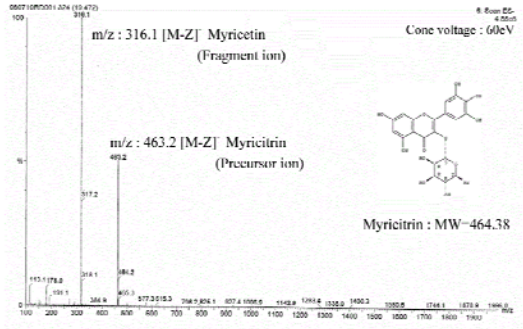
2206 2-Isopropyl-4-methyl-3-thiazoline (1H-NMR)



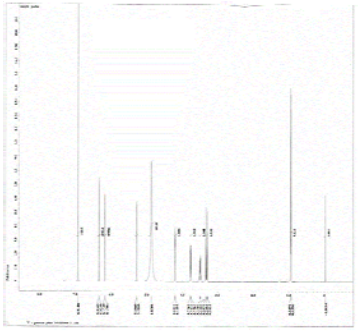
2207 Myricitrin (IR)



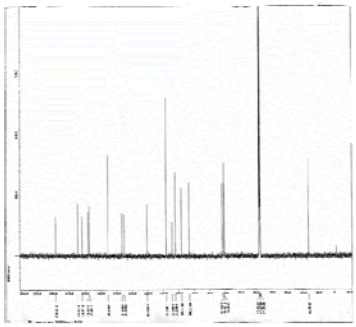
2207 Myricitrin (MS)



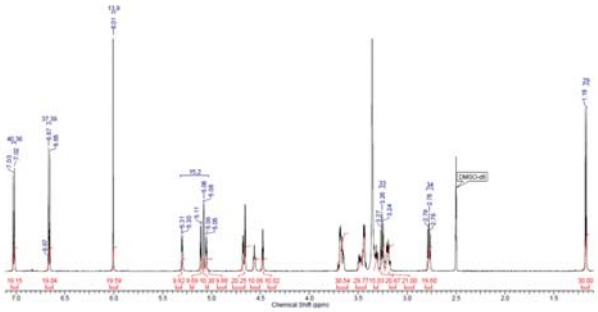
2207 Myricitrin (1H-NMR)



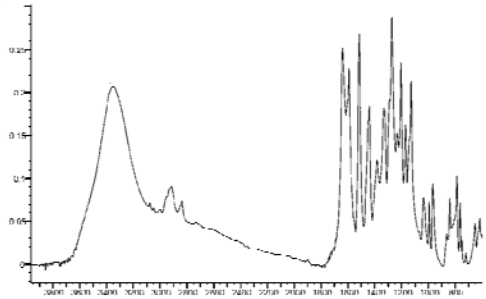
2207 Myricitrin (13C-NMR)



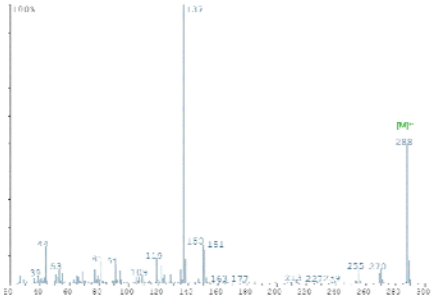
2208 Naringin dihydrochalcone (1H-NMR)



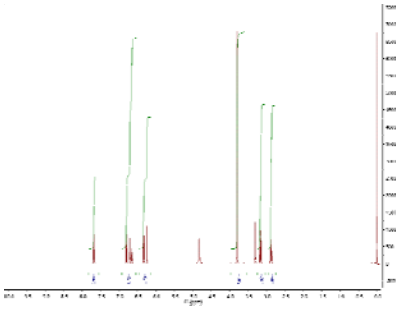
2209 1-(2,4-Dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (IR)



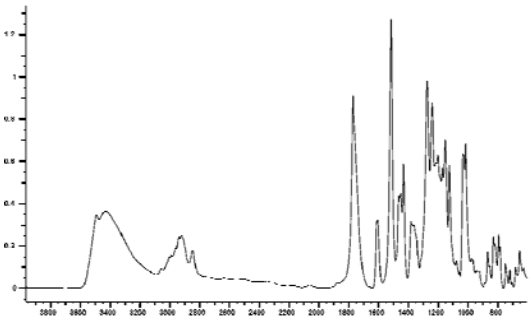
2209 1-(2,4-Dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (MS)



