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FAO JECFA Monographs 11

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

74th Meeting 2011

World Health Organization

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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SPECIAL NOTE

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Rome, 14 – 23 June, 2011

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 74th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome on 14 - 23 June 2011. 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 consist 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/ag/agn/jecfa-additives/search.html . 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 74th meeting, are available in the new format online searchable database at the JECFA website at FAO: http://www.fao.org/ag/agn/jecfa-flav/search.html. 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/ag/agn/agns/jecfa_archive_cta_en.asp .

Contact and Feedback

More information on the work of the Committee is available from the FAO homepage of JECFA at: http://www.fao.org/ag/agn/agns/jecfa_index_en.asp . 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), revised (R) or maintained (M) specifications monographs were prepared for the following food additives and these are provided in this publication:

Benzoe tonkinensis (N, T)
Caramel colours (R)
β-apo-8'-Carotenal (R)
β-apo-8'-Carotenoic acid ethyl ester (R)
β-Carotene, synthetic (R)
Glycerol ester of gum rosin (R, T)
Glycerol ester of tall oil rosin (R, T)
Glycerol ester of wood rosin (R, T)
Hydroxypropylmethyl cellulose (R)
Magnesium silicate, synthetic (R)
Modified starches (R)
Nitrous oxide (R)
Octenyl succinic acid modified gum arabic (R)
Polydimethylsiloxane (M)
Ponceau 4R (R)
Potassium aluminium silicate (N, T)
Potassium aluminium silicate-based pearlescent pigments (N, T)
Pullulan (R)
Pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis* (N)
Quinoline Yellow (R, T)
Sodium carboxymethyl cellulose (R)
Sucrose monoesters of lauric, palmitic or stearic acid (R)
Sunset Yellow FCF (M)

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

The specifications monographs that were maintained as such include new information on the ADI established at the 74th meeting, and these specifications monographs are provided in their entirety in this publication.

As a result of the revised PTWI of 2 mg/kg for aluminium established at this meeting, all existing specifications monographs for aluminium containing food additives have been revised to include this information. These are as follows: Aluminium ammonium sulfate; Aluminium lakes of colouring matters; Aluminium potassium sulfate; Aluminium powder; Aluminium silicate; Aluminium sulfate, anhydrous; Calcium aluminium silicate; Sodium aluminium phosphate, acidic; and Sodium aluminium phosphate, basic. In addition, minor editorial revisions have been made to these specifications. All these revisions have been introduced in the corresponding JECFA food additives monographs in the on-line database and are not reproduced in this volume.

New and revised INS numbers assigned to food additives by the Codex Alimentarius Commission at its 34th session in 2011, (REP11/FA, Appendix XII), have been introduced in the corresponding JECFA food additive specifications monographs in the on-line database, as appropriate, and these are not reproduced in this publication.

BENZOE TONKINENSIS
(TENTATIVE)

New tentative specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011). No ADI was allocated at the 74th JECFA (2011).

Information required on at least five different batches of commercially available product on the following:

- *Identification of substances other than benzoic acid, coniferyl benzoate, vanillin and benzyl benzoate in the ethanol extract;*
- *Data on microbiological contaminants (E. coli, Salmonella spp);*
- *Data on levels of inorganic contaminants (lead, arsenic, antimony, chromium, mercury and cadmium).*

A method for distinguishing between Benzoe tonkinensis and Sumatra benzoin should also be provided.

SYNONYMS Siam Benzoin gum; Siam Benzoin Laos; Styrax tonkinensis

DEFINITION Benzoe tonkinensis is a natural complex balsamic resin obtained from a native tree in Laos: *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 (15-45%) and coniferyl benzoate (15-60%). Lesser amounts of vanillin (<5%), benzyl benzoate (<2%), 2-hydroxy-1 phenyl ethanone and 1-(4-hydroxy-3-methoxyphenyl)-2 propanone are also present.
These specifications do not cover Sumatra benzoin (resin obtained from *Styrax benzoi*n Dryander and *Styrax paralleloneurum*).

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 chromatogram.
See description under TESTS.

Coniferyl benzoate The sample contains coniferyl benzoate identified by its characteristic peak in the chromatogram.

	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 or ICP-AES 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 contamination	Information required

TESTS

IDENTIFICATION AND PURITY	
Benzoic acid, vanillin and benzyl benzoate	Benzoic acid, vanillin and benzyl benzoate are identified and benzoic acid is quantified by gas chromatography. Reagents Ethanol 96% Reference standard of benzoic acid (purity >99%) Reference standard of vanillin (purity > 95%) Reference standard of benzyl benzoate (purity > 95%) Gas Chromatographic system Detector: Flame ionization detector Column: RESTEK, Rxi-5HT (30 m x 0.25 mm I.D., 0.25 µm-film) or

equivalent
Carrier gas: helium
Flow rate: 1 ml/min
Temperatures:
- Injector: 250°
- Oven: 5 min at 80°; to 320° at 10°/min; then hold for 61 min
- Detector: 250°
Injection mode: split 1/10
Run time: 90 min
Benzoic acid elutes at 9.8 min. Peaks at 13.3 and 17.6 corresponds to 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 in the concentration range of 0.25 to 20 mg/ml.

Vanillin and benzyl benzoate solution: Prepare a 10 mg/ml solution containing 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 solution and record the peak areas. Inject 1 µl of the 10 mg/ml solution containing 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 vanillin and benzyl benzoate, compare the retention times of the corresponding peaks of 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 (15 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).

CARAMEL COLOURS

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 55th JECFA (2000), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI for Class I 'Not specified' was established at the 29th JECFA (1985), for Class II of 0-160 mg/kg bw was established at the 55th JECFA (2000) and an ADI for Class III of 0-200 mg/kg bw (0-150 mg/kg bw on solids basis) was established at the 29th JECFA (1985) and an ADI for Class IV of 0-200 mg/kg bw (0-150 mg/kg bw on solids basis) was established at the 29th JECFA (1985).

SYNONYMS

Caramel colours are divided into four classes. The synonyms for each class are:

Class I: Plain caramel; INS No.150a
Class II: Sulfite caramel; INS No.150b
Class III: Ammonia caramel; INS No.150c
Class IV: Sulfite ammonia caramel; INS No.150d

DEFINITION

Complex mixtures of compounds, some of which are in the form of colloidal aggregates, manufactured by heating carbohydrates either alone or in the presence of food-grade acids, alkalis or salts; classified according to the reactants used in their manufacture as follows:

Class I: Prepared by heating carbohydrates with or without acids or alkalis; no ammonium or sulfite compounds are used.
Class II: Prepared by heating carbohydrates with or without acids or alkalis in the presence of sulfite compounds; no ammonium compounds are used.
Class III: Prepared by heating carbohydrates with or without acids or alkalis in the presence of ammonium compounds; no sulfite compounds are used.
Class IV: Prepared by heating carbohydrates with or without acids or alkalis in the presence of both sulfite and ammonium compounds.

In all cases the carbohydrate raw materials are commercially available food-grade nutritive sweeteners consisting of glucose, fructose and/or polymers thereof. The acids and alkalis are food-grade sulfuric or citric acids and sodium, potassium or calcium hydroxides or mixtures thereof.

Where ammonium compounds are used they are one or any of the following: ammonium hydroxide, ammonium carbonate and ammonium hydrogen carbonate, ammonium phosphate, ammonium sulfate, ammonium sulfite and ammonium hydrogen sulfite.

Where sulfite compounds are used they are one or any of the following: sulfurous acid, potassium, sodium and ammonium sulfites and hydrogen sulfites.

Food-grade anti-foaming agents may be used as processing aids during manufacture.

DESCRIPTION Dark brown to black liquids or solids having an odour of burnt sugar

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Miscible with water

Identification of colouring matters (Vol. 4) — Passes test

Classification — Class I: Not more than 50% of the colour is bound by DEAE Cellulose and not more than 50% of the colour is bound by Phosphoryl Cellulose.
Class II: More than 50% of the colour is bound by DEAE Cellulose and it exhibits an Absorbance Ratio of more than 50.
Class III: Not more than 50% of the colour is bound by DEAE Cellulose and more than 50% of the colour is bound by Phosphoryl Cellulose.
Class IV: More than 50% of the colour is bound by DEAE Cellulose and it exhibits an Absorbance Ratio of not more than 50.
See description under TESTS

PURITY — Note: Arsenic and lead metals limits apply to all classes of caramel and are expressed on the basis of the product as is:
Other limits and ranges apply to the individual classes as indicated and, unless otherwise stated, are expressed on a solids basis.

Solid content — Class I: 62-77%
Class II: 65-72%
Class III: 53-83%
Class IV: 40-75%
See description under TESTS

Colour intensity — Class I: 0.01-0.12
Class II: 0.06-0.10
Class III: 0.08-0.36
Class IV: 0.10-0.60
See description under TESTS

Total nitrogen (Vol. 4) — Class I: max 0.1%
Class II: max 0.2%
Class III: 1.3 -6.8%
Class IV: 0.5-7.5%

Determine as directed under Nitrogen Determination (Kjeldahl Method) using Method II

Total sulfur	Class I: max 0.3% Class II: 1.3 -2.5% Class III: max 0.3% Class IV: 1.4-10.0% See description under TESTS
Sulfur dioxide	Class I: - Class II: max 0.2% Class III: - Class IV: max 0.5% See description under TESTS
Ammoniacal nitrogen	Class I: - Class II: - Class III: max 0.4% Class IV: max 2.8% See description under TESTS
4-Methylimidazole (MEI)	Class I: - Class II: - Class III: max 300 mg/kg & max 200 mg/kg on an equivalent colour basis Class IV: max 1000 mg/kg & max 250 mg/kg on an equivalent colour basis See description under TESTS
2-Acetyl-4-tetrahydroxy-butylimidazole (THI)	Class I: - Class II: - Class III: max 40 mg/kg & max 25 mg/kg on an equivalent colour basis. Class IV: - See description under TESTS
Arsenic (Vol.4)	Not more than 1 mg/kg (Method II)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption 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, “Instrumental Methods”.

TESTS

IDENTIFICATION TESTS

Classification/Colour bound by DEAE Cellulose	For the purposes of this specification, colour bound by DEAE cellulose is defined as the percentage of decrease in absorbance of a caramel colour solution at 560 nm after treatment with DEAE Cellulose.

Special reagent
DEAE (diethylaminoethyl) Cellulose of 0.7 meq/gram capacity, e.g. Cellex D from Bio-Rad or equivalent DEAE Celluloses of higher or lower capacities in proportionately higher or lower quantities.

Procedure
Prepare a caramel colour solution of approximately 0.5 absorbance unit at 560 nm by transferring an appropriate amount of caramel colour into a 100-ml volumetric flask with the aid of 0.025 N hydrochloric acid. Dilute to volume with 0.025 N hydrochloric acid and centrifuge or filter, if solution is cloudy. Take a 20 ml aliquot of the caramel colour solution, add 200 mg of DEAE Cellulose, mix thoroughly for several min, centrifuge or filter, and collect the clear supernatant. Determine the absorbance of the caramel colour solution and the supernatant in a 1-cm cell at 560 nm, with a suitable spectrophotometer previously standardized using 0.025 N hydrochloric acid as reference. Calculate the percentage of Colour Bound by DEAE Cellulose by the formula:

$$\frac{(X_1 - X_2)}{X_1} \times 100$$

where
X_1 is the absorbance of the caramel colour solution at 560 nm; and
X_2 is the absorbance of the supernatant after DEAE Cellulose treatment at 560 nm.

Classification/Colour bound by phosphoryl cellulose

For the purposes of this specification colour bound phosphoryl cellulose is defined as the percentage of decrease in absorbance of a caramel colour solution at 560 nm after treatment with Phosphoryl Cellulose.

Special reagent
Phosphoryl Cellulose of 0.85 meq/gram capacity, e.g. Cellex P from Bio-Rad or equivalent Phosphoryl Celluloses of higher or lower capacities in proportionately higher or lower quantities.

Procedure
Transfer 200-300 mg of caramel colour into a 100-ml volumetric flask, dilute to volume with 0.025 N hydrochloric acid, and centrifuge or filter, if solution is cloudy. Take a 40 ml aliquot of the caramel colour solution, add 2.0 g Phosphoryl Cellulose and mix thoroughly for several min. Centrifuge or filter, and collect the clear supernatant. Determine the absorbance of the caramel colour solution and the supernatant in a 1-cm cell at 560 nm, with a suitable spectrophotometer previously standardized using 0.025 N hydrochloric acid as reference. Calculate the percentage of Colour Bound by Phosphoryl Cellulose by the formula:

$$\frac{(X_1 - X_2)}{X_1} \times 100$$

where
X_1 is the absorbance of the caramel colour solution at 560 nm;

and
X_2 is the absorbance of the supernatant after Phosphoryl Cellulose treatment at 560 nm.

Classification/ Absorbance ratio

For the purposes of this specification, Absorbance Ratio is defined as the absorbance of caramel colour at 280 nm divided by the absorbance of caramel colour at 560 nm.

Procedure

Transfer 100 mg of caramel colour into a 100-ml volumetric flask with the aid of water, dilute to volume, mix and centrifuge if solution is cloudy. Pipet a 5.0 ml portion of the clear solution into a 100-ml volumetric flask, dilute to volume with water, and mix. Determine the absorbance of the 0.1% solution in a 1-cm cell at 560 nm and that of the 1:20 diluted solution at 280 nm with a suitable spectrophotometer previously standardized using water as reference. (A suitable spectrophotometer is one equipped with a monochromator to provide a band width of 2 nm or less and of such quality that the stray-light characteristic is 0.5% or less.) Calculate the Absorbance Ratio of the caramel colour by dividing the absorbance units at 280 nm multiplied by 20 (dilution factor) by the absorbance units at 560 nm.

PURITY TESTS

Solids content

The solids content of Caramel Colour is determined by drying a sample upon a carrier composed of pure quartz sand that passes a No. 40 but not a No. 60 sieve and has been prepared by digestion with hydrochloric acid, washed acid-free, dried and ignited. Mix 30.0 g of prepared sand accurately weighed with 1.5-2.0 g Caramel Colour accurately weighed and dry to constant weight at 60° under reduced pressure 50 mm/Hg (6.7 kPa). Record the final weight of the sand plus caramel. Calculate the % solids as follows:

$$\% \text{ solids} = \frac{(w_F - w_S)}{w_C} \times 100$$

where
w_F is the final weight of sand plus caramel;
w_S is the weight of sand; and
w_C is the weight of caramel initially added.

Calculation on a solids basis

The contents of Total Nitrogen, Total sulfur, Ammoniacal nitrogen, sulfur dioxide, 4-MEI and THI are expressed on a solids basis. The concentration (C_I) of each impurity is determined on an "as is" basis; the concentration (C_S) on a solid basis is then calculated using the formula:

$$C_S = \frac{C_i \times 100}{\% \text{ solids}}$$

Colour Intensity

For the purpose of this specification, Colour Intensity is defined as the absorbance of a 0.1% (w/v) solution of Caramel Colour solids in water in a 1 cm cell at 610 nm.

Procedure

Transfer 100 mg of Caramel Colour into a 100 ml volumetric flask, dilute to volume with water, mix and centrifuge if the solution is cloudy. Determine the absorbance (A_{610}) of the clear solution in a 1 cm cell at 610 nm with a suitable spectrophotometer previously standardized using water as a reference. Calculate the Colour Intensity of the Caramel Colour as follows:

$$\text{Colour intensity} = \frac{A_{610} \times 100}{\%\ \text{solids}}$$

Determine % solids as described under Solids content.
Calculation on an equivalent colour basis: Where additional limits for 4-MEI and THI are expressed on an equivalent colour basis the concentrations are first calculated on a solids basis as directed under "Calculations on a solids basis", and then expressed on an equivalent colour basis according to the formula:

$$\text{Equivalent colour basis} = \frac{C_S}{\text{Colour intensity}} \times 0.1$$

where

C_S is the concentration on a solids basis.

This gives content expressed in terms of a product having a Colour Intensity of 0.1 absorbance units.

Total sulfur

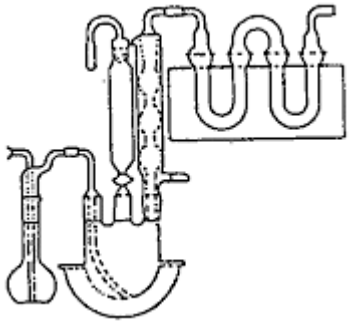
In the largest available casserole that fits in an electric muffle furnace, place 1-3 g MgO or equivalent quantity of $Mg(NO_3)_2 \cdot 6H_2O$ (6.4 - 19.2 g), 1 g powdered sucrose, and 50 ml HNO_3. Add 5-10 g caramel colour. Place same quantities of reagents in another casserole for blank. Evaporate on steam bath to paste. Place casserole in cold electric muffle (25º) and gradually heat until all NO_2 fumes are driven off. Cool, dissolve and neutralize with HCl (1+2.5), adding excess of 5 ml. Filter, heat to boiling, and add 5 ml 10% $BaCl_2 \cdot 2H_2O$ solution dropwise. Evaporate to 100 ml, let stand overnight, filter, wash, ignite, and weigh the $BaSO_4$. Correct result for $BaSO_4$ obtained in blank and report as mg S/100 g. Commercial instruments that analyse for total sulfur such as, the Leco-Combustion/Titration procedure can also be used and are recommended for sample amounts of about 200 mg.

Sulfur dioxide

Apparatus

Use a modified Monier-Williams apparatus (available from 5GA Scientific, Inc., Bloomfield, N.J., USA) for the determination of sulfurous acid, or construct the apparatus as shown in the figure. The assembly consists of a 1000-ml three-neck round-bottom distillation flask having 24/40 standard-taper ground-glass joints. A 30-cm Allihn condenser is attached in the reflux position to an outer neck of the flask, and the other end of the condenser is connected with 1/4-inch. Tygon or silicon tubing (preboiled with 1 in 20 hydrochloric acid

solution and rinsed with water) to the absorption tube assembly (having 35/20 ball joints or the equivalent). Connect the centre neck of the flask with a 125-ml cylindrical separator, and attach a piece of tubing to a short U-tube inserted through a rubber stopper in the neck of the separator. Attach a curved glass inlet tube, reaching nearly to the bottom of the flask, to the other outer neck of the flask, and connect the inlet tube to a 250-ml gas-washing bottle with a piece of the tubing. The gas-washing bottle, in turn, is connected by tubing to a nitrogen cylinder.



Grind 4.5 g of pyrogallol (pyrogallic acid) with 5 ml of water in a small mortar, and transfer the slurry to the gas-washing bottle. Grind the residue again, and transfer it quantitatively to the bottle with two 5-ml portions of water. Pass nitrogen from the cylinder to the bottle to flush out air, and then add to the bottle, through a long-stem funnel, a cooled solution of 65 g of potassium hydroxide in about 85 ml of water. Place the head of the bottle in position, and bubble nitrogen through it to remove air from the headspace. Clamp off the tubing on both sides of the bottle, and connect it to the glass inlet tube of the distillation flask. The gas-washing bottle must be prepared with fresh pyrogallol solution as described on the day of use.

To each U-tube of the absorption tube assembly add the following: two pieces of 8-mm glass rod about 25 mm in length, 10 ml of 3-mm glass beads at the exit side, 10.0 ml of 3% hydrogen peroxide solution, and 1 drop of methyl red TS.

Assemble all pieces of the apparatus, and check for leaks by blowing gently into the tubing attached to the neck of the separator. While blowing, close the stopcock of the separator. Let stand for a few min; if the liquid levels in the U-tubes equalize, reseal all connections and test again. If the system is airtight proceed as directed below.

Procedure

Disperse about 25 g of the sample, accurately weighed, in 300 ml of recently boiled and cooled water, and transfer the slurry to the flask with the aid of water, using a large-bore funnel. Dilute to about 400 ml with water, and reseal the separator. Add 90 ml of 4 N hydrochloric acid to the separator, and force the acid into the flask by blowing gently into the tube in the neck of the separator. Close the stopcock of the separator.

Unclamp the tubing on both sides of the gas-washing bottle, and start the nitrogen flow at a steady stream of bubbles. Heat the distilling flask with a heating mantle to cause refluxing in approximately 20 min. When steady refluxing is reached, apply the line voltage to the mantle and reflux for 1.75 h. Turn off the water in the condenser, and continue heating until the inlet joint of the first U-tube shows

condensation and slight warming. Remove the separator and turn off the heat.

When the joint at the top of the condenser cools, remove the connecting assembly and rinse it into the second U-tube, leaving the crossover tube attached to the exit joint of the first U-tube but disconnected from the entrance of the second U-tube. Rotate the crossover tube until the free end almost touches the entrance of the first U-tube. Add 1 drop of methyl red TS to the first U-tube, and titrate with 0.1 N sodium hydroxide just to a clear yellow colour, mixing with a gentle rocking motion. After titrating the first U-tube, remove the crossover tube, attach it to the second U-tube exit, and titrate similarly. Record the sum of the two titers as S, in ml.