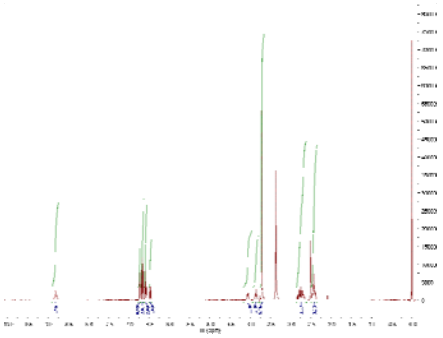
2209 1-(2,4-Dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (1H-NMR)



2210 (−)-Matairesinol (IR)



2210 (−)-Matairesinol (1H-NMR)



List of new flavourings evaluated in alphabetical order

4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid	2204
4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid hemisulfate monohydrate salt	2204.1
dl-Camphor	2199
Cassyrane	2189
1-Cyclopropanemethyl-4-methoxybenzene	2190
trans-alpha-Damascone	2188
1-(2,4-Dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one	2209
Ethyl 3-(2-hydroxyphenyl)propanoate	2202
l-Fenchone	2200
Beta-Isomethylionone	2186
2-Isopropyl-4-methyl-3-thiazoline	2206
3-[3-(2-Isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester	2203
(−)-Matairesinol	2210
Mixture of methyl cyclohexadiene and methylene cyclohexene	2197
4-Methyl-*cis*-2-pentene	2194
Myricitrin	2207
Naringin dihydrochalcone	2208
Nerolidol oxide	2137
2,4-Nonadiene	2192
1-Nonene	2195
1-Octene	2191
Pseudoionone	2187
2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol	2198
2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one	2201
Triethylthialdine	2205
1,3,5,7-Undecatetraene	2196

Annex I: Summary of recommendations from the 79th JECFA

Food additives considered for specifications only

Food additive	Specifications
Citric acid	R[a]
Gellan gum	R[b]
Polyoxyethylene (20) sorbitan monostearate	R[c]
Potassium aluminium silicate	R[d]
Quillaia extract (Type 2)	R[e]

R: existing specifications revised

[a] The method for the oxalate limit test was amended.

[b] The method of assay in the specifications refers to the alginates assay method. This method was replaced by a method without the use of mercury.

[c] Criteria for saponification and hydroxyl values were revised.

[d] The Committee reviewed the existing data as well as new information received from the sponsor and noted that potassium aluminium silicate (PAS) stabilizes the formed layers of titanium dioxide and/or iron oxide in the PAS-based pearlescent pigments. Therefore, the Committee concluded that PAS exerts a technological effect in the PAS-based pearlescent pigments; as a result, PAS could not be considered to function as a carrier according to the Codex definition for carrier. Hence, the Committee decided to delete the functional use as carrier in the specifications.

[e] The upper limit in the loss on drying specification was increased from 80% to 90%.

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations
Benzoe tonkinensis	R[a]	Given the no-observed-adverse-effect level (NOAEL) of 500 mg/kg body weight (bw) per day for Benzoe tonkinensis identified in a 90-day oral toxicity study in rats and the previously established ADIs for the major components of Benzoe tonkinensis (benzoic acid, benzyl benzoate and vanillin), **the Committee confirmed the conclusion from the seventy-fourth meeting that Benzoe tonkinensis would not be of safety concern at current estimated dietary exposures**, provided that it complies with the specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice.
Carrageenan (for use in infant formula and formula for special medical purposes intended for infants)	R	The margins of exposure (MOEs) between the NOAEL of 430 mg/kg bw per day (2250 mg/kg formula), the highest dose tested, from a neonatal pig study and human infant exposures at 2–4 weeks of age range from 2 to 12 on a body weight basis and from 2 to 8 on a concentration basis. The Committee noted that although the MOEs are small in magnitude, they are derived from a neonatal pig study in which the highest dose tested was without adverse effects on the gut or on immune parameters, supported by a neonatal minipig study. These new studies allay the earlier concerns that carrageenan, which is unlikely to be absorbed, may have a direct effect on the immature gut. The Committee also took account of the previous toxicological database on carrageenan, which did not indicate other toxicological concerns. It also noted that at carrageenan concentrations higher than 2500 mg/kg, formula becomes highly viscous, which adversely affects palatability and growth. **The Committee concluded that the use of carrageenan in infant formula or formula for special medical purposes at concentrations up to 1000 mg/L is not of concern.** The Committee recognized that there is variability in medical conditions among infants requiring formulas for special medical purposes that contain the higher levels of carrageenan, and the Committee noted that these infants would normally be under medical supervision.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations
Citric and fatty acid esters of glycerol (CITREM) (for use in infant formula and formula for special medical purposes intended for infants)	R	The Committee considered it unlikely that consumption of formulas containing typical levels of CITREM used in powdered formulas (up to 2.7 g/L as reconstituted), which is equivalent to an exposure to citrate of 440 mg/kg bw per day for the very young infant at the 95th percentile energy intake, would cause diarrhoea. At the high end of the requested range for use (up to 9 g/L), which is equivalent to an exposure to citrate of 1140 mg/kg bw per day for the very young infant at the 95th percentile energy intake, diarrhoea might occur in some infants. **The Committee concluded that there are no toxicological concerns about the use of CITREM in infant formula and formula for special medical purposes at concentrations up to 9 g/L.** At the higher use levels, there is a possibility of diarrhoea from free citric acid released from formula containing CITREM. Given the paucity of clinical data and the fact that exposure assumptions for citric acid have been maximized, it is difficult to estimate the risk of diarrhoea, but it is considered to be low.
Gardenia yellow	No[b]	Given the deficiencies in the toxicological and specifications databases, including incomplete data on the manufacturing process and composition of the material, missing toxicological studies (e.g. long-term toxicity, carcinogenicity, reproductive toxicity and developmental toxicity), the inadequate characterization of the test material in the available toxicological studies and limited reporting of the available studies, **the Committee was unable to evaluate gardenia yellow proposed for use as a food colour.**
Lutein esters from *Tagetes erecta*	N, T[c]	The Committee concluded that there was no need to establish a numerical ADI. This decision was based on a number of factors, including the absence of any observed toxicity of lutein or lutein esters in any of the available toxicological studies in animals; the absence of any adverse effects in humans consuming lutein or lutein esters; the large MOE (>1500) between the NOAEL for lutein in a new 13-week study in rats and the estimated dietary exposure of 0.32 mg/kg bw per day (from additive and natural sources); a 2-fold increase in the NOAEL for lutein as a result of the new 13-week study; and the fact that lutein esters from *Tagetes erecta* are considered to be substitutional for other lutein extracts. **The Committee established a temporary ADI "not specified"[e] for lutein esters from *Tagetes erecta*.** The ADI was made temporary because the specifications for lutein esters from *Tagetes erecta* were tentative. The Committee 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, but this would be possible only when the specifications for lutein esters from *Tagetes erecta* are finalized.
Octenyl succinic acid (OSA)–modified gum arabic	R, T[c]	The tentative status of the specifications was maintained pending the submission of additional data. The Committee noted that additional safety data may also be needed to complete the evaluation of OSA-modified gum arabic. **The Committee decided that the temporary ADI "not specified" will be withdrawn unless adequate data to complete the safety evaluation are submitted by the end of 2015.**