Perform a blank determination, and record the volume of 0.1 N sodium hydroxide required as B. Calculate the percentage of sulfur dioxide in the sample by the formula:

$$SO_2\ \% = \frac{(S - B) \times 0.0032 \times 100}{W}$$

where

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

Ammonium nitrogen

Add 25 ml of 0.1 N sulfuric acid to a 500-ml receiving flask, and connect it to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser such that the condenser delivery tube is immersed beneath the surface of the acid solution in the receiving flask. Transfer about 2 g of caramel colour, accurately weighed, into an 800-ml long-neck Kjeldahl digestion flask, and to the flask add 2 g of magnesium oxide (carbonate-free), 200 ml of water, and several boiling chips. Swirl the digestion flask to mix the contents, and quickly connect it to the distillation apparatus. Heat the digestion flask to boiling, and collect about 100 ml of distillate in the receiving flask. Wash the tip of the delivery tube with a few ml of water, collecting the washings in the receiving flask, then add 4 or 5 drops of methyl red indicator (500 mg of methyl red in 100 ml of alcohol), and titrate with 0.1 N sodium hydroxide, recording the volume, in ml, required as S. Conduct a blank determination, and record the volume, in ml, of 0.1 N sodium hydroxide required to neutralize as B. Calculate the percentage of ammoniacal nitrogen in the sample by the formula:

$$\text{Ammoniacal nitrogen} = \frac{(B - S) \times 0.0014 \times 100}{W}$$

where

W is the weight of caramel colour taken, in g.

4-Methylimidazole

NB: Information on an improved method is sought.
The following materials and reagents are required (the reagents should be ACS grade or equivalent where applicable).

Materials

Pyrex glasswool, 22 x 300 mm chromatography column with PTFE stopcock (e.g. Kimax 17800); 150 ml polypropylene beaker (e.g.

Nalge 1201); 250 ml round-bottom flask (e.g., Pyrex 4320); 75 mm powder funnel; 5 cm spatula; rotary vacuum evaporator; hot plate; pan for water bath; disposable Pasteur pipets; 5 ml volumetric flask.

Reagents
Acetone; Celite 545; methylene chloride; sodium hydroxide; and tetrahydrofuran.

Procedure
After thoroughly mixing the caramel colour sample by shaking or stirring, weigh a 10.00 g aliquot into a 150 ml polypropylene beaker. Polypropylene is considered superior to glass because of its hydrophobic surface which facilitates quantitative sample transfer. A 5.0 g portion of 3.0 N NaOH is added and thoroughly mixed to ensure that the pH of the entire sample exceeds 12. A 20 g portion of Celite 545 is added to the beaker, and the contents are mixed until a semi-dry mixture is obtained. This normally requires approximately 2 to 3 min. With samples of unusually high water content, the resultant caramel colour-Celite 545 mixture may be overly wet. In such cases, a 5.00 g aliquot of caramel colour may be mixed with 2.5 g of 3.0 N NaOH and 15 g of Celite 545 and carried through the remainder of the analysis.

A plug of Pyrex glasswool is placed in the bottom of a 22 x 300 mm chromatographic column with PTFE stopcock. The caramel colour-Celite 545 mixture is placed in the column with the aid of a 75 mm powder funnel. The column contents are settled by repeatedly allowing the column to fall vertically about 10 cm to a padded surface. When properly settled, the caramel colour-Celite 545 mixture should occupy approximately the lower 250 mm of the column. Care should be exercised at this point to avoid a column bed which is either too loosely or too tightly packed. Loose packing will allow too rapid elution of the methylene chloride and possibly incomplete extraction. A too tightly packed column, e.g., the result of tamping down the column contents, can result in regions of the bed which are relatively inaccessible to the extraction solvent. This can also result in incomplete extraction.

With the stopcock open, the column is filled with methylene chloride poured from the sample beaker. When the solvent reaches the glasswool plug, the stopcock is closed and the solvent is allowed to stand in contact with the bed for 5 min. The stopcock is then opened and the column is further eluted with methylene chloride until 200 ml have been collected in a 250 ml round-bottom flask. A 1.00 ml aliquot of 2 MEI internal standard solution (50.0 mg of 2 MEI/50.0 ml of methylene chloride) is added to the collected eluate. The 2 MEI is well separated from the 4 MEI under the GLC conditions employed and has not been found in caramel colour.

The bulk of the solvent is then removed from the eluate on a rotary vacuum evaporator operated at 45-50 kPa and with the round-bottom flask maintained at 35° in a water bath. The extracted residue is transferred quantitatively to a 5 ml volumetric flask with a disposable Pasteur pipet, by rinsing the round-bottom flask several times with small (ca. 0.75 ml) portions of either tetrahydrofuran or acetone. Both solvents have been used with equal success. After mixing the contents thoroughly by several inversions of the flask, the extract is

ready for GLC analysis. The extracts should be analysed as soon as possible after their preparation, because stability problems have occasionally been encountered with extracts more than 1 day old. The GLC analysis is carried out using a gas chromatograph equipped with a hydrogen flame detector. The column is glass, 1 mm x 6 mm o.d. x 4 mm i.d., filled with 7.5% Carbowax 20M + 2% K0H on 90/100 mesh Anakrom ABS. The GLC parameters are as follows: carrier, nitrogen, 50 ml/min; hydrogen, 50 ml/min; oxygen, 80 ml/min; injection port, 200°; column isothermal, 180°; detector, 250°; sample size, 5 µl. All quantitation is done by using the internal standard technique.

2-Acetyl-4-tetrahydroxy-butylimidazole (THI)

NB: Information on an improved method is sought.
THI is converted into its 2,4-dinitrophenylhydrazone (THI-DNPH). This derivative is separated from excess reagent and carbonylic contaminants by HPLC on RP-8, then determined by its absorbance at 385 nm.

Procedure
Caramel colour (200-250 mg) is weighed accurately, then dissolved in water (3 ml). The solution is transferred quantitatively to the upper part of a Combination Column. Elution with water is started, and a total of about 100 ml of water is passed through the columns.

The upper column is then disconnected. The lower column is eluted with 0.5 N HCl. The first 10.0 ml of eluate are discarded, then a volume of 35 ml is collected.

The solution is concentrated to dryness at 40^{o} and 15 torr. The syrup residue is dissolved in carbonyl-free methanol (250 µl) and the 2,4-dinitrophenylhydrazine reagent (250 µl) is added. The reaction mixture is transferred to a septum-capped vial and stored for 5 h at room temperature.
A volume of 5 µl (but also from 1 to 25 µl) is injected onto a LiChrosorb RP-8 (10 µm) HPLC column. The mobile phase is MeOH/0.1 M H_3PO_4 50/50 (v/v). Adjustments in mobile phase composition may be needed as column characteristics vary, depending upon the manufacturer. (Use of LiChrosorb RP-8, 10 µm, 250 x 4 mm "Vertex" column manufactured by Knauer, Bad Homburg, F.R.G. is strongly recommended). At a mobile phase flow rate of 2 ml/min and column dimensions of 250 x 4.6 mm, THI-DNPH is eluted at about 6.3±0.1 min. It is detected at 385 nm and the peak height is measured. The amount is calculated from a calibration curve prepared with THI-DNPH in methanol.

Materials
- 2,4,-Dinitrophenylhydrazine hydrochloride reagent: Commercial 2,4-dinitrophenylhydrazine (5 g) is added to concentrated hydrochloric acid (10 ml) in a 100-ml Erlenmeyer flask, and the latter is gently shaken until the free base (red) is converted to the hydrochloride (yellow). Ethanol (100 ml) is added and the mixture is heated on a steam bath until all the solid has dissolved. After crystallization at room temperature, the hydrochloride is filtered off, washed with ether, dried at room temperature and stored in a desiccator. On storage the hydrochloride is slowly converted to the free base. The latter can be removed by washing with dimethoxyethane. The reagent is prepared

by mixing 0.5 g of 2,4-dinitrophenylhydrazine hydrochloride in 15 ml of 5% methanol in dimethoxyethane for 30 min. It should be stored in the refrigerator and be checked periodically by HPLC.
- Cation-exchange resin (strong): Dowex 50 AG x 8, H^+, 100-200 mesh.
- Cation-exchange resin (weak): Amberlite CG AG 50 I, H^+, (100-200 mesh). (Sediment two or three times prior to use).
- Methanol, carbonyl-free: Methanol is prepared after Y. Peleg and C.H. Mannheim, J. Agr. Fd. Chem, 18 (1970) 176, by treatment with Girard P reagent.
- Dimethoxyethane: If impure, dimethoxyethane is purified by distillation from 2,4-dinitrophenylhydrazine in the presence of acid and redistilled from sodium hydroxide. Immediately prior to use it is passed through a column of neutral aluminium to remove peroxides.

Instrumental

Combination Columns: Similar to the set-up described in J. Agr. Fd. Chem. 22 (1974) 110. The upper column (150 x 12.5 mm, filling height max. 9 cm, or 200 x 10 mm, filling height max. 14 cm, with capillary outlet of 1 mm i.d.) is filled with weakly acidic cation-exchanger; bed height, approx. 50-60, or 80-90 mm, respectively. The lower column (total length 175 mm, i.d. 10 mm, with capillary outlet and Teflon stopcock) is filled with strongly acidic cation-exchanger to a bed-height of 60 mm. As a solvent reservoir, a dropping funnel (100 ml) with Teflon stopcock is used. All parts are connected by standard ground-glass joints (14.5 mm).

HPLC: With the column specified above and an ultraviolet detector capable of reading at 385 nm.

Calibration: THI-DNPH is dissolved in absolute, carbonyl-free methanol (about 100 mg/l; calculated concentration of THI: 47.58 ng/µl). A portion of this solution is diluted tenfold with methanol (4.7 ng THI/µl). THI-DNPH standard solutions are stable for at least twenty weeks when stored in the refrigerator.

ß-apo-8'-CAROTENAL

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011), superseding specifications prepared at the 28th JECFA (1984), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0-5 mg/kg bw expressed as the sum of carotenoids including β-carotene, β-apo-8'-carotenal, and the methyl and ethyl esters of β-apo-8'-carotenoic acid was established at the 18th JECFA (1974).

SYNONYMS CI Food Orange 6; CI (1975) No. 40820; INS No. 160e

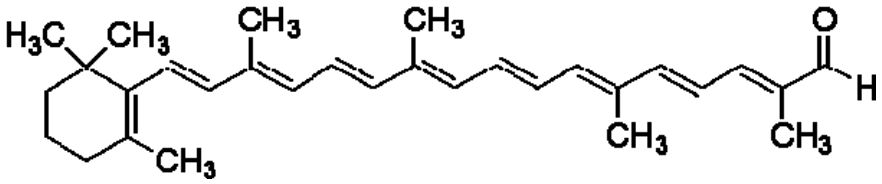
DEFINITION These specifications apply to β-apo-8'-carotenal which consists predominantly of all-trans- β-apo-8'-carotenal and may also contain minor quantities of other carotenoids such as all-trans-crocetindialdehyde, all-trans-β-apo-12'-carotenal and all-trans-β-carotene. Commercial preparations of β-apo-8'-carotenal intended for use in food are prepared from β-apo-8'-carotenal meeting these specifications and are formulated as suspensions in edible oil, emulsions and water dispersible powders. These preparations may also contain cis isomers.

Chemical names ß-Apo-8'-carotenal, 8'-apo-ß-carotene-al
2E,4E,6E,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethyl-1-cyclohexenyl)heptadeca-2,4,6,8,10,12,14,16-octaenal

C.A.S. number *1107-26-2*

Chemical formula $C_{30}H_{40}O$

Structural formula All-*trans*- β-apo-8'-carotenal (main compound)



Formula weight 416.65

Assay Not less than 96% of total colouring matters

DESCRIPTION Deep violet crystals with metallic lustre or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, slightly soluble in ethanol, sparingly soluble in vegetable oils.
Spectrophotometry (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay at 461 nm and 488 nm. The ratio A_{488}/A_{461} is between 0.80 and 0.84.
Test for carotenoid	The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 0.5 M sulfuric acid.

PURITY

Sulfated ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Subsidiary colouring matters	Carotenoids other than ß-apo-8'-carotenal: Not more than 3% of total colouring matters. See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES 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

Subsidiary colouring matters

Carotenoids other than ß-apo-8'-carotenal

Subsidiary colouring matters (carotenoids other than ß-apo-8'-carotenal) are determined by high performance liquid chromatography (HPLC) using the following conditions:

Chromatographic system

- HPLC equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler and integrator
- Detector wavelength: 463 nm
- Column: reverse phase C18, Suplex pkb-100 (250 x 4.6 mm, 5 µm) from Supelco or equivalent
- Mobile phase: In a 1000 ml volumetric flask, dissolve 50 mg BHT in 20 ml 2-propanol and add 0.2 ml N-ethyldiisopropyl-amine, 25 ml 0.2% aqueous ammonium acetate solution, 455 ml acetonitrile, and approx. 450 ml methanol. Mixture cools and contracts. Allow to reach room temperature and dilute to volume with methanol. Discard after 2 days.
- Isocratic elution

- Column temperature: 30°
- Flow rate: 0.6 ml/min
- Injection volume: 10 µl
- Temperature of the autosampler: (approx. 15°)
- Run time: approx. 35 min

Reagents
- Butylated hydroxytoluene (BHT), reagent grade
- 2-Propanol, HPLC grade
- N-ethyldiisopropyl-amine, reagent grade
- Ammonium acetate, reagent grade
- Acetonitrile, HPLC grade
- Methanol, HPLC grade
- Ethanol, HPLC grade
- Tetrahydrofuran, HPLC grade

Sample solution
Weigh accurately (to ±0.1 mg) 0.010 g of the sample and dissolve in tetrahydrofuran (stabilized with 0.025% BHT). Transfer to a 100 ml volumetric flask and bring to volume with tetrahydrofuran. Dilute to the ratio of 1:10 with ethanol.

Procedure
Inject the sample solution using the conditions detailed under *Chromatographic system*. The retention time for all-*trans*-ß-apo-8'-carotenal is in the range of 7-9 min and corresponds to the largest peak in the chromatogram. The relative retention times of minor carotenoids with respect to the retention time of all-*trans*-ß-apo-8'-carotenal are: all-*trans*-crocetindialdehyde (0.54); all-*trans*-β-apo-12'-carotenal (0.84); all-*trans*-β-carotene (2.55). Integrate the areas of the peaks in the chromatogram.

Calculation
Calculate the percentage of carotenoids other than ß-apo-8'-carotenal (%, w/w) using the following formula:

$$\text{Carotenoids other than ß - apo - 8'-carotenal (\%, w/w)} = \left(\frac{A_{total} - A_{\text{β-apo-8'-carotenal}}}{A_{total}} \right) \times 100$$

where

A_{total}, is the sum of the area of all the peaks in the chromatogram, excluding the solvent peak (area units); and

$A_{\text{ß-apo-8'-carotenal}}$ is the area of the peak of ß-apo-8'-carotenal in the chromatogram (area units).

METHOD OF ASSAY (Vol. 4)

Total colouring matters content by spectrophotometry

Proceed as directed under Total Colouring Matters Content – Colouring Matters Contents by Spectrophotometry, Procedure 2, using the following conditions:

Sample weight (W): 0.08 g (±0.01 g)

Volume of the three volumetric flasks: $V_1 = V_2 = V_3 = 100$ ml

Volume of the two pipets: $v_1 = v_2 = 5$ ml

Specific absorbance of the standard: $A^{1\%}_{1\ cm} = 2640$

Wavelength of maximum absorption: λ_{max} about 461nm

Calculation

Calculate the percentage of total colouring matters using the following formula:

$$\text{Total colouring matters } (\%, w/w) = \frac{A \times V_1 \times D}{A^{1\%}_{1cm} \times W}$$

where

A is the absorbance of the twice-diluted sample solution at 461 nm; and

D is the dilution factor $(V_2 x V_3)/(v_1 x v_2)$.

ß-apo-8'-CAROTENOIC ACID ETHYL ESTER

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011), superseding specifications prepared at the 28th JECFA (1984), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0-5 mg/kg bw expressed as the sum of carotenoids including β-carotene, β-apo-8'-carotenal, and the methyl and ethyl esters of β-apo-8'-carotenoic acid was established at the 18th JECFA (1974).

SYNONYMS CI Food Orange 7; CI (1975) No. 40825; INS No. 160f

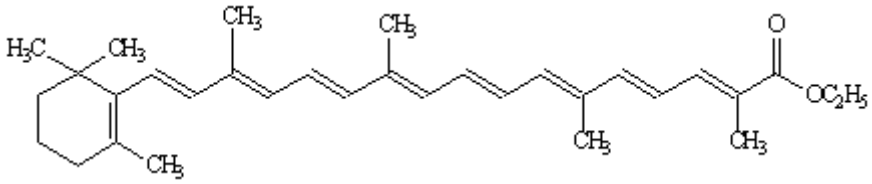
DEFINITION These specifications apply to β-apo-8'-carotenoic acid ethyl ester which consists predominantly of all-*trans*-β-apo-8'-carotenoic acid ethyl ester and may also contain minor quantities of all-*trans*- β-apo-12'-carotenal, methyl-all-*trans*-β-apo-8'-carotenoate, all-*trans*-ethyl 4'-apo-β-carotenate and all-*trans*-β-carotene. Commercial preparations of β-apo-8'-carotenoic acid ethyl ester intended for use in food are prepared from β-apo-8'-carotenoic acid ethyl ester meeting these specifications and are formulated as suspensions in edible oil, emulsions and water dispersible powders. These preparations may also contain cis isomers.

Chemical names All-*trans*-ß-apo-8'-carotenoic acid ethyl ester, ethyl 8'-apo-ß-caroten-8'-oate, ethyl (2E,4E,6E,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohexen-1-yl)heptadeca-2,4,6,8,10,12,14,16-octaenoate

C.A.S. number 1109-11-1

Chemical formula $C_{32}H_{44}O_2$

Structural formula All-*trans*- ß-Apo-8'-carotenoic acid ethyl ester (main compound)



Formula weight 460.70

Assay Not less than 96% of total colouring matters

DESCRIPTION Red to violet-red crystals or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water, very slightly soluble in ethanol, slightly soluble in vegetable oils.

Spectrophotometry (Vol. 4): Determine the absorbance of the diluted sample solution used in the Method of Assay at 449 nm and 475 nm. The ratio A_{475}/A_{449} is between 0.82 and 0.86.

Test for carotenoid: The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 0.5 M sulfuric acid.

PURITY

Sulfated ash (Vol. 4): Not more than 0.1%
Test 2 g of the sample (Method I)

Subsidiary colouring matters: Not more than 3% of total colouring matters
See description under TESTS

Lead (Vol. 4): Not more than 2 mg/kg
Determine using an AAS/ICP-AES 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

Subsidiary colouring matters

Carotenoids other than ß-apo-8'-carotenoic acid ethyl ester

Subsidiary colouring matters (carotenoids other than ß-apo-8'-carotenoic acid ethyl ester) are determined by high performance liquid chromatography (HPLC) using the following conditions:

Chromatographic system

- HPLC system equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler
- Detector wavelength: 446 nm
- Column: reverse phase C18, Suplex pkb-100 (250 x 4.6 mm, 5 µm) from Supelco or equivalent
- Mobile phase: In a 1000 ml volumetric flask, dissolve 50 mg BHT in 20 ml 2-propanol and add 0.2 ml N-ethyldiisopropyl-amine, 25 ml 0.2% aqueous ammonium acetate solution, 455 ml acetonitrile, and approx. 450 ml methanol. Mixture cools and contracts. Allow to reach room temperature and dilute to volume with methanol. Discard after 2 days.

- Isocratic elution
- Column temperature: 30°
- Flow rate: 0.6 ml/min
- Injection volume: 10 µl
- Temperature of the autosampler: (approx. 15°)
- Run time: approx. 35 min

Reagents
- Butylated hydroxytoluene (BHT), reagent grade
- 2-Propanol, HPLC grade
- N-ethyldiisopropyl-amine, reagent grade
- Ammonium acetate, reagent grade
- Acetonitrile, HPLC grade
- Methanol, HPLC grade
- Ethanol, HPLC grade
- Tetrahydrofuran, HPLC grade

Sample solution
Weigh accurately (to ±0.1 mg) 0.010 g of the sample and dissolve in tetrahydrofuran (stabilized with 0.025% BHT). Transfer to a 100 ml volumetric flask and bring to volume with tetrahydrofuran. Dilute to the ratio of 1:10 with ethanol.

Procedure
Inject the sample solution using the conditions detailed under *Chromatographic system*. The retention time for all-*trans*-apo-8'-carotenoic acid ethyl ester is in the range of 9-11 min and corresponds to the largest peak in the chromatogram. The relative retention times of carotenoids with respect to the retention time of all-*trans*-ß-apo-8'-carotenoic acid ethyl ester are: all-*trans*- β-apo-12'-carotenal (0.73); methyl all-*trans*-β-apo-8'-carotenoate (0.97); all-*trans*-ethyl 4'-apo-β-carotenate (1.22), all-*trans*-β-carotene (2.23).
Integrate the areas of the peaks in the chromatogram.

Calculation
Calculate the percentage of carotenoids other than ß-apo-8'-carotenoic acid ethyl ester (%, w/w) using the following formula:

$$\text{Carotenoids other than ß-apo-8'-carotenoic acid ethyl ester (\%, w/w)} = \left(\frac{A_{\text{total}} - A_{\beta\text{-apo ester}}}{A_{\text{total}}} \right) \times 100$$

where
A_{total} is the sum of the area of all the peaks in the chromatogram, excluding the solvent peak (area units); and
$A_{ß\text{-apo-ester}}$ is the area of the peak of ß-apo-8'-carotenoic acid ethyl ester in the chromatogram (area units).

METHOD OF ASSAY (Vol. 4)

Total colouring matters content by spectrophotometry
Proceed as directed under Total Colouring Matters Content – Colouring Matters Contents by Spectrophotometry, Procedure 2, using the following conditions:
Sample weight (W): 0.08 g (±0.01 g)
Volume of the three volumetric flasks: $V_1 = V_2 = V_3 = 100$ ml

Volume of the two pipets: $v_1 = v_2 = 5$ ml
Specific absorbance of the standard: $A^{1\%}_{1\ cm} = 2550$
Wavelength of maximum absorption: λ_{max} about 449 nm

Calculation
Calculate the percentage of total colouring matters using the following formula:

$$\text{Total colouring matters } (\%, w/w) = \frac{A \times V_1 \times D}{A^{1\%}_{1cm} \times W}$$

where
A is the absorbance of the twice-diluted sample solution at 449 nm; and
D is the dilution factor $(V_2 x V_3)/(v_1 x v_2)$.

β-CAROTENE, synthetic

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011), superseding specifications prepared at the 31st JECFA (1987), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0-5 mg/kg bw for beta carotene, synthetic and from Blakeslea trispora, was established at the 57th JECFA (2001).

SYNONYMS CI Food Orange 5; INS No. 160a(i); CI (1975) No. 40800

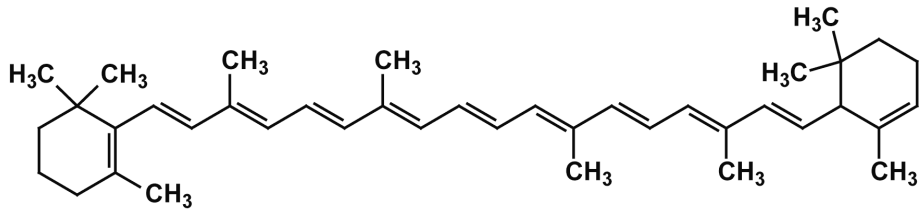
DEFINITION These specifications apply to synthetic β-carotene which consists predominantly of all-*trans*-β-carotene. Synthetic β-carotene may also contain minor amounts of *cis*-isomers and other carotenoids such as all-*trans*-retinal, β-apo-12'-carotenal, and β-apo-10'-carotenal. Commercial preparations of β-carotene intended for use in food are prepared from β-carotene meeting these specifications and are formulated as suspensions in edible oils or water-dispersible powders. These preparations may have different ratio of trans/cis isomers.

Chemical names ß-Carotene, ß,ß-carotene
1,1'-(3,7,12,16-tetramethyl-1,3,5,7,9,11,13,15,17-octadecanonaene-1,18-diyl)bis[2,6,6-trimethylcyclohexene]

C.A.S. number 7235-40-7

Chemical formula $C_{40}H_{56}$

Structural formula All-*trans*-β-carotene (main compound)



Formula weight 536.88

Assay Not less than 96% total colouring matters, expressed as ß-carotene.

DESCRIPTION Red to brownish-red crystals or crystalline powder; sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water; practically insoluble in ethanol; slightly soluble in vegetable oils.
Test for carotenoids	The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 0.5 M sulfuric acid.
Spectrophotometry (Vol. 4)	Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 483 nm. The ratio A_{455}/A_{483} is between 1.14 and 1.19. Determine the absorbance of the diluted sample solution used in the Method of Assay at 455 nm and 340 nm. The ratio A_{455}/A_{340} is not lower than 15.

PURITY

Sulfated Ash (Vol. 4)	Not more than 0.1% Test 2 g of the sample (Method I)
Subsidiary colouring matters	Carotenoids other than ß-carotene: Not more than 3% of total colouring matters. See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES 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

Subsidiary colouring matters

Carotenoids other than β-carotenes

Subsidiary colouring matters (carotenoids other than β-carotenes) are determined by high performance liquid chromatography (HPLC) using the following conditions:

Chromatographic system

- HPLC equipped with a UV/Vis detector or a photodiode array detector, refrigerated auto sampler and integrator
- Detector wavelength: 453 nm
- Column: Reverse phase C18, Suplex pkb-100 (250 x 4.6 mm, 5 µm) from Supelco or equivalent
- Mobile phase: In a 1000 ml volumetric flask, dissolve 50 mg BHT in 20 ml 2-propanol and add 0.2 ml N-ethyldiisopropyl-amine, 25 ml 0.2% aqueous ammonium acetate solution, 455 ml acetonitrile, and approx. 450 ml methanol. Mixture cools and contracts. Allow to reach room temperature and dilute to volume with methanol. Discard after 2 days.
- Isocratic elution
- Column temperature: 30°

- Flow rate: 0.6 ml/min
- Injection volume: 10 µl
- Temperature of the autosampler: (approx. 15°)
- Run time: approx. 35 min

Reagents
- Butylated hydroxytoluene (BHT), reagent grade
- 2-Propanol, HPLC grade
- N-ethyldiisopropyl-amine, reagent grade
- Ammonium acetate, reagent grade
- Acetonitrile, HPLC grade
- Methanol, HPLC grade
- Ethanol, HPLC grade
- Tetrahydrofuran, HPLC grade

Sample solution
Weigh accurately (to ±0.1 mg) 0.010 g of the sample and dissolve in tetrahydrofuran (stabilized with 0.025% BHT). Transfer to a 100 ml volumetric flask and bring to volume with tetrahydrofuran. Dilute to the ratio of 1:10 with ethanol.

Procedure
Inject the sample solution using the conditions detailed under *Chromatographic system*. The retention times for all-*trans*-β-carotene and cis-isomers are in the range of 20-25 min. The largest peak in the chromatogram corresponds to all-*trans*-β-carotene. The relative retention times of minor carotenoids and cis-isomers of β-carotene with respect to the retention time of all-*trans*-β-carotene are: all-*trans*-retinal (0.26), all-*trans*-β-apo-12'-carotenal (0.33), all-*trans*-β-apo-10'carotenal (0.34), all-*trans*-γ-carotene (0.85), all-*trans*-α-carotene (0.95), 9-cis-β-carotene (1.05), 13-cis-β-carotene (1.15) and 15-cis-β-carotene (1.18).
Integrate the areas of the peaks in the chromatogram.

Calculation
Calculate the percentage of carotenoids other than β-carotenes (%, w/w) using the following formula:

$$\text{Carotenoids other than } \beta\text{-carotenes (\%, w/w)} = \left(\frac{A_{\text{total}} - A_{\beta-\text{carotenes}}}{A_{\text{total}}} \right) \times 100$$

where

$A_{\text{total}'}$ is the sum of the area of all the peaks in the chromatogram, excluding the solvent peak (area units); and

$A_{\beta\text{-carotene}}$ is the sum of the areas of the peaks of all β-carotenes (all-*trans*-β-carotene, 9-cis-β-carotene, 13-cis-β-carotene and 15-cis-β-carotene) in the chromatogram (area units).

METHOD OF ASSAY (Vol. 4)

Total colouring matters content by spectrophotometry

Proceed as directed under Total Colouring Matters Content – Colouring Matters Content by Spectrophotometry, Procedure 2, using the following conditions:
Sample weight (W): 0.08 g (±0.01 g);
Volume of the three volumetric flasks: $V_1 = V_2 = V_3 = 100$ ml;

Volume of the two pipets: $v_1 = v_2 = 5$ ml;
Specific absorbance of the standard: $A^{1\%}_{1\ cm} = 2500$;
Wavelength of maximum absorption: λ_{max} about 455 nm.

Calculation
Calculate the percentage of total colouring matters using the following formula:

$$\text{Total colouring matters } (\%, w/w) == \frac{A \times V_1 \times D}{A^{1\%}_{1cm} \times W}$$

where
A is the absorbance of the twice-diluted sample solution at 455 nm; and
D is the dilution factor $(V_2 x V_3)/(v_1 x v_2)$.

GLYCEROL ESTER OF GUM ROSIN
(TENTATIVE)

Tentative specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding tentative specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). A temporary group ADI of 0-12.5 mg/kg bw for glycerol ester of gum rosin and glycerol ester of wood rosin was established at the 74th JECFA (2011).

Updated information required:

- *Composition of the refined gum rosin currently used as the source rosin with regard to the levels of resin acids and neutrals (non-acidic saponifiable and unsaponifiable substances)*
- *Composition of the unsaponified glycerol ester of gum rosin with regard to the levels of:*
  - *a) glycerol esters;*
  - *b) free resin acids; and*
  - *c) neutrals (non-acidic saponifiable and unsaponifiable substances)*
- *Composition of the total glycerol ester of resin acids with regard to the levels of:*
  - *a) monoglycerol esters;*
  - *b) (1,2) diglycerol esters;*
  - *c) (1,3) diglycerol esters;*
  - *d) triglycerol esters; and*
  - *e) dimeric esters*
- *Composition of the neutral fraction, including identification of the major classes of compounds and the concentration of the major constituents within the classes*

NOTE:
Validated methods should be used for the analysis of substances considered in the specification. Detailed information on the methods used, including sample preparation and validation parameters should be provided. It is recommended that representative samples of commercially available glycerol ester of gum rosin be analysed by independent laboratories.

SYNONYMS

INS No. 445(i)

DEFINITION

Glycerol ester of gum rosin is a complex mixture of tri- and diglycerol esters of resin acids from gum rosin, with a residual fraction of monoglycerol esters. Besides these esters, up to x % resin acids (data on percentage required) and up to x % other non-acidic saponifiable and unsaponifiable substances (data on percentage required) are present. It is obtained by the esterification of refined gum rosin under a nitrogen atmosphere with food-grade glycerol, and purified by countercurrent steam distillation.

Refined gum rosin is obtained by extracting oleoresin gum from living pine trees (*Pinus oocarpa* Schiede*) and* refining it through washing, filtration and distillation. It is composed of x% resin acids (data on percentage required) and x% neutrals (non-acidic saponifiable and unsaponifiable substances) (data on percentage required). The resin-acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, of which the main component is abietic acid.

These specifications do not cover substances derived from wood rosin, obtained by the solvent extraction of aged pine stumps, and substances derived from tall oil rosin, a by-product of kraft (paper) pulp processing.

Assay — Sum of tri- and diglycerol esters: information required

DESCRIPTION

Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES

Emulsifier, density adjustment agent (flavouring oils in beverages)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Insoluble in water; soluble in acetone

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

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

PURITY

Monoglycerol esters: — Information required

Neutrals (non-acidic saponifiable and unsaponifiable substances) — Information required

Specific gravity (Vol. 4) — d^{20}_{25}: Not less than 0.935 (50% solution in d-limonene)

Ring and ball softening point (Vol. 4)	Not less than 82° (see "Specific Methods, Glycerol Esters of Rosins")
Acid value (Vol. 4)	Between 3 and 9 (see "Specific Methods, Fats, Oils, and Hydrocarbons")
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES 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

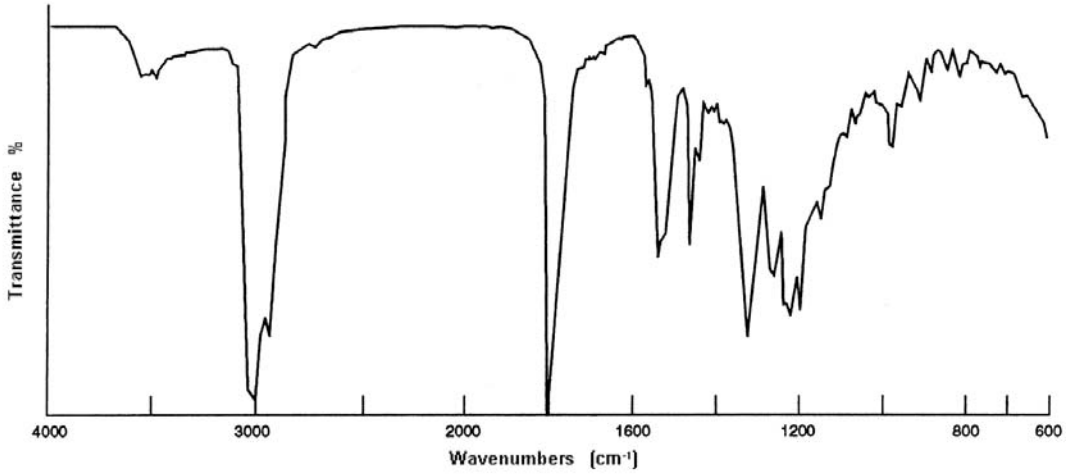
PURITY TESTS

Monoglycerol esters	Information required
Neutrals (non-acidic saponifiable and unsaponifiable substances)	Information required

METHOD OF ASSAY

Tri- and diglycerol esters	Information required

Infrared spectrum



NOTE: The IR spectrum for glycerol ester of gum rosin is referenced from the Food Chemicals Codex, 7th Edition, 2010, p. 443. Reprinted with permission from the US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

GLYCEROL ESTER OF TALL OIL ROSIN
(TENTATIVE)

Tentative specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding tentative specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). No ADI was allocated at the 74th JECFA (2011).

Updated information required:

- *Composition of the refined tall oil rosin currently used as the source rosin with regard to the levels of resin acids and "neutrals" (non-acidic saponifiable and unsaponifiable substances)*
- *Composition of the glycerol ester of tall oil rosin with regard to the levels of:*
  - *a) glycerol esters;*
  - *b) free resin acids; and*
  - *c) neutrals (non-acidic saponifiable and unsaponifiable substances)*
- *Composition of the total glycerol ester of resin acids with regard to the levels of:*
  - *a) monoglycerol esters;*
  - *b) (1,2) diglycerol esters;*
  - *c) (1,3) diglycerol esters;*
  - *d) triglycerol esters; and*
  - *e) dimeric esters*
- *Composition of the neutrals, including identification of the major classes of compounds and the concentration of the major constituents within the classes*

NOTE:
Validated methods should be used for the analysis of substances considered in the specification. Detailed information on the methods used, including sample preparation and validation parameters should be provided. It is recommended that representative samples of commercially available glycerol ester of gum rosin be analysed by independent laboratories.

SYNONYMS INS No. 445(ii)

DEFINITION Glycerol ester of tall oil rosin is a complex mixture of tri- and diglycerol esters of resin acids from tall oil rosin with a residual fraction of monoglycerol esters. Besides these esters, up to x % resin acids (data on percentage required) and up to x % non-acidic saponifiable and unsaponifiable substances (data on percentage required) are present. It is obtained by the esterification of tall oil rosin under a nitrogen atmosphere with food-grade glycerol, and purified by steam-stripping.

Tall oil rosin is obtained by distillation of crude tall oil, a by-product of kraft (paper) pulp processing. It is composed of x% resin acids (data on percentage required) and x% neutrals (non-acidic saponifiable and unsaponifiable substances) (data on percentage required). The resin-acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, of which the main component is abietic acid.

These specifications do not cover substances derived from wood rosin, obtained by the solvent extraction of aged pine stumps, and substances derived from gum rosin, an exudate of living pine trees.

Assay — Sum of tri- and diglycerol esters: information required

DESCRIPTION — Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES — Emulsifier, density adjustment agent (flavouring oils in beverages)

CHARACTERISTICS

IDENTIFICATION

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

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

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

PURITY

Monoglycerol esters — Information required

Neutrals (non-acidic saponifiable and unsaponifiable substances) — Information required

Specific gravity (Vol. 4) — d^{20}_{25}: Not less than 0.935 (50% solution in d-limonene)

Ring and ball softening point (Vol. 4) — Not less than 80° (see "Specific Methods, Glycerol Esters of Rosins")

Acid value (Vol. 4) — Between 3 and 9 (see "Specific Methods, Fats, Oils, and Hydrocarbons")

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an AAS/ICP-AES 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

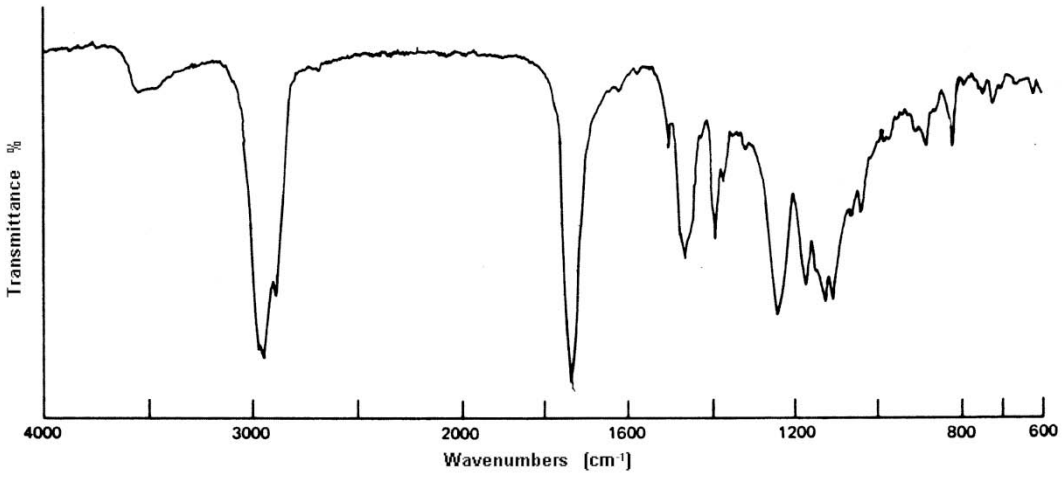
Monoglycerol esters: — Information required

Neutrals (non-acidic saponifiable and unsaponifiable substances) — Information required

METHOD OF ASSAY

Tri- and diglycerol esters — Information required

Infrared spectrum



NOTE: The IR spectrum for glycerol ester of tall oil rosin is referenced from the Food Chemicals Codex, 7th Edition, 2010, p. 448. Reprinted with permission from the US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

GLYCEROL ESTER OF WOOD ROSIN
(TENTATIVE)

Tentative specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding tentative specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). A temporary group ADI of 0-12.5 mg/kg bw for glycerol ester of gum rosin and glycerol ester of wood rosin was established at the 74th JECFA (2011).

Updated information required:

- *Composition of the refined wood rosin currently used as the source rosin with regard to the levels of resin acids and "neutrals" (non-acidic saponifiable and unsaponifiable substances)*
- *Composition of the glycerol ester of wood rosin with regard to the levels of:*
  - *a) glycerol esters;*
  - *b) free resin acids; and*
  - *c) neutrals (non-acidic saponifiable and unsaponifiable substances)*
- *Composition of the total glycerol ester of resin acids with regard to the levels of:*
  - *a) monoglycerol esters;*
  - *b) (1,2) diglycerol esters;*
  - *c) (1,3) diglycerol esters;*
  - *d) triglycerol esters; and*
  - *e) dimeric esters*
- *Composition of the neutrals, including identification of the major classes of compounds and the concentration of the major constituents within the classes*

NOTE:
Validated methods should be used for the analysis of substances considered in the specification. Detailed information on the methods used, including sample preparation and validation parameters should be provided. It is recommended that representative samples of commercially available glycerol ester of gum rosin be analysed by independent laboratories.

SYNONYMS INS No. 445(iii)

DEFINITION Glycerol ester of wood rosin is a complex mixture of tri- and diglycerol esters of resin acids from wood rosin, with a residual fraction of monoglycerol esters. Besides these esters, up to x % resin acids (data on percentage required) and up to x % non-acidic saponifiable and unsaponifiable substances (data on percentage required) are present. It is obtained by the solvent extraction of aged pine stumps (*Pinus palustris* (longleaf) and *Pinus elliottii* (slash) species) followed by a liquid-liquid solvent refining process.

The refined wood rosin composed of x% resin acids (data on percentage required) and x% neutrals (non-acidic saponifiable and unsaponifiable substances) (data on percentage required). The resin-acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, of which the main component is abietic acid. The substance is purified by steam stripping or by countercurrent steam distillation.