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations
Octenyl succinic acid (OSA)–modified starch (starch sodium octenyl succinate) (for use in infant formula and formula for special medical purposes intended for infants)	R[d]	Taking into account the overall low toxicity of OSA-modified starch, the conservatism in the NOAEL, which was the highest dose tested in a study in neonatal animals, and in the exposure assessments, as well as the supporting evidence from clinical trials and post-marketing surveillance, **the Committee concluded that the consumption of OSA-modified starch in infant formula or formula for special medical purposes intended for infants is not of concern at use levels up to 20 g/L.** New data available since the twenty-sixth meeting confirm the very low toxicity of OSA-modified starch, and **the Committee confirmed the ADI "not specified" established at that meeting for its use as a food additive for the general population.**
Paprika extract	M	**The Committee established an ADI for paprika extract used as a food colour of 0–1.5[1] mg/kg bw, expressed as total carotenoids**, with the application of an uncertainty factor of 100 to the NOAEL of 153 mg/kg bw per day from a 2-year toxicity and carcinogenicity study in rats. The Committee concluded that dietary exposure to paprika extract used as a food colour does not present a health concern.
Pectin (for use in infant formula and formula for special medical purposes intended for infants)	M	In a 3-week study in neonatal pigs fed pectin-containing milk replacer, the NOAEL was 847 mg/kg bw per day, with decreased feed intake and body weight gain observed at 3013 mg/kg bw per day. Using the NOAEL from this study, the MOEs were estimated to be 0.9 for infants with median energy intake and 0.8 for infants with high (95th percentile) energy intake. The Committee concluded that estimated exposure to pectin from its use in infant formula is in the region of the NOAEL derived from the neonatal pig study and close to the LOAEL based on decreased feed intake and body weight gain. While no overt toxicological effects were observed in the neonatal pigs, decreased food intake and body weight gain would be considered an undesirable effect in human infants. The available clinical studies were mainly conducted with pectin or pectin-derived oligosaccharides at concentrations of 0.2% or less and therefore do not provide support for tolerance and normal growth at the proposed use level. Therefore, **the Committee concluded that the use of pectin in infant formulas at the maximum proposed use level (0.5%) is of concern.**

M: existing specifications maintained; N: new specifications; No: no specifications prepared; R: existing specifications revised; T: tentative specifications

[a] The tentative qualification of the specifications was removed.

[b] No specifications were prepared. Information is required to prepare specifications (see Annex 3).

[c] Additional information is required to finalize the specifications (see Annex 3).

[d] The analytical method for the determination of the octenyl succinyl group in starch sodium octenyl succinate was amended.

[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.

[1] The Committee noted that although derived values, such as health-based guidance values, should be rounded to a single significant figure, it decided to use two significant figures in the present case, as the impact of rounding to one significant figure would be more than 30%.

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

A. Aliphatic and alicyclic hydrocarbons

The Committee determined that the flavouring agent α-ionene (No. 2193), which was submitted for evaluation as part of this flavouring agent group, did not fit into this group on the basis of its chemical structure and did not evaluate α-ionene.

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
1-Octene	2191	N	No safety concern
2,4-Nonadiene	2192	N	No safety concern
4-Methyl-*cis*-2-pentene	2194	N	No safety concern
1-Nonene	2195	N	No safety concern
1,3,5,7-Undecatetraene	2196	N	No safety concern
Mixture of methyl cyclohexadiene and methylene cyclohexene	2197	N	No safety concern

N: new specifications

B. Aliphatic and aromatic ethers

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
Cassyrane	2189	N	No safety concern
1-Cyclopropanemethyl-4-methoxybenzene	2190	N	No safety concern
Nerolidol oxide	2137	M	No safety concern

M: existing specifications maintained; N: new specifications

C. Ionones and structurally related substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
β-Isomethylionone	2186	N	No safety concern
Pseudoionone	2187	N	No safety concern
trans-α-Damascone	2188	N	Additional data required to complete evaluation

N: new specifications

D. Miscellaneous nitrogen-containing substances

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class III			
3-[3-(2-Isopropyl-5-methylcyclohexyl)-ureido]-butyric acid ethyl ester	2203	N	No safety concern

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
4-Amino-5-(3-(isopropylamino)-2,2-dimethyl-3-oxopropoxy)-2-methylquinoline-3-carboxylic acid (and its hemisulfate monohydrate salt)	2204	N	No safety concern
	2204.1	N	No safety concern

N: new specifications

E. Monocyclic and bicyclic secondary alcohols, ketones and related esters

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-ol	2198	N	No safety concern
dl-Camphor	2199	N	No safety concern
l-Fenchone	2200	N	No safety concern
2,2,6,7-Tetramethylbicyclo[4.3.0]nona-4,9(1)-dien-8-one	2201	N	No safety concern

N: new specifications

F. Phenol and phenol derivatives

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
Myricitrin	2207	N	No safety concern
Structural class III			
Naringin dihydrochalcone	2208	N	No safety concern
1-(2,4-Dihydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)propan-1-one	2209	N	No safety concern
(−)-Matairesinol	2210	N	No safety concern

N: new specifications

G. Phenyl-substituted aliphatic alcohols and related aldehydes and esters

The Committee concluded that the Procedure could not be applied to (±)-2-phenyl-4-methyl-2-hexenal (No. 2069) until concerns regarding genotoxicity are resolved. In addition, the evaluations of the other α,β-unsaturated aldehydes in this group (Nos 1472–1494 and 1476) should be reconsidered at a future meeting, given the potential genotoxicity of 2-phenyl-2-butenal (No. 1474).