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

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

Assay Sum of tri- and diglycerol esters: information required

DESCRIPTION Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES Emulsifier, density adjustment agent (flavouring oils in beverages), stabilizer, chewing gum base component

CHARACTERISTICS

IDENTIFICATION

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

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

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

PURITY

Monoglycerol esters Information required

Neutrals (non-acidic saponifiable and unsaponifiable substances) Information required

Specific gravity (Vol. 4) d^{20}_{25}: Not less than 0.935 (50% solution in d-limonene)

Ring and ball softening point (Vol. 4) Not less than 82° (see “Specific Methods, Glycerol Esters of Rosins”)

Acid value (Vol. 4) Between 3 and 9 (see “Specific Methods, Fats, Oils, and Hydrocarbons”)

Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES 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

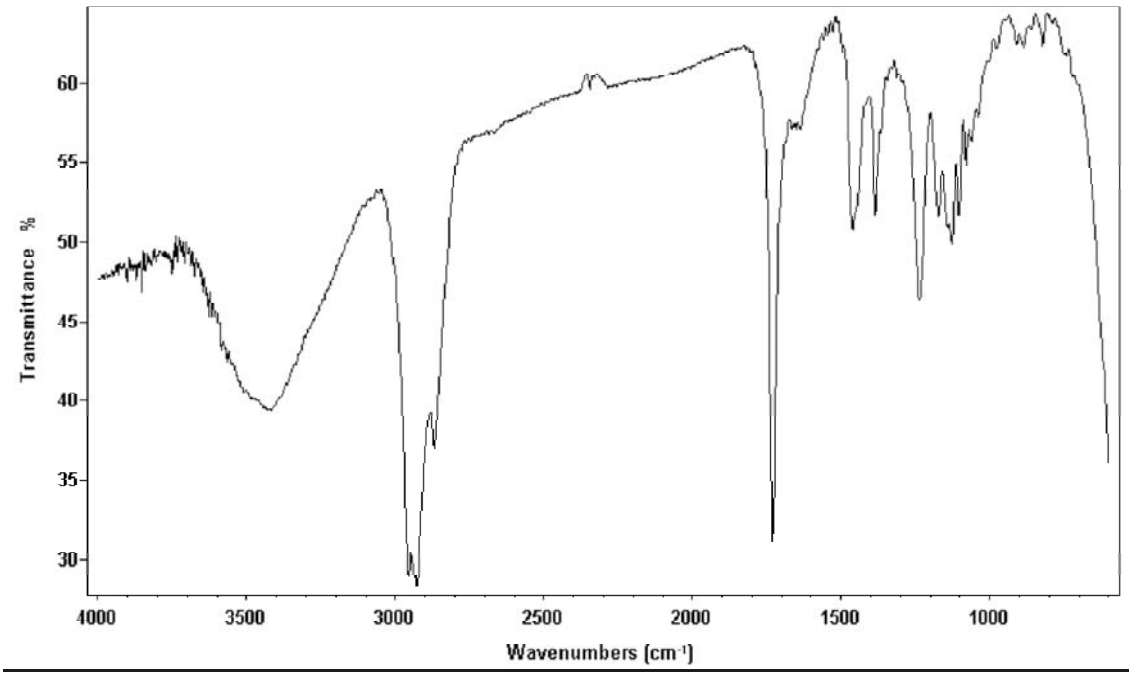
PURITY TESTS

Monoglycerol esters:	Information required
Neutrals (non-acidic saponifiable and unsaponifiable substances)	Information required

METHOD OF ASSAY

Tri- and diglycerol esters	Information required

Infrared spectrum



NOTE: The IR spectrum for glycerol ester of wood rosin is referenced from the Food Chemicals Codex, 7th Edition, 2010, p. 449. Reprinted with permission from the US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

HYDROXYPROPYLMETHYL CELLULOSE

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding the specifications prepared at the 63rd JECFA (2004), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI "not specified" for modified celluloses (ethyl cellulose, ethyl hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, methyl cellulose, methyl ethyl cellulose, and sodium carboxymethyl cellulose) was established at the 35th JECFA (1989).

SYNONYMS INS No. 464

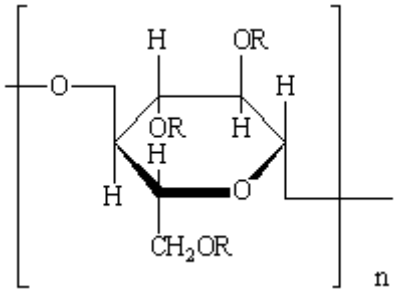
DEFINITION Hydroxypropylmethyl cellulose is a methyl cellulose modified by treatment with alkali and propylene oxide by which a small number of 2-hydroxypropyl groups are attached through ether links to the anhydroglucose units of the cellulose. The article in commerce may be further specified by viscosity.

Chemical names: Hydroxypropylmethyl cellulose, 2-hydroxypropyl ether of methyl cellulose

C.A.S. number: 9004-65-3

Chemical formula: $[C_6H_7O_2(OH)_x(OCH_3)_y(OCH_2CHOHCH_3)_z]_n$
where
z = 0.07 - 0.34
y = 1.12 - 2.03
x = 3-(z + y): (z + y = degree of substitution)

Structural formula



where R = H or CH_3 or $CH_2CHOHCH_3$

Formula weight: Unsubstituted structural unit: 162.14
Structural unit with 1.19 degree of substitution: approx. 180
Structural unit with 2.37 degree of substitution: approx. 210
Macromolecules: from about 13,000 (n about 70) up to about 200,000 (n about 1000)

Assay: Not less than 19% and not more than 30% of methoxy groups (-OCH_3) and not less than 3% and not more than 12% hydroxypropoxy groups (-$OCH_2CHOHCH_3$), on the dried basis

DESCRIPTION Hygroscopic white or off-white powder, or granules or fine fibres

FUNCTIONAL USES Emulsifier, thickening agent, stabiliser

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Swells in water, producing a clear to opalescent, viscous colloidal solution; insoluble in ethanol
Foam formation	A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.
Precipitate formation	To 5 ml of a 0.5% solution of the sample, add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers.
Substituents	See description under METHOD OF ASSAY

PURITY

Loss on drying (Vol. 4)	Not more than 10.0% (105° to constant weight)
pH (Vol. 4)	Not less than 5.0 and not more than 8.0 (1 in 100 solution)
Sulfated ash (Vol. 4)	Not more than 1.5% for products with viscosities of 50 centipoise or above, and not more than 3% for products with viscosities below 50 centipoise Test 1 g of the sample
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES 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”).
Propylene chlorohydrins	Not more than 1 mg/kg See description under TESTS

TESTS

PURITY TESTS

Propylene chlorohydrins	Determine by Gas Chromatography–Mass Spectrometry (GC-MS) (Vol. 4) using the following procedure. **Note:** Propylene chlorohydrins (PCH) are present as 2 isomers namely: 1-chloro-2-propanol (1C2P) and 2-chloro-1-propanol (2C1P). Internal standard solutions *Internal Standard Stock Solution #1 (1 mg/ml):* Weigh 0.1 g to nearest 0.1 mg (approximately 100 µl) of o-xylene-d_{10} (CAS 56004-61-6) into a 100 ml volumetric flask and make up to volume with methanol.

Internal Standard Stock Solution #2 (100 µg/ml): Pipette 5ml of Internal Standard Stock Solution #1 into a 50 ml volumetric flask and make up to volume with methanol.

Internal Standard Stock Solution #3 (4 µg/ml): Pipette 1 ml of Internal Standard Stock Solution #2 into a 25 ml volumetric flask and make up to volume with methanol:

Internal Standard Solution #4 (16 ng/ml): Add 1 ml of Internal Standard Stock Solution #3 into a 250 ml volumetric flask and dilute to volume with diethyl ether.

Internal Standard Solution #5 (8 ng/ml): Pipette 25 ml of Internal Standard Stock Solution #4 into a 50 ml volumetric flask and dilute to volume with diethyl ether.

Standards

Stock Standard Solution #1 (1mg/ml): Weigh 0.1 g to the nearest 0.1 mg of propylene chlorohydrin, mixture of 1-Chloro-2-propanol-75% and 2-Chloro-1-propanol-25%, Eastman Kodak, Cat. # P1325 or equivalent) into a 100 ml volumetric flask and make up to volume with diethyl ether.

Stock Standard Solution #2 (100 µg/ml): Pipette 5 ml of Standard Stock Solution #1 into a 50 ml volumetric flask and make up to volume with diethyl ether.

Stock Standard Solution #3 (10 µg/ml): Pipette 5 ml of Standard Stock Solution #2 into a 50 ml volumetric flask and make up to volume with diethyl ether.

Stock Standard Solution #4 (500 ng/ml): Pipette 5 ml of Standard Stock Solution #3 into a 100 ml volumetric flask and make up to volume with diethyl ether.

Note: All standard solutions should be prepared with diethyl ether of the highest purity available.

Prepare working standard solutions by pipetting the volumes shown in the table below in to a 10 ml volumetric flask and make up to volume with diethyl ether.

Vol. of Stock Standard Solution #4, ml	Vol. of Internal standard solution #4, ml	Vol. made up, ml	Conc. of Standard (ng/ml)	Conc. of Internal Standard (ng/ml)
0.50	5.0	10.0	25	8
1.0	5.0	10.0	50	8
2.0	5.0	10.0	100	8
4.0	5.0	10.0	200	8
5.0	5.0	10.0	300	8

Instrument:

A gas chromatograph with a mass selective detector (GCMS) in Selective Ion Monitoring (SIM) mode, Electron impact ionisation (EI)

source, pulsed-splitless injector and a data station.

GCMS Conditions:

	Inlet temperature	225°
	Pulse pressure	50 psi until 2 min
	Inlet purge flow	40 ml/min at 2 min
	Injection volume	5 µl
Guard Column	Deactivated fused silica, 10 m x 0.25 mm i.d. x 0.35 mm o.d.	
Column	30 m x 0.25 mm i.d. x 1.4 µm film DB-624 or equivalent	
Temperature programming:	Initial temperature	40°
	Initial hold Time	5.0 min
	Ramp rate	10°/min
	Temperature 2	80°
	Hold time	3.0 min
	Ramp rate	25°/min
	Final temperature	230°
	Final hold time	5.0 min
Carrier	Gas	Helium
	Flow rate	1.4 ml/min
	Column he d pressure	11.5 psi
Detector	Ion source temperature.	230°
	Transfer line temperature	260°
SIM ions:	o-Xylene-d_{10}	Target ion m/z = 116
		Qualifier ion m/z = 98
	1-Chloro-2-Propanol	Target ion m/z = 79
		Qualifier ion m/z = 81
	2-Chloro-1-Propanol	Target ion m/z= 58
		Qualifier ion m/z= 31
Retention times	o-Xylene-d_{10}	13.7 min
	1-Chloro-2-Propanol	9.5 min
	2-Chloro-1-Propanol	10.4 min

Procedure:

Weigh about 1.00 g, to nearest 0.1 mg, of sample into a glass vial. Pipette 5.0 ml of Internal Standard Solution #5 into the vial, securely close the vial and sonicate for 10 minutes. Centrifuge the vial to separate the mixture. Remove a portion of the diethyl ether layer for GCMS analysis.

Calculations:

Calculate the ratios of detector responses for 1C2P and 2C1P versus detector response for o-xylene-d_{10} at each working standard concentration using the following equation:

$$AR_{(std)} = R_{(std)}/R_{(IS)}$$

where

$AR_{(std)}$ is the ratio of detector response for 1C2P or 2C1P versus the detector response for o-xylene-d_{10} in the standard;

$R_{(std)}$ is the detector response of the target ion for 1C2P or 2C1P in the standard; and

$R_{(IS)}$ is the detector response of the target ion for o-xylene-d_{10} in the standard.

Prepare standard curves for 1C2P and 2C1P by plotting the concentration of 1C2P or 2C1P (ng/ml) versus the ratios of detector response ($AR_{(std)}$) for each isomer in the working standards

Calculate the ratio of detector response for 1C2P and 2C1P versus the detector response for o-xylene-d_{10} in the sample using the following equation:

$$AR_{(sample)} = R_{(Sample)}/R_{(IS)}$$

where

$AR_{(sample)}$ is the ratio of detector response for 1C2P or 2C1P versus the detector response for o-xylene-d_{10} in the sample;

$R_{(sample)}$ is the detector response of the target ion for 1C2P or 2C1P in the sample; and

$R_{(IS)}$ is the detector response of the target ion for o-xylene-d_{10} in the sample.

From the linear regression of the standard curves for each isomer, calculate ng/g using the following equation:

$$ng/g = (V \times (AR_{(sample)} - b)/m)/W$$

where

$AR_{(sample)}$ is the Ratio of detector response for 1C2P or 2C1P versus the detector response for o-xylene-d_{10} in the sample;

b is the y-intercept of the linear regression curve;

m is the slope of the linear regression curve;

V is the final volume (5.0 ml); and

W is the weight of the sample in grams.

Report the PCH content in mg/kg as the sum of the 2 isomers (1C2P and 2C1P).

METHOD OF ASSAY **Determination of the content of hydroxypropoxy groups**

Apparatus

The apparatus for hydroxypropoxy group determination is shown in the accompanying diagram. The boiling flask, D, is fitted with an aluminium foil-covered Vigreux column, E, on the sidearm and with a bleeder tube through the neck and to the bottom of the flask for the introduction of steam and nitrogen. A steam generator, B, is attached to the bleeder tube through Tube C, and a condenser, F, is attached to the Vigreux column. The boiling flask and steam generator are immersed in an oil bath, A, equipped with a thermo-regulator such that a temperature of 155° and the desired heating rate may be maintained. The distillate is collected in a 150 ml beaker, G, or other suitable container.

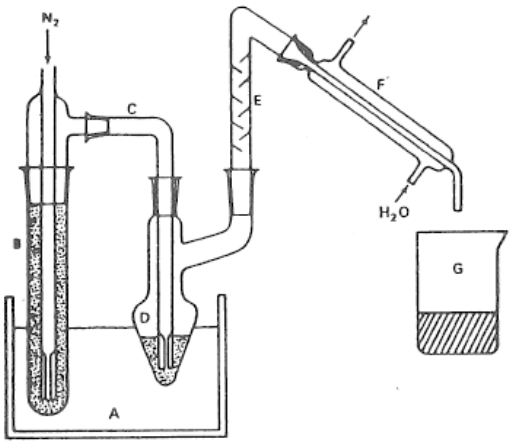


Figure. Apparatus for hydroxypropyl determination

Procedure

Accurately weigh about 100 mg of the sample, previously dried at 105° for 2 h, transfer into the boiling flask and add 10 ml of chromium trioxide solution (60 g in 140 ml of water). Immerse the steam generator and the boiling flask in the oil bath (at room temperature) to the level of the top of the chromium trioxide solution. Start cooling water through the condenser and pass nitrogen gas through the boiling flask at the rate of one bubble per sec. Starting at room temperature, raise the temperature of the oil bath to 155° over a period of not less than 30 min, and maintain this temperature until the end of the determination. Distil until 50 ml of the distillate is collected. Detach the condenser from the Vigreux column, and wash it with water, collecting the washings in the distillate container. Titrate the combined washings and distillate with 0.02 N sodium hydroxide to a pH of 7.0, using a pH meter set at the expanded scale.

Note: Phenolphthalein TS may be used for this titration instead of pH meter, if it is also used for all standards and blanks.

Record the volume, V_a of the 0.02 N sodium hydroxide used. Add

500 mg of sodium bicarbonate and 10 ml of dilute sulfuric acid TS, and then after evolution of carbon dioxide has ceased, add 1 g of potassium iodide. Stopper the flask, shake the mixture, and allow it to stand in the dark for 5 min. Titrate the liberated iodine with 0.02 N sodium thiosulfate to the sharp disappearance of the yellow colour, confirming the end-point by the addition of a few drops of starch TS. Record the volume of 0.02 N sodium thiosulfate required as Y_a.

Make several reagent blank determinations, using only the chromium trioxide solution in the above procedure. The ratio of the sodium hydroxide titration (V_b) to the sodium thiosulfate titration (Y_b), corrected for variation in normalities, will give the acidity-to-oxidizing ratio, $V_b/Y_b = K$, for the chromium trioxide carried over in the distillation. The factor K should be constant for all determinations.

Make a series of blank determinations using 100 mg of methyl cellulose (containing no foreign material) in place of the sample, recording the average volume of 0.02 N sodium hydroxide required as V_m and the average volume of 0.02 N sodium thiosulfate required as Y_m.

Calculate the content of hydroxypropoxy groups (in mg) in the sample using the formula:

$$75.0 \times [N_1 (V_a - V_m) - k\, N_2 (Y_a - Y_m)]$$

where

N_1 is the exact normality of the 0.02 N sodium hydroxide solution;
N_2 is the exact normality of the 0.02 N sodium thiosulfate solution; and
k is $V_b N_1 / Y_b N_2$

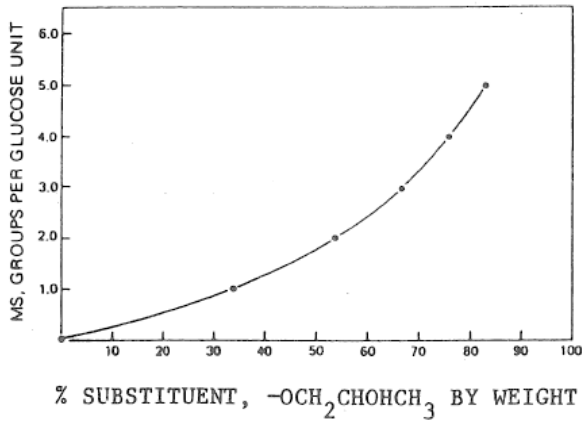


Chart for converting percentage of substitution, by weight, of hydroxypropoxy groups to molecular substitution per glucose unit.

Determination of the content of methoxy groups

Volume 4, under ASSAY METHODS, Cellulose Derivatives Assay, *Ethoxyl and Methoxyl Group Determination*.

See Apparatus and Procedure in *Ethoxy and Methoxy Group Determination* and determine the content of methoxy groups ($-OCH_3$).

Calculation

Calculate as percentage. Correct the % of methoxy groups thus determined by the formula:

$$A - (B \times 0.93 \times 31 / 75)$$

where

A is the total % of $-OCH_3$ groups determined;

B is the % of $-OCH_2CHOHCH_3$ determined in the Method of Assay for hydroxypropoxy group content; and

0.93 is an average obtained by determining, on a large number of samples, the propylene produced from the reaction of hydriodic acid with hydroxypropoxy groups during the Method of Assay for methoxy groups ($-OCH_3$).

MAGNESIUM SILICATE, synthetic

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011) superseding specifications prepared at the 61st JECFA (2003), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established at the 25th JECFA (1981).

SYNONYMS INS No. 553(i)

DEFINITION Magnesium silicate (synthetic) is manufactured by the precipitation reaction between sodium silicate and a soluble magnesium salt. The aqueous suspension of the precipitate is filtered and the collected solid washed, dried, classified for particle size and packaged. The finest material is intended for use as an anticaking agent and the coarser particles are for use as a filtering aid. The moisture content of the material meant for use as an anticaking agent is kept to less than 15%. Although magnesium silicate is of variable composition, the molar ratio of MgO to SiO_2 is approximately 2:5.

Chemical name: Magnesium silicate

C.A.S. number: 1343-88-0

Assay: Not less than 15% of MgO and not less than 67% of SiO_2, calculated on the ignited basis.

DESCRIPTION Very fine, white, odourless powder, free from grittiness

FUNCTIONAL USES Anticaking agent, filtering aid

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water

pH (Vol. 4): 7.0-11.0 (1 in 10 slurry)

Magnesium (Vol. 4): Mix about 0.5 g of the sample with 10 ml of dilute hydrochloric acid TS, filter, and neutralize the filtrate to litmus paper with ammonia TS. The neutralized filtrate gives a positive test for magnesium.

Silicate: Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with the sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a web-like structure.

PURITY

Loss on drying (Vol. 4): Not more than 15% (for material used as an anticaking agent (105°, 2 h)).
Retain the dried sample for determination of loss on ignition.

Loss on ignition (Vol. 4) — Not more than 15% on the dried basis
Weigh to the nearest 0.1 mg, 1 g of the dried sample in a tared platinum crucible provided with a cover. Gradually apply heat to the crucible at first, then strongly ignite at 900/1000° for 20 min. Cool, weigh and calculate as percentage.

Free alkali — Not more than 1% (as NaOH)
Add 2 drops of phenolphthalein TS to 20 ml of dilute filtrate prepared in the test for Soluble salts (see below), representing 1 g of the sample. Not more than 2.5 ml of 0.1 N hydrochloric acid should be required to discharge the pink colour produced.

Soluble salts — Not more than 3%
Boil 10 g of the sample with 150 ml of water for 15 min. Cool to room temperature, and add water to restore the original volume. Allow the mixture to stand for 15 min, and filter until clear. Retain 20 ml of the filtrate for the test Free alkali. Evaporate 75 ml of the filtrate, representing 5 g of the sample in a tared platinum dish on a steam bath to dryness, and ignite gently to constant weight. Cool, weigh and calculate as percentage (the weight of the residue should not exceed 150 mg).

Fluoride (Vol. 4) — Not more than 10 mg/kg
Weigh 2.5 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method I or III).

Lead (Vol. 4) — Not more than 5 mg/kg
Determine using an AAS/ICP-AES 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

Magnesium oxide and silicon dioxide: 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 hot deionized water and remove it. Add 50 ml hydrochloric acid and transfer the contents into a 250-ml volumetric flask. Wash the beaker three times with hot deionized 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. Analyze magnesium and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer and use the analytical lines for Si (251.611 nm) and Mg (279.553 nm). Read the concentration (as µg/ml) of Mg and Si from respective standard curves. Calculate the magnesium oxide and silicon dioxide content of the sample, on the ignited basis, using the formula:

$$\%MgO \text{ (on the ignited basis)} = \frac{4.1458 \times C \times DF}{W \times [100 - (\%LOD + \%LOI)]}$$

$$\%SiO_2 \text{ (on the ignited basis)} = \frac{5.3504 \times C \times DF}{W \times [100 - (\%LOD + \%LOI)]}$$

where

C is concentration of Mg or Si in the test solution, µg/ml;
DF is dilution factor (dilution of Solution A to get test solution);
W is weight of sample, g;
%LOD is % loss on drying; and
%LOI is % loss on ignition.

MODIFIED STARCHES

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). 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

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/ICP-AES 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).

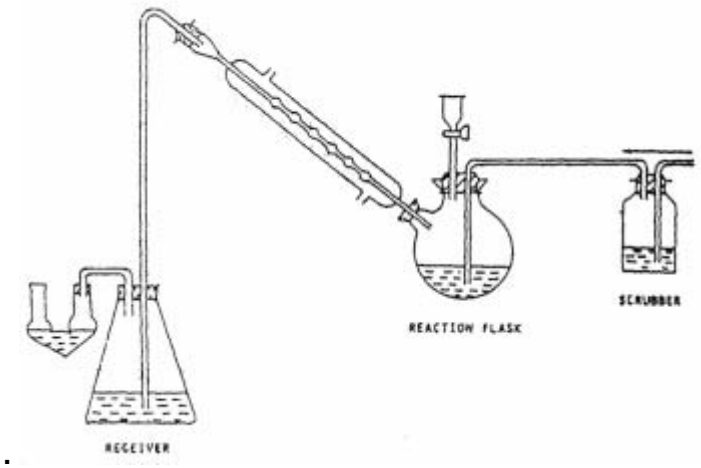


Figure 1

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

Modification	Process limitations	End-product specifications
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
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 gelatinization 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

Modification	Process limitations	End-product specifications
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	Esterification with propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg
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%

Modification	Process limitations	End-product specifications
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^{o} as some starches may gelatinize). Remove the stopper, wash the stopper and sides of the flask with a few ml of water, and titrate the excess alkali with 0.2 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.

Degree of substitution of starch sodium octenyl succinate

The degree of esterification is determined by the amount of alkali consumed after acidification and thorough washing of the sample.

Procedure

Weigh 5.0 g (to nearest 0.1 mg), of the sample in a 150-ml beaker and wet it with a few ml of isopropanol. Pipette 25.0 ml of 2.5 N hydrochloric acid in isopropanol and 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 for another 10 min. Filter the sample 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). Transfer the filtrate to a 600-ml beaker, rinse the Buchner flask and bring to a 300-ml volume with distilled water. Place the beaker on the top of a boiling water bath for 10 min, while stirring. While hot, titrate with 0.1 N sodium hydroxide using phenolphthalein TS as an indicator.

Calculation

$$\text{Degree of substitution} = \frac{0.162 \times A}{1 - 0.210 \times A}$$

where

A is milliequivalents of sodium hydroxide required per 1g of starch octenyl succinate.

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.

NITROUS OXIDE

Prepared at the 74th JECFA (2011) and published in FAO Monographs 11(2011), superseding the tentative specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). An ADI "Acceptable" was established at the 29th JECFA (1985).

SYNONYMS Dinitrogen oxide; Dinitrogen monoxide; INS No. 942

DEFINITION Nitrous oxide, a colourless and non-flammable gas, commonly known as laughing gas, is manufactured by the controlled heating of ammonium nitrate, at temperatures 170-240°, either using superheated steam or other thermal decomposition processes. The hot, corrosive mixture of gases are cooled to condense the steam and filtered to remove higher oxides of nitrogen. The gas is further purified in a train of three gas washes with base, acid and base again. Nitric oxide impurity, if present, is chelated out with ferrous sulfate, or reduced with iron metal, or oxidised and absorbed in a base as a higher oxide.

Chemical names	Nitrous oxide
C.A.S. number	10024-97-2
Chemical formula	N_2O
Formula weight	44.01
Assay	Not less than 99 % (v/v)

DESCRIPTION Colourless, odourless gas

FUNCTIONAL USES Propellant, antioxidant, packaging gas, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	1 volume dissolves in 1.4 volumes of water (20° 760 mm Hg). Freely soluble in alcohol; soluble in ether and in oils.
Infrared absorption	The infrared absorption band of the sample corresponds with the typical infrared absorption band of nitrous oxide
Carbon dioxide test	Passes test See description under TESTS

PURITY

Carbon monoxide	Not more than 10 µl/l See description under TESTS
Nitric oxide	Not more than 1 µl/l See description under TESTS
Nitrogen dioxide	Not more than 1 µl/l See description under TESTS
Halogens (as Cl)	Not more than 5 µl/l See description under TESTS
Ammonia	Not more than 25 µl/l See description under TESTS

TESTS

NOTE 1: The carbon dioxide identification test and all of the purity tests are referenced from the Food Chemicals Codex, 7th Edition, 2011, p. 719-720.
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NOTE 2: The identification and purity tests given below are designed to reflect the quality of nitrous oxide in both its vapour and its liquid phases, which are present in previously unopened cylinders. Reduce the sample cylinder pressure with a regulator. Withdraw the samples for the tests with the least possible release of sample gas consistent with proper purging of the sample apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination of or change to the samples. Perform the tests in the sequence in which they are listed below.

NOTE 3: Detector tubes referenced under identification and purity tests are available from National Draeger Inc., P.O. Box 120, Pittsburgh, PA 15205-0120, USA.

IDENTIFICATION TESTS

Carbon dioxide test	Pass 100 ml of sample gas released from the vapour phase of the contents of the sample gas cylinder through a carbon dioxide detector tube (Draeger CH 30801 or equivalent) at the rate specified for the tube. No colour change occurs.

PURITY TESTS

Carbon monoxide	Pass 1000 ml of sample gas released from the vapour pressure of the contents of the sample gas cylinder, through a carbon monoxide detector tube (Draeger CH 25601 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 10 µl of carbon monoxide.

Nitric oxide

Pass 500 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through a nitric oxide-nitrogen dioxide detector tube (Draeger CH 29401 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 0.5 µl of nitrogen monoxide.

Nitrogen dioxide

Arrange a sample gas cylinder so that when its valve is opened, a portion of the liquid phase of the contents is released through a piece of tubing of sufficient length to allow all of the liquid to vaporize during passage through it and to prevent frost from reaching the inlet of the detector tube. Release a flow of liquid into the tubing sufficient provide 500 ml of the vaporized sample plus any excess necessary to ensure adequate flushing of air from the system.

Pass 500 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through a nitric oxide-nitrogen dioxide detector tube (Draeger CH 29401 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 0.5 µl of nitrogen dioxide.

Halogens (as Cl)

Pass 1000 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through a chlorine detector tube (Draeger CH 24301 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 5 µl.

Ammonia

Pass 1000 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through an ammonia detector tube (Draeger CH 20501 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 25 µl.

METHOD OF ASSAY

Determine using an Infrared gas analyzer or conventional IR/FTIR fitted with a suitable gas cell to analyze nitrous oxide in the range of 85% to 100% v/v.

Reference gas standards: (a) nitrous oxide standard (99.9%) and (b) a mixture containing 5%v/v nitrogen and 95%v/v nitrous oxide.

Method using Infrared gas analyzer:
Infrared gas analyzers consist of a light source emitting broad band radiation, an optical device, a gas sample cell and a detector. Set up the instrument and select the filter for nitrous oxide. Calibrate the instrument using reference gas standards (a) and (b). Flush the sample cell using the gas to be examined and read the nitrous oxide concentration from the analyzer.

Method using conventional IR/FTIR:
Set up the instrument, following manufacturer's instructions, and set the wave number at the highest absorption band (2218 cm^{-1}). Construct standard curve, using a set of standard gases containing 5% v/v of nitrogen in 95% nitrous oxide to pure nitrous oxide (>99.9% nitrous oxide). Flush the sample cell using the gas to be examined and read the nitrous oxide concentration from the standard curve.

OCTENYL SUCCINIC ACID MODIFIED GUM ARABIC

Prepared at the 74th JECFA (2011) and published in the FAO Monographs 11 (2011), superseding specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). A temporary ADI "not specified" was established at the 71st JECFA (2009).

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

Degree of esterification Not more than 0.6%
See description under TESTS

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

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

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

Water-insoluble matter (Vol. 4) Not more than 1.0%

Starch or dextrin Boil a 1 in 50 aqueous solution of the sample, add about 0.1 ml iodine

TS. No bluish or reddish colour should be produced.

Tannin-bearing gums — To 10 ml of a 1 in 50 aqueous solution of the sample add about 0.1 ml ferric chloride TS. No blackish coloration or blackish precipitate should be formed.

Residual octenyl succinic acid — Not more than 0.3%
See description under TESTS

Microbiological criteria (Vol. 4) — *Salmonella* species: absent in 25 g
Escherichia coli: absent in 1 g

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an AAS/ICP-AES 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

Degree of esterification — The degree of esterification is determined by the amount of alkali consumed after acidification and thorough washing of the sample.

Procedure
Weigh 5.0 g (to nearest 0.1 mg), of the sample in a 150-ml beaker and wet it with a few ml of isopropanol. Pipette 25.0 ml of 2.5 N hydrochloric acid in isopropanol and stir the mixture for 30 min with a magnetic stirrer. Using a graduated measuring cylinder, add 100 ml of 90% isopropanol in water and stir for another 10 min. Filter the sample 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). Transfer the filtrate to a 600-ml beaker, rinse the Buchner flask and bring to a 300-ml volume with distilled water. Place the beaker on top of a boiling water bath for 10 min, while stirring. While hot, titrate with 0.1 N sodium hydroxide using phenolphthalein TS as an indicator.

Calculation

$$\text{Degree of esterification} = \frac{0.162 \times A}{1 - 0.210 \times A}$$

where
A is milliequivalents of sodium hydroxide required per 1g of the sample.

Residual octenyl succinic acid — Determine by HPLC on the 2-bromoacetophenone-derivatised methanolic extract of the sample.

Extraction and Preparation of Sample Solution
Accurately weigh 500 mg (to nearest 0.1 mg) of the sample in a 25 ml Erlenmeyer flask, add 15 ml of methanol, stopper the flask and shake it on a shaker overnight. Filter the extract using a filter paper, wash the residue, three times with 7 ml portions of methanol and combine the filtrate (about 80% of the OSA residues is extracted by this

procedure). Add 1 ml of 0.16 N KOH in methanol to the combined filtrate. Dry the extract using a flash evaporator at 30° and dissolve the residue in 2 ml of methanol. Pipette 0.5 ml of this solution into a reaction vial, add 0.5 ml of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml CH_3CN]. Add 2 ml CH_3CN to the reaction vial, cap the vial and heat at 80° for 30 min. Allow the vial to reach room temperature and analyse the reaction product by HPLC within 24 h.

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

Preparation of Standard Curve
Prepare a 105.14 mg/ml solution of 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 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.

POLYDIMETHYLSILOXANE

Prepared at the 69th JECFA (2008), published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 37th JECFA (1990), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-1.5 mg/kg bw was established at the 74th JECFA (2011).

SYNONYMS Poly(dimethylsiloxane); dimethylpolysiloxane; dimethylsilicone fluid; dimethylsilicone oil; dimethicone; INS No. 900a

DEFINITION Polydimethylsiloxane consists of fully methylated linear siloxane polymers containing repeating units of the formula $[(CH_3)_2SiO]$ with trimethylsiloxy end-blocking units of the formula $(CH_3)_3SiO$-. The additive is produced by hydrolysis of a mixture of dimethyldichlorosilane and a small quantity of trimethylchlorosilane. The average molecular weights of the linear polymers range from approximately 6,800 to 30,000.

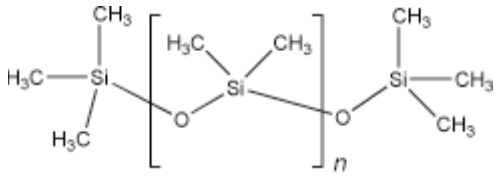
(NOTE: In commerce, polydimethylsiloxane is frequently used in preparations usually containing silica gel. The pure substance described in this monograph can be isolated from silica gel-containing liquids by centrifuging at about 20,000 rpm. Before testing the Polydimethylsiloxane for *Identification, Refractive index*, *Specific gravity*, and *Viscosity*, any silica gel present must be removed by centrifugation.)

(NOTE: This monograph does not apply to aqueous formulations of Polydimethylsiloxane containing emulsifying agents and preservatives, in addition to silica gel.)

Chemical names α-(Trimethylsilyl)-ω-methylpoly(oxy(dimethylsilylene))

C.A.S. number 9006-65-9

Structural formula



n ranges from 90 to 410

Assay Silicon content not less than 37.3% and not more than 38.5% of the total

DESCRIPTION Clear, colourless, viscous liquid.

FUNCTIONAL USES Antifoaming agent, anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water and in ethanol; soluble in most aliphatic and aromatic hydrocarbon solvents
Specific gravity (Vol. 4)	d^{25}_{25} : 0.964 - 0.977
Refractive index (Vol. 4)	n^{25}_{D} : 1.400 - 1.405
Infrared absorption	The infrared absorption spectrum of a liquid film of the sample between two sodium chloride plates exhibits relative maxima at the same wavelengths as those of a similar preparation of USP Dimethylpolysiloxane Reference Standard (available through http://www.usp.org/referenceStandards/catalog.html or by mail to USP 12601 Twinbrook Pkwy, Rockville, MD 20852 USA).

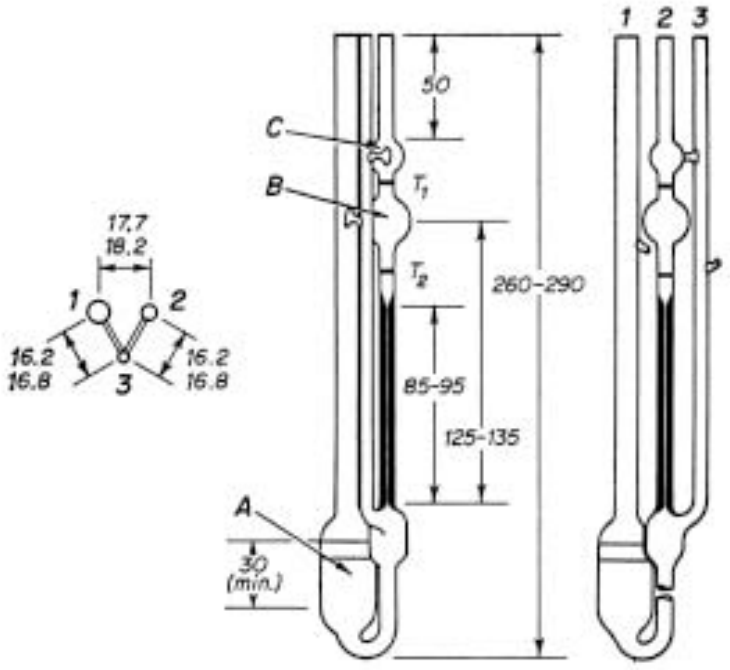
PURITY

Loss on drying (Vol.4)	Not more than 0.5% (150°, 4h)
Viscosity	100 - 1500 cSt at 25 ° See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES 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

Viscosity	The Ubbelohde suspended level viscometer, shown in the accompanying diagram, is preferred for the determination of the viscosity.



(Dimensions in mm)

For use in the range of 100 to 1,500 centistokes, a No. 3 size viscometer, having a capillary diameter of 2.00 + 0.04 mm, is required. The viscometer should be fitted with holders that satisfy the dimensional positions of the separate tubes as shown in the diagram, and that hold the viscometer vertical. Filling lines in bulb *A* indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb *B* is approximately 5 ml.

Calibration of the viscometer

Determine the viscosity constant, *k*, for each viscometer by using an oil of known viscosity. [NOTE: Choose an oil with a viscosity as close as possible to that of the sample to be tested.] Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb *A* below the capillary, and then introduce enough of the sample into tube *1* to bring the level up to the lower filling line. The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube *1.* Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air.

After the viscometer has been in a constant-temperature bath long enough for the sample to reach temperature equilibrium, place a finger over tube *3* and apply suction to tube *2* until the liquid reaches the centre of bulb *C*. Remove suction from tube *2*, then remove the finger from tube *3* and place it over tube *2* until the sample drops away from the lower end of the capillary. Remove the finger from tube *2,* and measure the time, to the nearest 0.1 sec required for the meniscus to pass from the first time mark (T_1) to the second (T_2). In order to obtain accurate results within a reasonable time, the apparatus should be adjusted to give an elapsed time of from 80 to 100 sec.

Calculate the viscometer constant k by the equation

$$k = v/t_1$$

where
v is the viscosity, in centistokes; and
t_1 is the efflux time, in sec, for the standard liquid.

Viscosity determination of Polydimethylsiloxane
Charge the viscometer with the sample in the same manner as described for the calibration procedure; determine the efflux time, t_2; and calculate the viscosity of the sample, v_s, by the equation

$$v_s = kt_2$$

METHOD OF ASSAY

Principle
Silicon in the sample is converted to a soluble form by fusion with sodium peroxide. Soluble silicon is measured in the percent range as total silicon by atomic absorption spectrophotometry.

Apparatus
- Fusion apparatus: Parr-type fusion cup; 500-ml nickel beaker; and nickel lid for beaker - or equivalent (avoid use of glassware during fusion and solubilization).
- Instrument: atomic absorption spectrophotometer with silicon hollow cathode lamp; nitrous oxide - acetylene burner, or equivalent.

Reagents
- Sodium peroxide, glacial acetic acid, silica (of known purity for use as standard).

Procedure
[CAUTION: Normal safe laboratory practices for Parr-type bomb fusion should be followed.]

Equivalent fusions must be performed on sample(s), reagent blank(s) and silica standards for each series of samples. For each sample weigh a portion (W) not to exceed 0.3 g into a Parr-type fusion cup (use gelatine capsules for liquid samples). Add 15.0±0.5 g of sodium peroxide.

Assemble the fusion apparatus and place it in a protective ignition rack. Fill the cavity above the cap with water and keep it full during ignition to prevent the gasket from melting. Heat the bottom of the cup with a blast lamp until the cup becomes cherry red about 100 mm up from the bottom within 90 sec. Quench the apparatus in ice water and disassemble the apparatus. Place the cup in the nickel beaker containing 150 to 200 ml of distilled water. Rinse any material adhering to the inside of the assembly cap into the beaker with distilled water. Cover the beaker with the nickel lid. When dissolution is complete and the solution has cooled, remove the cup from the beaker and rinse it with distilled water into the beaker. Add 55.0 ml of reagent grade glacial acetic acid to the beaker. Cool the solution to room temperature and transfer it to a 500 ml volumetric flask. Dilute to volume with distilled water. The solution should contain about 100 µg

silicon/ml for a sample weight of about 0.13 g. This method performs best if the silicon concentration of the final analysis solution is 1 to 200 µg/ml. Prepare a series of standards using the same fusion technique that brackets the sample.

Measure the absorbance of sample(s), reagent blank and standards at 251.6 nm with the spectrophotometer according to the manufacturer's operating instructions to obtain optimum analysis conditions for maximum lamp output and fuel and oxidant flow rate to the burner (or equivalent procedures for other vaporizing techniques). Adjust the zero absorbance while aspirating the solvent blank (water) used to dilute the samples. Measure the absorbance of sample(s), reagent blank and standards. Estimate the concentration of silicon in the sample solution from the standards, correcting for the reagent blank. Calculate the percent total silicon in the sample by the equation

$$\%\text{Silicon} = 0.05 \times C/W$$

where

C is the silicon concentration of the sample solution, µg/ml; and
W is the weight of sample taken, g.

PONCEAU 4R

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 28th JECFA (1984), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-4 mg/kg bw was established at the 27th JECFA (1983) and maintained at the 74thJECFA (2011).

SYNONYMS CI Food Red 7; Cochineal Red A; New Coccine; Brilliant Scarlet; CI (1975) No. 16255; INS No. 124

DEFINITION Ponceau 4R consists essentially of trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate, and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

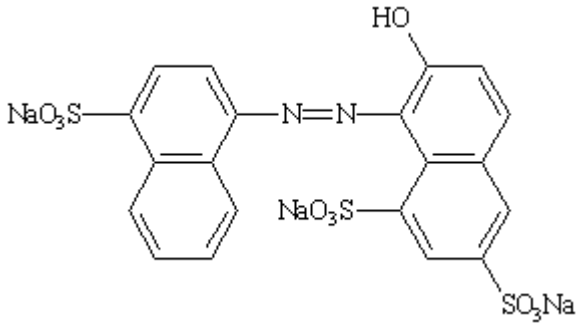
May be converted to the corresponding aluminium lake, in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.

Chemical names Trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate

C.A.S. number 2611-82-7

Chemical formula $C_{20}H_{11}N_2Na_3O_{10}S_3$

Structural formula



Formula weight 604.48

Assay Not less than 80% total colouring matters

DESCRIPTION Reddish powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol
Spectrophotometry	Maximum wave length: Between 505 and 510 nm Determine the UV-visible absorption spectrum of the sample solution dissolved in 0.02 mol/l ammonium acetate.