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class I			
Ethyl 3-(2-hydroxyphenyl)propanoate	2202	N	No safety concern

N: new specifications

H. Sulfur-containing heterocyclic compounds

The Committee concluded that 2,5-dimethyl-3-acetylthiophene (No. 1051) is mutagenic in vitro and in vivo and considered that it is inappropriate for such a compound to be used as a flavouring agent or for any other food additive purpose. It therefore withdrew the previous conclusion of the Committee. The Committee is also aware that the flavouring industry has already taken steps to remove this compound from the market. Specifications established at the fifty-ninth meeting for No. 1051 were also withdrawn based on toxicological concerns.

Flavouring agent	No.	Specifications	Conclusion based on current estimated dietary exposure
Structural class II			
Triethylthialdine	2205	N	No safety concern
Structural class III			
2-Isopropyl-4-methyl-3-thiazoline	2206	N	No safety concern

N: new specifications

Annex 2. Further information required or desired

Limits for lead in specifications of food additives for use in infant formulas

The Committee referred back to the Codex Committee on Food Additives (CCFA) on whether specific purity criteria for additives for use in infant formulas should be considered and appropriate ways to present these criteria (e.g. establishing specifications for additives for use in infant formulas only; establishing different purity limits for additives for use in infant formulas in existing specifications).

Citric and fatty acid esters of glycerol (CITREM)

The Committee noted that the test method for the determination of total citric acid in the specifications monograph for CITREM currently employs a gas chromatographic method using a packed column. The Committee recommended the submission of data for a suitable method using a capillary/wide-bore column to replace the current method for consideration at a future meeting.

Gardenia yellow

The Committee noted that it is not clear whether the material tested toxicologically was representative of gardenia yellow. In addition, the available toxicity studies have not been conducted following internationally recognized guidelines, and a number of studies were performed using non-relevant routes of administration. Finally, there are no long-term toxicity, carcinogenicity, reproductive toxicity or developmental toxicity studies available.

In order to establish specifications, the Committee requires:

- information on the manufacturing process, including purification steps;
- analytical data on the composition of the substance, including the total amount of colouring matter and relevant compounds of known biological activity, such as geniposide and genipin;
- data on loss on drying;
- information on a method of assay;
- analytical data on at least five different batches of commercial materials supporting the specifications; and
- data on stability in food.

Lutein esters from **Tagetes erecta**

New tentative specifications were prepared. The Committee requested the following information, **by the end of 2015**, to complete the safety assessment:

- details on the manufacturing process, including purification steps;
- detailed analytical data on the full composition of at least five different batches of commercially available product to support the specifications;
- method of analysis to determine carotenoid composition; and
- method of analysis to determine the composition of the non-carotenoid lipidic fraction.

Octenyl succinic acid (OSA)–modified gum arabic

The existing specifications were revised and their tentative status was maintained, pending the submission of the following information, **by the end of 2015**:

- data on the manufacturing process, including purification steps;
- chemical characterization of the product in commerce;
- updated analytical methods for the determination of esterified (bound) and residual (free) OSA;
- results of the analysis of at least five batches of product in commerce; and
- applicability of the high-performance liquid chromatographic method for the determination of residual OSA.

Modified starches

The existing specifications monograph for modified starches includes 16 different modified starches, which complicates revisions of the specifications for any individual modified starch. Therefore, the Committee recommended that the specifications monograph for the modified starches be split into 16 individual specifications monographs.

The Committee, as noted at its seventy-sixth meeting, considered that it would also be necessary to revise the specifications for all the modified starches, including test methods, at future meetings.

Pectin

The Committee requested additional data to support the safety evaluation of pectin in infant formula, including an explanation for the decreased feed intake and body weight gain in neonatal pigs.

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

79th Meeting 2014

This document contains food additive specification monographs, analytical methods, and other information prepared at the seventy-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, Switzerland, from 17 – 26 June 2014. 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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