PURITY

Loss on drying (Vol. 4)	Not more than 20% at 135° together with chloride and sulfate calculated as sodium salts Determine using Loss on Drying under “GENERAL METHODS”, Chloride as Sodium Chloride and Sulfate as Sodium Sulfate under “SPECIFIC METHODS, Food Colours” in Volume 4.
Water-insoluble matter (Vol. 4)	Not more than 0.2%
Subsidiary colouring matters	Not more than 1% See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5% of sum of 4-amino-1-naphthalenesulfonic acid, 7-hydroxy-1,3-naphthalenedisulfonic acid, 3-hydroxy-2,7-naphthalenesulfonic acid, 6-hydroxy-2-naphthalenesulfonic acid, and 7-hydroxy-1,3,6-naphthalenetrisulfonic acid. (See Volume 4 under “SPECIFIC METHODS, Food Colours”) Proceed as directed under *Determination by High Performance Liquid Chromatography* using the conditions of Subsidiary colouring matters except detector wavelength (238 nm).
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline (See Volume 4 under “SPECIFIC METHODS, Food Colours”)
Ether-extractable matter (Vol. 4)	Not more than 0.2% (See Volume 4 under “SPECIFIC METHODS, Food Colours, Method II”) Use 2 g of sample for the test.
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES 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

Subsidiary colouring matters Determine by HPLC using the following conditions:

Chromatography conditions
- HPLC system with a UV/VIS detector or a diode array detector
- Detector wavelength: 510 nm
- Column: C18 on silica gel (250 x 4.6 mm, 5 µm)
- Mobile phase: solvent A: 0.02 mol/l ammonium acetate and solvent B: acetonitrile:water (7:3 v/v)
- Gradient elution: A:B 100:0 v/v to A:B 40:60 v/v (0-30 min); hold at A:B 40:60 v/v (30-35 min).
- Column temperature: 40°
- Flow rate: 1.0 ml/min

Procedure
Accurately weigh 10 mg of the sample into a 100-ml volumetric flask. Dissolve and make to volume with 0.02 mol/l ammonium acetate. Filter through a 0.45 µm membrane filter. Inject 20 µl of the sample solution into HPLC.

Calculation
Calculate the percentage of subsidiary colouring matters from;

$$\text{Subsidiary colouring matters } (\%) = \left(\frac{A_{total} - A_{main}}{A_{total}} \right) \times D \times 100$$

where
D is the total colouring matters content of sample (%);
A_{total} is the sum of the area of all the peaks in the chromatogram between 2 and 40 min; and
A_{main} is the area of main peak.

METHOD OF ASSAY Proceed as directed under *Colouring Matters Content by Titration with Titanous Chloride* in Volume 4 (under "Specific Methods, Food Colours), using the following:

Weight of sample: 0.7-0.8 g
Buffer: 10 g sodium citrate
Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N $TiCl_3$: 0.01511 g

POTASSIUM ALUMINIUM SILICATE
(TENTATIVE)

New tentative specifications prepared at the 74th JECFA (2011), published in FAO Monographs 11 (2011). A PTWI of 2 mg/kg bw for aluminium was established at the 74th JECFA (2011).

Information required:

- *On the preparation and purification methods of potassium aluminium silicate to be used in the manufacture of pearlescent pigments.*
- *Methods for the identification of silicate and aluminium in potassium aluminium silicate*
- *Data from five batches of potassium aluminium silicate on particle size distribution and the levels of the contaminants (arsenic, mercury, lead, antimony, cadmium, zinc, barium, chromium, copper and nickel).*
- *Method for the determination of lead, antimony, cadmium, zinc, barium, chromium, copper and nickel in potassium aluminium silicate using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Indicate if method involves digestion or only extraction of the impurities from the sample.*
- *Suitability of proposed method for the determination of mercury using digestion under a closed system followed by a cold vapour generation technique and analysis with atomic absorption spectrometry.*
- *Suitability of proposed Assay method which incorporates alkali fusion coupled with ICP-AES for the determination of levels of aluminium for use in the assay of potassium aluminium silicate.*

SYNONYMS Mica; Muscovite

DEFINITION Potassium aluminium silicate is mined from natural sources and then further purified. It is used as a carrier substrate for pearlescent pigments made with titanium dioxide and/or iron oxide.

Chemical names Potassium aluminium silicate

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

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

Formula weight 398.31

Assay Not less than 98%

DESCRIPTION Colourless needles or white free flowing powder, having a slight characteristic odour and showing no change in colour after heating for 90 minutes at 105°.

FUNCTIONAL USES Carrier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water, dilute acids and alkali
Aluminium	Information required
Silicate	Information required

PURITY

Loss on drying (Vol. 4)	Not more than 0.5% (105°, 2 h)
Arsenic (Vol. 4)	Information required Mix 1 g of substance in a conical flask with 5 g of potassium hydrogen sulfate and add 5 ml of sulfuric acid (98%). Heat carefully until white fumes are formed and allow to cool to room temperature. Add 10 ml of water and 4.5 ml of hydrochloric acid (25%). Filter through filter paper and wash residue with hot water. Dilute filtrate with water to 50 ml in a volumetric flask. Determine arsenic using atomic absorption hydride technique.
Mercury (Vol. 4)	Information required Digest 0.5 g of sample under closed conditions by heating under reflux with sulfuric and nitric acids and make up to a known volume with deionized water. Alternatively, a closed vessel microwave digestion system may be used for the digestion of samples. Determine the mercury content by cold vapour atomic absorption technique.
Lead (Vol. 4)	Information required
Antimony (Vol. 4)	Information required
Cadmium (Vol. 4)	Information required

Zinc (Vol. 4)	Information required
Barium (Vol. 4)	Information required
Chromium (Vol. 4)	Information required
Copper (Vol. 4)	Information required
Nickel (Vol. 4)	Information required

TESTS

METHOD OF ASSAY Information required as to suitability of Assay method proposed below.

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.

$$\% \text{ (w/w) Potassium aluminium silicate} = \frac{0.123 \times C_{Al} \times DF}{W}$$

where

C is the concentration of Al in the test solution, µg/ml;
DF is the Dilution Factor (dilution of Solution A to get test solution); and
W is the weight of sample, g.

POTASSIUM ALUMINIUM SILICATE-BASED PEARLESCENT PIGMENTS
(TENTATIVE)

New tentative specifications prepared at the 74th JECFA (2011) and published in FAO Monographs 11 (2011). A PTWI of 2 mg/kg bw for aluminium was established at the 74th JECFA (2011). An ADI 'not limited' was established for titanium dioxide at the 13th JECFA (1969) and an ADI of 0-0.5 mg/kg bw was established for iron oxides at the 53rd JECFA (1999).

Information required:

- *On the manufacture of all types of potassium aluminium silicate-based pearlescent pigments currently used in food.*
- *On the stability of potassium aluminium silicate-based pearlescent pigments in food.*
- *Suitable description for inclusion in the specifications monograph for the three versions of potassium aluminium silicate-based pearlescent pigments used in food: 1) potassium aluminium silicate with titanium dioxide; 2) potassium aluminium silicate with iron oxide; 3) potassium aluminium silicate with titanium dioxide and iron oxide).*
- *On the complete particle size distribution for all available types of potassium aluminium silicate-based pearlescent pigments used in food.*
- *On the pH of a 10% aqueous slurry for each type of potassium aluminium silicate-based pearlescent pigment available for use in food.*
- *Method for the identification of iron, titanium and aluminium in potassium aluminium silicate-based pearlescent pigments.*
- *Data from the analysis of five batches of each type of potassium aluminium silicate-based pearlescent pigment available for use in food for the following contaminants: arsenic, mercury, lead, antimony, cadmium, zinc, barium, chromium, copper and nickel.*
- *Method for the determination of lead, antimony, cadmium, zinc, barium, chromium, copper and nickel in potassium aluminium silicate-based pearlescent pigments using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Indicate if the method involves digestion or only extraction of the impurities from the sample.*
- *Suitability of proposed method for the determination of mercury using digestion under a closed system followed by a cold vapour generation technique and analysis with atomic absorption spectrometry.*
- *Suitability of proposed Assay method which incorporates alkali fusion coupled to ICP-AES for the determination of the percentage of titanium, iron and aluminium.*

- *If x-ray fluorescence is preferred for the Assay for titanium dioxide and iron oxide, provide a detailed description of the method, validation data, and data from the analysis of 5 batches for each type of pigment.*
- *Specific filtration methods for pigments with particle sizes below 60 micrometers.*

SYNONYMS Mica-based pearlescent pigments

DEFINITION Potassium aluminium silicate-based pearlescent pigments are produced by reaction of potassium aluminium silicate with soluble salts of titanium and/or iron followed by calcination at high temperatures. They can be produced with a variety of different pearlescent colour effects depending upon particle size and the combination of titanium dioxide and/or iron oxide deposited on the mica.

The mica-based pearlescent pigments consist of one of the following types:

Type I: Potassium aluminium silicate with titanium dioxide
Type II: Potassium aluminium silicate with iron oxide
Type III: Potassium aluminium silicate with titanium dioxide and iron oxide

Chemical names
Potassium aluminium silicate (mica)
Titanium dioxide
Iron oxide

Assay

Type I	Information required
Type II	Information required
Type III	Information required

DESCRIPTION

Type I	Information required
Type II	Information required
Type III	Information required

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, dilute acids and alkali

pH

Type I	Information required
Type II	Information required
Type III	Information required

Titanium Information required

Iron Information required

Aluminium — Information required

Identification test requirements for each type of pigment

	Titanium	Iron	Aluminium
Type I	Positive	Negative	Positive
Type II	Negative	Positive	Positive
Type III	Positive	Positive	Positive

PURITY

Loss on drying (Vol. 4) — Not more than 0.5% (10 g sample, 105°, 2 h)

Particle size distribution — Information required

Measurement of the particle size distribution by laser diffraction
Instrument: Malvern Mastersizer 2000 with HydroG 2000
Dispersant: Water
Dispersant aid: Extran Ma 02 (Merck)
Detection range: from 0.02 μm to 2000 μm
Test solution: Add 1 drop of Extran to the pigment suspension.

Arsenic (Vol. 4) — Information required

Mix 1 g of substance in a conical flask with 5 g of potassium hydrogen sulphate and add 5 ml of sulphuric acid (98%). Heat carefully until white fumes are formed and allow to cool to room temperature. Add 10 ml of water and 4.5 ml of hydrochloric acid (25%). Filter through filter paper and wash residue with hot water. Dilute filtrate with water to 50 ml in a volumetric flask.
Determine arsenic using atomic absorption hydride technique.

Mercury (Vol. 4) — Information required

Digest 0.5 g of sample under closed conditions by heating under reflux with sulfuric and nitric acids and make up to a known volume with deionized water. Alternatively, a closed vessel microwave digestion system may be used for the digestion of samples. Determine the mercury content by cold vapour atomic absorption technique.

Lead (Vol. 4) — Information required

Antimony (Vol.4) — Information required

Cadmium (Vol. 4) — Information required

Zinc (Vol. 4) — Information required

Barium (Vol. 4) — Information required

Chromium (Vol. 4) — Information required

Copper (Vol. 4) — Information required

Nickel (Vol. 4) Information required

TESTS

METHOD OF ASSAY Information required as to the suitability of the method proposed, below.

Determination of percent titanium, iron and aluminium: 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, titanium and iron in the test solution using ICP-AES (Vol. 4). Set instrument parameters as specified by the instrument manufacturer and use the analytical lines for titanium (334.941 nm), iron (259.940 nm) and aluminium (396.152 nm). Determine the concentration (as µg/ml) of titanium, iron and aluminium from the respective standard curves. Calculate the percentage of titanium, iron and aluminium using the formulas below:

$$\%\ \text{Titanium (w/w) in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{0.025 \times C_{Ti} \times DF}{W}$$

$$\%\ \text{Iron (w/w) in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{0.025 \times C_{Fe} \times DF}{W}$$

$$\%\ \text{Aluminium (w/w) in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{0.025 \times C_{Al} \times DF}{W}$$

Where

C is the concentration of Ti, Fe or Al in the test solution, µg/ml;
DF is the Dilution Factor (dilution of Solution A to get test solution); and
W is the weight of the sample, g.

PULLULAN

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 65th JECFA (2005), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established at the 65th JECFA (2005) and maintained at the 74th JECFA (2011).

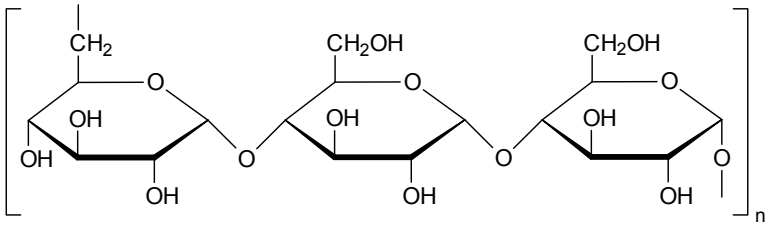
SYNONYMS INS No. 1204

DEFINITION Linear, neutral glucan consisting mainly of maltotriose units connected by α-1,6 glycosidic bonds. It is produced by fermentation from a food grade hydrolysed starch using a non-toxigenic strain of *Aureobasidium pullulans*. After completion of the fermentation, the fungal cells are removed by microfiltration, the filtrate is heat-sterilized and pigments and other impurities are removed by adsorption and ion exchange chromatography.

C.A.S. number 9057-02-7

Chemical formula $(C_6H_{10}O_5)_x$

Structural formula



Assay Not less than 90% of glucan on the dried basis

DESCRIPTION White to off-white odourless powder

FUNCTIONAL USES Glazing agent, film-forming agent, thickener

CHARACTERISTICS

IDENTIFICATION

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

pH (Vol. 4) 5.0 - 7.0 (10% solution)

Precipitation with polyethylene glycol 600 Add 2 ml of polyethylene glycol 600 to 10 ml of a 2% aqueous solution of pullulan. A white precipitate is formed.

Depolymerization with pullulanase Prepare two test tubes each with 10 ml of a 10% pullulan solution. Add 0.1 ml pullulanase solution having activity 10 units/g (refer to pullulanase activity, under Methods for enzyme preparations in Volume 4) to one test tube, and 0.1 ml water to the other. After incubation at about 25° for 20 min, the viscosity of the pullulanase-treated solution is visibly lower than that of the untreated solution.

PURITY

Loss on drying (Vol. 4)	Not more than 6% (90°, pressure not more than 50 mm Hg, 6 h)
Mono-, di- and oligosaccharides	Not more than 10% (expressed as glucose) See description under TESTS
Viscosity	100-180 mm^2/s (10% w/w aqueous solution at 30°) See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES 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").
Microbiological criteria (Vol. 4)	Yeast and moulds: Not more than 100 CFU/g Coliforms: Negative in 25 g Salmonella: Negative in 25 g

TESTS

PURITY TESTS

Mono-, di- and oligosaccharides

Principle

The soluble mono-, di- and oligosaccharides of pullulan are measured using the anthrone-sulfuric acid method after pullulan has been precipitated with methanol and KCl.

Equipment

Spectrophotometer capable of measuring absorbance at 620 nm

Procedure

*Preparation of standard: W*eigh accurately 0.2 g glucose, dissolve in water and make up to 1 l.

Measurement of mono-, di- and oligosaccharides:
Weigh accurately 0.8 g sample and dissolve in water to make 100 ml (stock solution).
Place 1 ml of the stock solution in a centrifuge tube. Add 0.1 ml saturated potassium chloride solution. Add 3 ml methanol and mix vigorously for 20 sec. Centrifuge at 11000 rpm for 10 minutes. Add 0.2 ml of the supernatant to 5 ml modified anthrone solution (0.2 g anthrone in 100 g 75% (v/v) sulfuric acid, freshly prepared). Add 0.2 ml of glucose standard solution and 0.2 ml water (blank control) to separate 5 ml portions of modified anthrone solution. Mix rapidly. Place samples in a 90° water bath and incubate for 15 min. Measure absorbance of the test solution at 620 nm.

Calculate the percent of mono-, di- and oligosaccharides expressed as glucose, *C*, in the sample:

$$C(\%) = [(A_t - A_b)\times 0.41\times G\times 100]/(A_s - A_b)\times W$$

where

A_t is absorbance of the test solution;
A_b is absorbance of the water blank;
A_s is absorbance of the standard solution;
G is weight of the glucose (g); and
W is weight of the sample (g).

Viscosity

Dry the sample for 6 h at 90º under reduced pressure (50 mm Hg). Weigh 10.0 g of the sample and dissolve in water to yield 100 g of solution.

Use an Ubbelohde-type (falling-ball) viscometer. Charge the viscometer with sample in the manner dictated by the design of the instrument. Immerse the viscometer vertically in the thermostatic tank at 30 ± 0.1° and allow to stand for 20 min so that the sample equilibrates with the temperature in the tank. Adjust the meniscus of the column of liquid in the capillary tube to a position about 5 mm above of the first mark. With the sample flowing freely, measure, in seconds, the time required for the meniscus to pass from the first to the second mark. Calculate the viscosity, V:

$$V\ (mm^2/s) = C \times t$$

where

C is the calibration constant of the viscometer (mm^2/s^2); and
t is the flow time (s).

METHOD OF ASSAY

Calculate the percentage of pullulan on dried basis, P, as the difference between 100% and the sum of the percentages of known impurities (mono-, di- and oligosaccharides and water).

$$P(\%) = 100 - (L+C)$$

where

L is loss on drying; and
C is taken from the calculation for mono-, di- and oligosaccharides.

PULLULANASE FROM *BACILLUS DERAMIFICANS* EXPRESSED IN *BACILLUS LICHENIFORMIS*

New specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011). An ADI "not specified" was established at the 74th JECFA (2011).

SYNONYMS Pullulan α-1,6-glucanohydrolase; amylopectin 6-glucanohydrolase, bacterial debranching enzyme; α–dextrin endo-1,6-glucosidase; debranching enzyme; R-enzyme

SOURCES Pullulanase is produced by submerged fed-batch fermentation of a genetically modified strain of *Bacillus licheniformis* which contains a gene coding for pullulanase from *Bacillus deramificans.* The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cellular biomass and concentration by ultrafiltration. The final product is formulated using food-grade stabilizing and preserving agents and is standardized to the desired activity.

Active principles	Pullulanase
Systematic names and numbers	Pullulan 6-α-glucanohydrolase; EC 3.2.1.41; CAS No. 9075-68-7
Reactions catalysed	Hydrolysis of (1→6)-α-D-glucosidic linkages in pullulan, amylopectin and glycogen, and in the α- and β-limit dextrins of amylopectin and glycogen
Secondary enzyme activities	No significant levels of secondary enzyme activities

DESCRIPTION Amber liquid

FUNCTIONAL USES Enzyme preparation.
Used in the hydrolysis of carbohydrates in the manufacture of starch hydrolysates (maltodextrins, maltose and glucose), high fructose corn syrup, beer, and potable alcohol.

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

CHARACTERISTICS

IDENTIFICATION

Pullulanase activity	The sample shows pullulanase activity. See description under TESTS.

TESTS

Enzyme activity

Principle

Pullulanase catalyses the hydrolysis of (1→6)-α-D-glucosidic linkages in pullulan, amylopectin and glycogen, and in the α- and β-limit dextrins of amylopectin and glycogen. The activity of pullulanase is determined by measuring a degree of hydrolysis of the insoluble Red Pullulan substrate, which is solubilized due to hydrolysis by pullulanase. The insoluble residue is precipitated with ethanol and removed by centrifugation. The absorbance of the supernatant is measured at 510 nm. The colour intensity is proportional to the enzyme activity. Pullulanase with known activity is used as a standard.

The enzyme activity is expressed in Acid Stable Pullulanase Units (ASPU). One ASPU is defined as the amount of pullulanase that liberates reducing sugars equivalent to 0.45 µmol of glucose per minute from pullulan at pH 5.0 and a temperature of 40°.

Apparatus

Spectrophotometer (510 nm)
Water bath with thermostatic control (40±1^{o})
pH meter
Microcentrifuge
Positive displacement pipette and tips (250 µl)
Repeater pipette with 12.5 ml or 50 ml tips

Reagents and solutions

(Note: use deionized water)

Sodium acetate buffer (0.2 M, pH 5.0): Weigh 16.406 g of anhydrous sodium acetate and dissolve in 900 ml of water. Adjust the pH to 5.00±0.05 with glacial acetic acid. Bring the volume to 1000 ml with water and mix. The solution can be stored at 4^{o} for one month.

Red Pullulan substrate solution (2%): Weigh 1.00 g of Red Pullulan (Megazyme) into a 50-ml conical tube and dissolve in 50 ml of the sodium acetate buffer by stirring with a magnetic stirring bar for approximately 20-30 min. The solution can be stored at 4^{o} for two weeks.

Standard pullulanase solution: Use pullulanase standard solution with certified activity expressed in ASPU/ml (available from Danisco US Inc., Genencor Division, Rochester, NY, USA). Dilute the solution with the sodium acetate buffer to obtain three working standard solutions with concentrations that fall within the linear range of the assay after subtracting blank (typically 0.95 – 1.80 ASPU/ml). The assay is linear within the absorbance range of 0.4 - 1.0. The standard solutions should be kept on ice and used within 2 h.

Sample solution: Dilute the pullulanase samples with the sodium acetate buffer to obtain a net absorbance (after subtracting blank) within the linear range of the assay. The solutions should be kept on ice and used within 2 h.

Procedure

1. Prepare duplicate microcentrifuge tubes in a rack for each standard solution, sample and blank. Using a positive displacement pipette, dispense to appropriate tubes 250 µl of standard solutions, control sample (pullulanase solution with known activity) and test sample. Add 250 µl of the sodium acetate buffer to the blank tube.
2. Add to each tube 250 µl of 2% Red Pullulan substrate solution with a repeater pipette and vortex for 3 sec.
3. Incubate all tubes in a water bath at 40±1^{o} for exactly 20 min.
4. Remove from the water bath and immediately add 1.0 ml of absolute ethanol using a repeater pipette. Vortex for 3 sec.
5. Leave all tubes at room temperature for 5-10 min.
6. Centrifuge the tubes in a benchtop centrifuge for 10 min.
7. Transfer the supernatant from each tube to a 1.5 ml cuvette. Zero the spectrophotometer with absolute ethanol and measure the absorbance for each cuvette at 510 nm. Subtract the absorbance of the blank from the absorbance readings for all standards and samples.
8. Prepare the standard curve using linear regression. The correlation coefficient must be ≥0.99.
9. Determine the pullulanase concentration of each sample solution from the standard curve.
10. Calculate the pullulanase activity for each sample of the pullulanase preparation in ASPU/g as follows:

$$Activity\ (ASPU/g) = C \times D$$

where

C is the pullulanase concentration of each sample determined from the standard curve (ASPU/ml); and

D is the dilution factor calculated by dividing the sample volume after dilution (ml) by the initial sample weight (g).

QUINOLINE YELLOW
(TENTATIVE)

Tentative specifications prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 28th JECFA (1984), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A temporary ADI of 0-5 mg/kg bw was established at the 74th JECFA (2011).

Information required on the commercially available products for use as food additives:

- *Range of mono-, di- and trisulfonated components*
- *Chemical name, C.A.S. number and structural formula for the principal component*
- *Maximum wavelengths for UV-visible absorption of mono-, di-, trisulfonated compounds and the absorptivities of the three sulfonate compounds together with the testing solvent*
- *Suitable test method for the separate determination of mono-, di- and trisulfonated compounds*
- *Data from five batches on organic impurities including subsidiary colouring matters and their levels*
- *Suitable test method for the determination of subsidiary colouring matters and other organic impurities using HPLC, including information on the availability of suitable standards*
- *Level of zinc in commercial products and explanation for this impurity*

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

DEFINITION Quinoline yellow is prepared by sulfonating 2-(2-quinolyl)-1,3-indandione. It consists essentially of sodium salts of a mixture of monosulfonates, disulfonates and trisulfonates of the above compounds and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

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

Chemical name Information required

C.A.S. number Information required

Chemical formula $C_{18}H_9NNa_2O_8S_2$ (principal component)

Structural formula	Information required
Formula weight	477.38 (Principal component)
Assay	Not less than 70% total colouring matters. Of the total colouring matters present: - not less than 80% of disodium 2-(2-quinolyl)-indan-1,3-dionedisulfonates; - not more than 15% of sodium 2-(2-quinolyl)-indan-1,3-dionemonosulfonates; - not more than 7% of trisodium 2-(2-quinolyl)-indan-1,3-dionetrisulfonate

DESCRIPTION

Yellow powder or granules

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol
Spectrophotometry	Maximum wavelength: Information required Determine the UV-visible absorption spectrum of the sample solution dissolved in a suitable solvent.

PURITY

Loss on drying (Vol. 4)	Not more than 30% at 135º together with chloride and sulfate calculated as sodium salts Determine using Loss on Drying under “GENERAL METHODS”, Chloride as Sodium Chloride and Sulfate as Sodium Sulfate under “SPECIFIC METHODS, Food Colours” in Volume 4.
Water-insoluble matter (Vol. 4)	Not more than 0.2%
Subsidiary colouring matters	Information required
Organic compounds other than colouring matters	Information required
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01% calculated as aniline (See Volume 4 under “SPECIFIC METHODS, Food Colours”)
Ether-extractable matter (Vol. 4)	Not more than 0.2% (See Volume 4 under “SPECIFIC METHODS, Food Colours, Method II”) Use 2 g of sample for the test.

Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES 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”).
Zinc (Vol. 4)	Information required

TESTS

PURITY TESTS

Subsidiary colouring matters (Vol. 4)	Information required
Organic compounds other than colouring matters	Information required

METHOD OF ASSAY

Determine using *Colouring Matters Content by Spectrophotometry* in Volume 4 (under “Specific Methods, Food Colours).
Determination of the percentages of mono-, di- and trisulfonates in Quinoline Yellow by HPLC: Information required.

SODIUM CARBOXYMETHYL CELLULOSE

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding specifications prepared at the 55th JECFA (2000), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established for modified celluloses at the 35th JECFA (1989).

SYNONYMS Sodium cellulose glycolate; Na CMC; cellulose gum; sodium CMC; INS No. 466

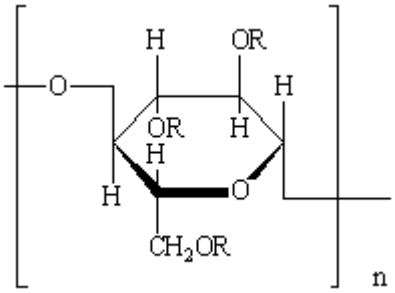
DEFINITION Prepared from cellulose by treatment with alkali and monochloro-acetic acid or its sodium salt. The article of commerce can be specified further by viscosity.

Chemical names: Sodium salt of carboxymethyl ether of cellulose

C.A.S. number: 9004-32-4

Chemical formula: $[C_6H_7O_2(OH)_x(OCH_2COONa)_y]_n$
where
n is the degree of polymerization
x = 1.50 to 2.80
y = 0.2 to 1.50
x + y = 3.0
(y = degree of substitution)

Structural formula



where R = H or CH_2COONa

Formula weight: Structural unit with a degree of substitution of 0.20: 178.14
Structural unit with a degree of substitution of 1.50: 282.18
Macromolecules: greater than about 17,000 (n about 100)

Assay: Not less than 99.5% of sodium carboxymethyl cellulose, calculated on the dried basis

DESCRIPTION White or slightly yellowish, almost odourless hygroscopic granules, powder or fine fibres

FUNCTIONAL USES Thickening agent, stabilizer, suspension agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Yield viscous colloidal solution with water; insoluble in ethanol
Foam test	Vigorously shake a 0.1% solution of the sample. No layer of foam appears. This test distinguishes sodium carboxymethyl cellulose from other cellulose ethers and from alginates and natural gums.
Precipitate formation	To 5 ml of a 0.5% solution of the sample add 5 ml of a 5% solution of copper sulfate or of aluminium sulfate. A precipitate appears. (This test permits the distinction of sodium carboxymethyl cellulose from other cellulose ethers, and from gelatine, carob bean gum and tragacanth gum).
Colour reaction	Add 0.5 g of powdered carboxymethylcellulose sodium to 50 ml of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. To 1 ml of the solution, diluted with an equal volume of water, in a small test tube, add 5 drops of 1-naphthol TS. Incline the test tube, and carefully introduce down the side of the tube 2 ml of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface.

PURITY

Loss on drying (Vol. 4)	Not more than 12% after drying (105°, to constant weight)
pH (Vol. 4)	6.0 - 8.5 (1 in 100 solution)
Sodium (Vol. 4)	Not more than 12.4% on the dried basis Determine total sodium content by *Atomic Absorption Spectroscopy* or *Flame Photometry*
Sodium chloride	Not more than 0.5% on the dried basis See description under TESTS
Free glycolate	Not more than 0.4% calculated as sodium glycolate on the dried basis See description under TESTS
Degree of substitution	Not less than 0.20 and not more than 1.50 See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES 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

Sodium chloride

Heat 5 g of the sample, weighed to the nearest 0.1 mg, in a platinum or porcelain crucible, first with a small flame so that the sample does not ignite and then, when the charring is complete, heat further in an electric oven for 15 min at about 500°. After cooling, pulverize the ashes thus obtained and extract several times with warm water. Filter the extracts into a 500-ml volumetric flask, acidify with nitric acid and dilute to the mark. Determine the NaCl content of 100 ml of this extract by the method of Volhard, using 0.02 N silver nitrate and 0.02 N ammonium thiocyanate. Each ml of 0.02 N silver nitrate is equivalent to 1.169 mg of NaCl. Calculate the sodium chloride content by the formula:

$$\% \text{ NaCl} = \frac{a \times 0.001169 \times 5}{b} \times 100$$

where
a is ml of 0.02 N silver nitrate used; and
b is the dry weight of 5 g of the sample.

Free glycolate

Weigh 0.5 g of the sample to the nearest 0.1 mg, and transfer to a 100-ml beaker. Moisten the sample thoroughly with 5 ml of glacial acetic acid, followed by 5 ml of water, and stir with a glass rod until the solution is complete (usually about 15 min are required). Slowly add 50 ml of acetone while stirring and then approximately 1 g of sodium sulfate. Continue the stirring for several min to ensure complete precipitation of the carboxymethyl cellulose. Filter through a soft, open-texture paper, previously wetted with a small amount of acetone, and collect the filtrate in a 100-ml volumetric flask. Use 30 ml of acetone to facilitate the transfer of the solids and to wash the filter cake. Make up to volume with acetone and mix.

Prepare a blank solution containing 5 ml of water, 5 ml of glacial acetic acid and acetone in another 100-ml volumetric flask. Pipet 2 ml of the sample solution and 2 ml of the blank solution into two 25-ml volumetric flasks. Remove the acetone by heating the uncovered flasks upright in a boiling water bath for exactly 20 min. Cool to room temperature and add 5 ml of naphthalenediol TS, mix thoroughly, then add 15 ml more of the TS and mix. Cover the mouth of the flask with a small piece of aluminium foil and heat upright in the boiling water bath for 20 min. Cool to room temperature and make up to volume with naphthalenediol TS.

Measure the absorbance of sample solution against blank solution at 540 nm using 1-cm cells. Read the corresponding mg of glycolic acid from the calibration curve obtained as follows:

Introduce 0, 1, 2, 3 and 4-ml aliquots of standard glycolic acid solution (1 mg per ml, prepared by weighing accurately 0.100 g of glycolic acid, previously dried in a vacuum desiccator for at least 16 hours, and then dissolving in 100 ml of water; do not keep the

solution longer than 30 days) into a series of five 100-ml volumetric flasks. Add water to each flask to a volume of 5 ml, then add 5 ml of glacial acetic acid and make up with acetone to mark and mix. Pipet 2 ml of each solution (containing, respectively, 0, 1, 2, 3, and 4 mg of glycolic acid per 100 ml) into a series of five 25-ml volumetric flasks and proceed in the same manner as described for the Test Solution. Plot the mg of glycolic acid in the original 100 ml of solution against absorbance to give a calibration curve.

Calculate the sodium glycolate (free glycolate) content by the formula:

$$\% \text{ Sodium glycolate} = \frac{a \times 0.129}{b}$$

where

a is mg of glycolic acid read from the calibration curve; and
b is g of dry-weight of the sample.

Degree of substitution

Sample preparation

Weigh 5 g of the sample to the nearest 0.1 mg, and transfer into a 500-ml conical flask. Add 350 ml of methanol or ethanol (80% by volume). Shake the suspension mechanically for 30 min. Decant through a tared glass filtering crucible under gentle suction. Avoid, at the end of the decanting, suction of air through the crucible. Repeat the treatment with the extraction liquid until the test for chloride ions with a solution of silver nitrate TS is negative. Normally three treatments are sufficient. Transfer the sodium carboxymethyl cellulose into the same crucible. Displace the extraction liquid that adheres to the substance with acetone. Dry the crucible in an oven at 110^{o} until constant in weight. Weigh the first time after 2 h. Cool the crucible every time in a desiccator and pay attention during weighing to the fact that sodium carboxymethyl cellulose is slightly hygroscopic.

Procedure

Weigh 2 g, to the nearest 0.1 mg, of the bone dry substance, obtained with the above-mentioned alcohol-extraction procedure, in a tared porcelain crucible. Initially, char carefully with a small flame and afterwards for 10 min, with a large flame. Cool and then moisten the residue with 3-5 ml of concentrate sulfuric acid. Heat cautiously until the fuming is finished. After some cooling add about 1 g of ammonium carbonate, distributing the powder over the whole contents of the crucible. Heat again, initially with a small flame until the fuming is finished and heat then at a dull red heat for 10 min. Repeat the treatment with sulfuric acid and ammonium carbonate if the residual sodium sulfate still contains some carbon. Cool the crucible in a desiccator and weigh. Instead of adding ammonium carbonate and heating further with a flame, the crucible can be placed for 1 h in an oven at about 600^{o}.

Calculate the sodium content of the alcohol-extracted sample by the formula:

$$\% \text{ Sodium} = \frac{a \times 32.38}{b}$$

where
a is the weight of residual sodium sulfate; and
b is the weight of the alcohol-extracted dry sample.

Calculate the degree of substitution by the formula:

$$\text{Degree of substitution} = \frac{162 \times \% \text{ sodium}}{2300 - (80 \times \% \text{ sodium})}$$

METHOD OF ASSAY Calculate the percentage of sodium carboxymethyl cellulose in the sample by subtracting from 100% the sum of the percentages of sodium chloride and sodium glycolate (free glycolate), determined separately by the procedures above.

$$\text{Content \%} = 100 - (\%\text{NaCl} + \% \text{ sodium glycolate})$$

SUCROSE MONOESTERS OF LAURIC, PALMITIC OR STEARIC ACID

Prepared at the 74th JECFA (2011) and published in FAO JECFA Monographs 11 (2011), superseding tentative specifications prepared at the 73rd JECFA (2010), published in FAO JECFA Monographs 10 (2010). A group ADI of 0 - 30 mg/kg bw for this substance together with sucrose esters of fatty acids, sucroglycerides and sucrose oligoesters type I and type II was established at the 73rd JECFA (2010).

DEFINITION The product consists of sucrose monoesters of individual fatty acids, namely lauric, palmitic or stearic acid. They are manufactured by a transesterification reaction of sucrose and vinyl esters of lauric, palmitic or stearic acids in dimethyl sulfoxide. Impurities are removed by a series of evaporation and solvent extraction steps. Only the following solvents may be used for the production: dimethyl sulfoxide and isobutanol.

Assay Total content of sucrose esters: not less than 85%
Content of monoesters: not less than 90% of total sucrose esters

DESCRIPTION White to off white powder

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4) Soluble in water and in ethanol

Fatty acids Add 1 ml of ethanol to 0.1 g of the sample, dissolve by warming, add 5 ml of dilute sulfuric acid TS, heat in a waterbath for 30 min and cool. A yellowish white solid or oil is formed, which has no odour of isobutyric acid, and which dissolves when 3 ml of diethyl ether are added. Use the aqueous layer separated from the diethyl ether in the Test for sugars.

Sugars To 2 ml of the aqueous layer separated from the diethyl ether in the test for fatty acids, carefully add 1 ml of anthrone TS down the inside of a test tube; the boundary surface of the two layers turns blue or green.

Composition of fatty acids The chromatogram obtained by following the procedure in TESTS represents mainly one peak, which corresponds to methyl laurate, methyl palmitate or methyl stearate.

PURITY

Sulfated ash (Vol.4) Not more than 2%
Test 1 g of the sample (Method I)

Acid value (Vol.4) Not more than 6

Free sucrose	Not more than 5% See description under TESTS
Dimethyl sulfoxide	Not more than 2 mg/kg See description under TESTS
Isobutanol	Not more than 10 mg/kg See description under TESTS
Vinyl laurate, vinyl palmitate and vinyl stearate	Not more than 10 mg/kg See description under TESTS
Acetaldehyde	Not more than 1 mg/kg See description under TESTS
Lead (Vol.4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES 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

IDENTIFICATION TESTS

Composition of fatty acids

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

Standard solutions
Dissolve 0.1 g each of methyl laurate, methyl palmitate and methyl stearate in heptane and dilute to 50.0 ml with heptane.

Chromatography conditions
Column: DB-WAX (10-30 m x 0.2-0.8 mm i.d. with 0.1-1.0 µm film) or equivalent
Carrier gas: Helium or hydrogen
Detector: Flame-ionization detector (FID)
Temperatures:
- injection port: 250°
- column: 200°
- detector: 250°

The peaks of methyl laurate, methyl palmitate and methyl stearate are separate under the conditions.

Procedure
Weigh 0.15 g of the sample and dissolve in 2 ml of a 20 g/l sodium hydroxide-methanol solution in a 25 ml pear-shape flask. Boil under a reflux condenser for 30 min. Add 2 ml of 14% boron trifluoride-methanol solution through the condenser and boil for 30 min. Add 4 ml heptane through the condenser and boil for 5 min. Cool and add 10 ml saturated sodium chloride solution and shake for 15 s. Add saturated sodium chloride solution to bring the upper phase into the neck of the flask. Collect 2 ml of the upper phase, wash 3 times with each 2 ml of water and dry over anhydrous sodium sulphate. Inject 1 µl into the chromatograph.

PURITY TESTS

Free sucrose

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

Standard solutions
Prepare a stock solution containing 5.0 mg/ml of sucrose in *N,N*-dimethylformamide. Prepare a range of standard solutions containing 0.5, 1.25 and 2.5 mg/ml of sucrose by diluting the stock solution with *N,N*-dimethylformamide.

Internal standard solution
Weigh accurately 0.25 g of octacosane into a 50-ml volumetric flask, add 25 ml of tetrahydrofuran to dissolve the octacosane, and add tetrahydrofuran to volume.

Chromatography conditions
Column: 100%-Dimethylpolysiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)
Carrier gas: Helium
Flow rate: 1.5 ml/min
Detector: Flame-ionization detector (FID)
Temperatures:
- injection port: 280°
- column: Hold for 1 min at 100°, then 100-300° at 12°/min, hold for 45 min at 300°
- detector: 320°

The retention times of free sucrose and octacosane measured under the above conditions are approx. 18.8 and 19.3 min, respectively.

Procedure
Weigh accurately 20-50 mg of the sample into a centrifugation tube, add 1 ml internal standard solution, 1 ml *N,N*-dimethylformamide, 0.4 ml of *N,O*-bis(trimethylsilyl)acetamide (BSA) and 0.2 ml trimethylchlorosilane (TMCS). After sealing the tube, shake and let stand for 5 min at room temperature. Inject 1 µl into the chromatograph.

Standard curve
Prepare silylated standard solutions following the above procedure using 1 ml of each standard solution in place of the sample and *N,N*-dimethylformamide. Draw a standard curve by plotting the concentration of sucrose in the standard solution (X-axis) vs. ratio of peak area of sucrose/internal standard (Y-axis).

Measure the peak areas for sucrose and internal standard. Calculate the ratio of their peak areas, and obtain the amount of sucrose from the standard curve.

Calculate the percentage of free sucrose using the following formula:

$$\% \text{ free sucrose} = \frac{\text{amount of sucrose determined (mg)}}{\text{weight of sample (mg)}} \times 100$$

Dimethyl sulfoxide

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

Standard solutions
Prepare a 0.25 mg/ml stock solution of dimethyl sulfoxide in tetrahydrofuran. Prepare a range of solutions containing 0.1, 0.2, 0.4 and 1.0 μg/ml of dimethyl sulfoxide by dilutions of the stock solution with tetrahydrofuran.

Chromatography conditions
Column: 10% PEG 20M and 3% KOH on Chromosorb W AW DMCS 60/80 mesh (2 m x 3 mm i.d.) or equivalent.
Carrier gas: Nitrogen
Flow rate: 30 ml/min
Detector: Flame photometric detector (using 394 nm sulfur filter)
Temperatures
- injection port: 210°
- column: 160°
The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3.4 min.
Note: Before using the column, raise the oven temperature to 180° at a rate of 10°/min and let stabilize for 24 to 48 h with 30 to 40 ml/min of nitrogen for the column conditioning.

Procedure
Weigh accurately 5 g of the sample into a 25-ml volumetric flask, add 10 ml of tetrahydrofuran to dissolve the sample, add tetrahydrofuran to the mark, and mix the solution well. Inject 3 μl of the sample solution into the chromatograph.

Standard curve
Prepare by injecting 3 μl of each standard solution into the chromatograph.

Calculate the concentration of dimethyl sulfoxide in mg/kg (C_{DMSO}) using the following formula:

$$C_{DMSO}\ (mg/kg) = C \times 25 / W$$

where
C is dimethyl sulfoxide concentration determined (μg/ml); and
W is weight of sample (g).

Isobutanol

Determined by gas chromatography (Vol.4) with a head space sampler under the following conditions.

Standard solutions
Prepare standard solution A containing 4000 mg/l of isobutanol by weighing accurately 0.2 g of isobutanol into a 50-ml volumetric flask containing approx. 20 ml of water, then adding water to volume. By dilutions of this solution, prepare solutions containing 2000 mg/l (standard solution B) and 1000 mg/l (standard solution C).

Procedure
Weigh accurately 1 g of the sample into each of four sample vials.

To one vial add 5 μl of water, to the second, third and fourth, add, respectively, standard solutions A, B and C, and seal them quickly with a septum. (The concentrations of each solvent after adding 5 μl of standard solutions A, B and C to 1 g of the sample are equal to 20, 10 and 5 mg/kg of isobutanol, respectively). Place the sample vials in a head space sampler and analyse using the following conditions:
Column: 100% Polydimethylsiloxane (30 m x 0.53 mm i.d. with 1.5 μm film)
Carrier gas: Nitrogen
Flow rate: 3.5 ml/min
Detector: FID
Temperatures
- injection port: 110°
- column: 40°
- detector: 110°
Head space sampler:
- sample heat insulating temperature: 80°
- sample heat insulating period: 40 min
- syringe temperature: 85°
- sample gas injection: 1.0 ml

Calculation
Plot the relationship between the added amount against the peak area for isobutanol using the analytical results. The relationship should be linear. Extrapolate and determine the x-intercept (w_i), and calculate the solvent concentrations (C_i) using the following formula:

$$C_i \text{ (mg/kg)} = w_i / W$$

where
w_i is x-intercept of relationship line using the standard addition method (μg); and
W is weight of sample (g).

Vinyl laurate, vinyl palmitate and vinyl stearate

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

Standard solutions
Prepare separate stock solutions each containing 100.0 μg/ml of vinyl laurate, vinyl palmitate or vinyl stearate in acetonitrile. Prepare a range of mixed standard solutions containing 0.5, 1, 2 and 5 μg/ml of vinyl laurate, vinyl palmitate and vinyl stearate in acetonitrile.

Procedure
Weigh accurately 0.5 g of the sample into a 5-ml volumetric flask. Dilute to volume with methanol and mix using Vortex until the sample dissolves. Inject 1 μl of the sample solutions into the chromatograph.

Chromatography conditions
Column: Nitroterephthalic acid modified polyethylene glycol (DB-FFAP or equivalent) (30 m x 0.32 mm i.d. with 0.5 μm film)
Carrier gas: Nitrogen

Pressure: 7.18 psi
Split ratio: 10:1
Detector: Flame-ionization detector (FID)
Temperatures:
- injection port: 230 °
- column: Hold for 4 min at 100°, then 100-230° at 45°/min, hold for 10 min at 230°
- detector: 250°

The retention times of vinyl laurate, vinyl palmitate and vinyl stearate measured under the above conditions are approx. 9.1, 12.0 and 14.4 min, respectively.

Calculation

Calculate the content of vinyl laurate, vinyl palmitate and vinyl stearate using the following formula:

$$\text{Content of vinyl laurate, vinyl palmitate and vinyl stearate (mg/kg)} = C \times 5 / W$$

where
C is concentration of vinyl laurate, vinyl palmitate and vinyl stearate determined (μg/ml); and
W is weight of sample (g).

Acetaldehyde

Principle

The volatile acetaldehyde is converted with an acidic solution of 2, 4- dinitrophenylhydrazine (DNPH) to a more stable compound, acetaldehyde-2, 4-dinitrophenylhydrazone (ADNPH) that absorbs in the UV region. ADNPH is determined by HPLC under the following conditions.

Standard solutions

Prepare ADNPH stock solution of 40 μg/ml from ADNPH standard (Sigma) with acetonitrile. Prepare a range of solutions containing 0, 0.05, 0.1, 0.2 and 0.5 μg/ml of ADNPH by dilutions of the stock solution with acetonitrile.

Chromatography conditions

Column: NUCLEOSIL 100-5 C18 (250 mm x 4.6 mm i.d., 5 μm) or equivalent
Mobile phase: Methanol - 1.0mM LiCl solution (80:20)
Flow rate: 1.0 ml/min
Detector: UV 360 nm
Column temperatures: 40°

The retention time of ADNPH measured under the above conditions is approx. 20 min.

Procedure

Accurately weigh 0.5 g of the sample into a 5-ml volumetric flask. Add 1.5mL of methanol to dissolve the sample, add 0.5ml of DNPH reagent and make to volume with acetonitrile. Stir the mixture with a magnetic stirrer for 10min. Centrifuge and collect the liquid layer. Filter through a 0.45 μm membrane filter. Inject 20 μl of the sample solution into HPLC.

Calculation
Calculate the content of acetaldehyde using the following formula:

$$\text{Content of acetaldehyde (mg/kg)} = C \times 5 / W$$

where
C is acetaldehyde concentration determined (μg/ml); and
W is weight of sample (g).

METHOD OF ASSAY Determine by HPLC using the following conditions:

Procedure
Accurately weigh 250 mg of the sample into a 50-ml volumetric flask. Dilute to volume with tetrahydrofuran and mix. Filter through a 0.45 μm membrane filter. Inject 80 μl of the sample into the pre-stabilized chromatograph.

Chromatography conditions
Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G1000HXL, G2000HXL, G3000HXL, G4000HXL (each 30 cm x 7.8 mm i.d., 5 μm) in series, Tosoh Co. or equivalent)
Mobile phase: HPLC-grade degassed tetrahydrofuran
Flow rate: 0.8 ml/min
Detector: Refractive index
Temperatures:
- Column: 40°
- Detector: 40°
Record the chromatogram for about 50 min.

Calculate the percentage of total sucrose esters in the sample using the following formula:

$$\% \text{ sucrose esters} = 100 (A_m + A_d) / T$$

Calculate the percentage of monoesters in total sucrose esters using the following formula:

$$\% \text{ monoesters} = 100 A_m / (A_m + A_d)$$

where
A_m is the peak area of the monoesters eluting at about 39.0-40.0 min;
A_d is the peak area of the diesters eluting at about 37.0-38.2 min; and
T is the sum of all peak areas eluting within 43 min.

SUNSET YELLOW FCF

Prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008), superseding specifications prepared at the 28th JECFA (1984), published in combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-4 mg/kg bw was established at the 74th JECFA (2011).

SYNONYMS CI Food Yellow 3; Orange Yellow S; CI (1975) No. 15985; INS No. 110

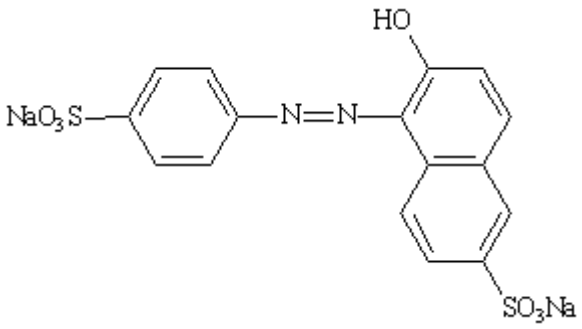
DEFINITION Sunset Yellow FCF consists principally of the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.
(NOTE: The colour may be converted to the corresponding aluminium lake, in which case only the *General Specifications for Aluminium Lakes of Colouring Matters* apply.)

Chemical names: Principal component:
Disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-sulfonate

C.A.S. number: 2783-94-0

Chemical formula: $C_{16}H_{10}N_2Na_2O_7S_2$ (Principal component)

Structural formula



(Principal component)

Formula weight: 452.38 (Principal component)

Assay: Not less than 85% total colouring matters

DESCRIPTION Orange-red powder or granules

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water; sparingly soluble in ethanol
Colour test	In water, neutral or acidic solutions of Sunset Yellow FCF are yellow-orange, whereas basic solutions are red-brown. When dissolved in concentrated sulfuric acid, the additive yields an orange solution that turns yellow when diluted with water.
Colouring matters, identification (Vol. 4)	Passes test

PURITY

Water content (Loss on drying) (Vol. 4)	Not more than 15% together with chloride and sulfate calculated as sodium salts
Water-insoluble matter (Vol. 4)	Not more than 0.2%
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES 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”).
Subsidiary colouring matter content (Vol. 4)	Not more than 5% Not more than 2% shall be colours other than trisodium 2-hydroxy-1-(4-sulfonatophenylazo)naphthalene-3,6-disulfonate Use the following conditions: Chromatography solvent: 2-Butanone:acetone:water:ammonia (s.g. 0.880) (700:300:300:2) Height of ascent of solvent front: approximately 17 cm
Sudan I (1-(Phenylazo)-2-naphthalenol)	Not more than 1 mg/kg See description under TESTS
Organic compounds other than colouring matters (Vol. 4)	Not more than 0.5%, sum of the: monosodium salt of 4-aminobenzenesulfonic acid, disodium salt of 3-hydroxy-2,7-naphthalenedisulfonic acid, monosodium salt of 6-hydroxy-2-naphthalenesulfonic acid, disodium salt of 7-hydroxy-1,3-naphthalenedisulfonic acid, disodium salt of 4,4'-diazoaminobis-benzenesulfonic acid, and disodium salt of 6,6'-oxybis-2-naphthalenesulfonic acid Proceed as directed under *Determination by High Performance Liquid Chromatography* using an elution gradient of 2 to 100% at 4% per min (linear) followed by elution at 100%.
Unsulfonated primary aromatic amines (Vol. 4)	Not more than 0.01%, calculated as aniline

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

TESTS

PURITY TESTS

Sudan I (1-(Phenylazo)-2-naphthalenol)

Principle
The additive is dissolved in water and methanol and filtered solutions are analysed by Reverse-Phase Liquid Chromatography (Volume 4 under "Analytical Techniques, Chromatography"), without extraction or concentration. (Based on *J.AOAC Intl* 90, 1373-1378 (2007).)

Mobile phase
Eluant A: Ammonium acetate (LC grade), 20 mM aqueous
Eluant B: Methanol (LC grade)

Sample solution
Accurately weigh 200 mg of Sunset yellow FCF and transfer it into a 10-ml volumetric flask. Dissolve the sample in 4 ml water via swirling or sonication. Add 5 ml of methanol and swirl. Allow the solution to cool for 5 min and adjust the volume to the mark with water. Filter a part of the solution for analysis through a 13 mm syringe filter with a 0.2 µm pore size PTFE membrane by using a 5 ml polypropylene/polyethylene syringe. (NOTE: Do not substitute a PVDF filter for the PTFE filter, as a PVDF filter adsorbs Sudan I.)

Standard
Sudan I (>97%, Sigma Aldrich, or equivalent), recrystallized from absolute ethanol (5g/150 ml)

Standard stock solution
Accurately weigh a sufficient quantity of the *Standard* to prepare a solution in methanol of 0.0100 mg/ml.

Standard solutions
Transfer 0, 20, 50, 100, 150, 200, and 250 µl aliquots of the *Standard stock solution* to seven 10-ml volumetric flasks. To each flask, add 5 ml of methanol, swirl to mix, and add 4 ml of water. Dilute to volume with water, mix, and filter each solution through a PTFE membrane syringe filter (see *Sample solution*, above) into LC vials for analysis. (NOTE: These solutions nominally contain 0, 0.02, 0.05, 0.10, 0.15, 0.20, and 0.25 µg of Sudan I/ml.)

Chromatographic system
Detector: Photodiode Array (485 nm)
Columns: 150 mm x 2.1 mm id, packed with 5 µm reversed-phase C18, or equivalent, with a guard column (10 mm x 2.1 mm i.d.) – Waters Corp., or equivalent
Column temperature: 25°
Flow rate: 0.25 ml/min
Injection volume: 50 µl
Elution: 50% *Eluant A*/50% *Eluant B* for 5 min; 50 to 100% *Eluant B* in 10 min; 100% *Eluant B* for 10 min. (NOTE: The column

should be requilibrated with 50% *Eluant A*/50% *Eluant B* for 10 min.)

System suitability: Inject three replicates of the *Standard solutions* with concentrations of 0.05 and 0.25 µg of Sudan I/ml. The responses for each set of three injections show relative standard deviations of not more than 2%.

Procedure

Separately inject the seven *Standard solutions* and the *Sample solution* into the chromatograph and measure the peak areas for Sudan I. From the chromatograms for the *Standard solutions*, prepare a standard curve of the concentration of Sudan I vs. the peak areas. (NOTE: The retention time for Sudan I is 19.0 min. Other peaks appearing at earlier retention times in the sample chromatograph are likely attributed to sulfonated subsidiary colours.) Determine the concentration of Sudan I in the *Sample solution* and convert it to mg/kg in the sample of Sunset Yellow FCF.

(NOTE: The limit of determination is 0.4 mg/kg.)

METHOD OF ASSAY

Proceed as directed under *Colouring Matters Content by Titration with Titanous Chloride* (Volume 4, under "Food Colours, Colouring Matters"), using the following:

Weight of sample: 0.5-0.6 g

Buffer: 10 g sodium citrate

Weight (*D*) of colouring matters equivalent to 1.00 ml of 0.1 N $TiCl_3$: 11.31 mg

ANALYTICAL METHODS

The analytical method for total colouring matter content was revised and made tentative by the Committee at the 74th meeting. This method will be made available in the on-line edition of Volume 4 of the Combined Compendium of Food Additive Specifications, including the required information to allow completing of the work on this method.

TOTAL COLOURING MATTERS CONTENT
(TENTATIVE)

Requested information:

Information on the wavelength of maximum absorbance, absorptivity and/or specific absorbance (including information on the solvent used) for the 17 synthetic colours for which the specifications monograph indicates that the colour is used to form a lake. Currently available data are included in the draft table attached to the revised method. The objective is to provide single values for each synthetic colour, thus recommendations to allow for the establishment of consensus values would be welcomed.

Total colouring matters content

Two general methods are used for determination of total colouring matters: 'Colouring Matters Content by Spectrophotometry' and 'Colouring Matters Content by Titration with Titanous Chloride.'

When using the spectrophotometric method, all colours present in the sample that absorb in the same region as that of the main colour will contribute to the absorbance figure used to calculate the results; subsidiary colouring matters of markedly different hue will not be accounted for by this method

The titanous chloride reduction method assumes that isomers and subsidiary colouring matters have the same titanous chloride equivalent as the main colouring matter.

Colouring Matters Content by Spectrophotometry

Three experimental procedures are described. Procedure 1 is used for water-soluble colouring matters. Procedure 2 is used for organic solvent-soluble colouring matters. Procedure 3 is used for lakes. Information pertaining to the wavelength of maximum absorbance, absorptivity or specific absorbance necessary for determination of percent colouring matters for the lakes of synthetic colours is included in Table 1.

Principle

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

Apparatus

- UV-visible range spectrophotometer capable of accurate (± 1% or better) measurement of absorbance in the region of 350 - 700 nm with an effective slit width of 10 nm or less
- Spectrophotometer cells, 1 cm path length

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

Accurately weigh 0.25 g (± 0.02 g) of the sample (W). Transfer to a 1-liter volumetric flask. Add the solvent prescribed in Table 1 and swirl to dissolve. Make up to volume and mix. Dilute the solution with the same solvent in order to obtain an absorbance between 0.3 and

0.7. Measure the absorbance (A) at the wavelength of maximum absorption in a 1 cm path length cell, using the prescribed solvent as the blank.

Calculation

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

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

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

where

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

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

Reagents

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

Accurately weigh 0.08 g (± 0.01 g) of the sample (W) into a 100-ml volumetric flask (V_1). Add 20 ml of chloroform and dissolve by swirling briefly. Make sure that the solution is clear. Make up to volume with cyclohexane and mix. Pipet 5.0 ml of the solution (v_1) into a second 100-ml volumetric flask (V_2) and make up to volume with cyclohexane. Pipet 5.0 ml of this diluted solution (v_2) into the final 100-ml volumetric flask (V_3) and make up to volume with cyclohexane. Measure the absorbance (A) of the twice-diluted solution at the wavelength of maximum absorption in a 1 cm cell, using cyclohexane as the blank.

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

Calculation

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

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

$$\% \text{ total colouring matter} = 1000 \times \frac{A \times V_1 \times V_2 \times V_3}{v_1 \times v_2 \times W \times A^{1\%}_{1cm} \times 10}$$

where

A is absorbance of the sample solution at the wavelength of maximum absorption;
$A^{1\%}_{1\ cm}$ is the specific absorbance of the standard indicated in the specification monograph;
V_1, V_2, and V_3 are the volumes of the three volumetric flasks (each 100 ml);
v_1 and v_2 are the volumes of the two pipets (each 5 ml);
a is absorptivity of the standard in liter/(g·cm); and
10^{-3} is the conversion factor.

Procedure 3 – Colouring matters content of lakes

Reagents

- Potassium dihydrogen phosphate, reagent grade
- Sodium hydroxide, reagent grade
- Phosphoric acid, reagent grade
- Hydrochloric acid, reagent grade

Prepare 0.1 M phosphate buffer pH 7 as follows: Weigh 13.61 g of potassium dihydrogen phosphate into a 2000-ml beaker and dissolve in about 900 ml of water. Add about 90 ml of 1 N sodium hydroxide. Measure the pH using a pH-meter and adjust the pH to 7.0 using 0.1 N sodium hydroxide or diluted phosphoric acid. Make to volume in a 1-liter volumetric flask

Accurately weigh a quantity of lake which will give an absorbance approximately equal to that of the parent colour when the latter is tested according to Procedure 1, above. Transfer to a 250- ml beaker containing 10 ml hydrochloric acid previously diluted with water to approximately 50 ml. Heat with stirring to dissolve the lake, and then cool to ambient temperature. Transfer to a 1-liter volumetric flask, make up to volume with pH 7 phosphate buffer, and mix. Proceed as detailed in Procedure 1, above, and in the specification monograph, using the values for wavelength of maximum absorbance and absorptivity or specific absorbance included in Table 1, and using the phosphate buffer as the spectrophotometric blank.

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

JECFA Colour	Wavelength of Maximum Absorbance (nm)	Absorptivity (l/(g·cm))	Specific absorbance $A^{1\%}_{1cm}$	Solvent
Allura Red AC	500 (FDA[1]), 504 (EU[2]), 502 (FCC), 497-501 (Japan)[3]	52.0 (FDA, FCC)	540 (EU)	
Amaranth	520 (EU), 518-522 (Japan)	44.0[4]	440 (EU)	
Azorubine	510 (EU)	51.6[4]	516 (EU)	
Brilliant Black PN	570 (EU)	53.0[4]	530 (EU)	
Brilliant Blue FCF	630 (FDA & EU), 628-632 (Japan)	164 (FDA)	1630 (EU)	
Brown HT	460 (EU)	40.3[4]	403 (EU)	
Erythrosine	527 (FDA), 526 (EU), 524-528 (Japan)	110 (FDA)	1100 (EU)	
Fast Green FCF	625 (FDA), 622-626 (Japan)	156 (FDA)	1560[5]	
Fast Red E				
Green S	632 (EU)	172[4]	1720 (EU)	
Indigotine	610 (FDA & EU), 610-614 (Japan)	47.8 (FDA)	480 (EU)	
Patent Blue V	638 (EU)	200[4]	2000 (EU)	
Ponceau 4R	505 (EU), 506-510 (Japan)	43.0[4]	430 (EU)	
Quinoline Yellow	415 (JECFA)	86.5 (JECFA)	865 (EU)	
Red 2G	532 (EU)	62.0[4]	620 (EU)	
Sunset Yellow FCF	484 (FDA), 485 (EU), 480-484 (Japan)	54.0 (FDA)	555 (EU)	
Tartrazine	428 (FDA), 426 (EU), 426-430 (Japan)	53.0 (FDA)	530 (EU)	

[1] Values based on information from the United States Food and Drug Administration (FDA)
[2] Values based on information obtained from the European Union (EU)
[3] Values based on information obtained from Japan
[4] Calculated from specific absorbance
[5] Calculated from absorptivity

WITHDRAWAL OF SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

Potassium bromate

The specifications for potassium bromate prepared at the forty-fourth meeting and published in FAO Food and Nutrition Paper No. 52, Addendum 3, although they had not been republished and included in the Combined Compendium of Food Additives Specifications (FAO JECFA Monographs No. 1), they were never formally withdrawn.

As bromates are genotoxic carcinogens, the Committee reiterated the general principle that bromates should not be present in food as consumed and withdrew the specifications for potassium bromate.

ANNEX 1: SUMMARY OF RECOMMENDATIONS FROM THE 74TH JECFA

Food additives evaluated toxicologically and assessed for dietary exposure

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Aluminium-containing food additives (including new food additives potassium aluminium silicate and potassium aluminium silicate–based pearlescent pigments)	N, T[b]	**The Committee established a provisional tolerable weekly intake (PTWI) of 2 mg/kg body weight** based on a no-observed-adverse-effect level (NOAEL) of 30 mg/kg body weight per day and application of a safety factor of 100. The PTWI applies to all aluminium compounds in food, including food additives. **The previous PTWI of 1 mg/kg body weight was withdrawn.** For adults, the estimates of mean dietary exposure to aluminium-containing food additives from consumption of cereals and cereal-based products are up to the PTWI. Estimates of dietary exposure of children to aluminium-containing food additives, including high dietary exposures (e.g. 90th or 95th percentile), can exceed the PTWI by up to 2-fold. For potassium aluminium silicate–based pearlescent pigments at the maximum proposed use levels and using conservative estimates, anticipated dietary exposure at the highest range of estimates is 200 times higher than the PTWI. The Committee emphasized that whereas substances that have long half-lives and accumulate in the body are not generally considered suitable for use as food additives, consumption of aluminium-containing food additives would not be a health concern, provided that total dietary exposure to aluminium is below the PTWI. The Committee recommended that provisions for food additives containing aluminium included in the Codex General Standard for Food Additives should be compatible with the revised PTWI for aluminium compounds of 2 mg/kg body weight as aluminium from all sources.
Benzoe tonkinensis	N, T	**The Committee concluded that the available data were inadequate to establish an acceptable daily intake (ADI)** because of the variability in composition of Benzoe tonkinensis and the inadequate characterization of the material tested. The margin of exposure between the conservative dietary exposure estimate of 0.2 mg/kg body weight per day and the NOAEL of 500 mg/kg body weight per day identified in a 90-day oral toxicity study in rats is 2500. Given this margin of exposure as well as the nature of the hepatic effects observed at doses above the NOAEL and the negative genotoxicity results, **the Committee concluded that Benzoe tonkinensis would not pose a health concern at current estimated dietary exposures, provided that it complies with the tentative specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice**. The Committee also noted that exposure to benzoic acid and benzyl benzoate from the use of Benzoe tonkinensis is well below the upper limit of the group ADI (0–5 mg/kg body weight) for benzyl derivatives, and exposure to vanillin is also well below the upper limit of its ADI (0–10 mg/kg body weight). The Committee further noted that

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
		benzoic acid, one of the major components of Benzoe tonkinensis, is used as a preservative, but that Benzoe tonkinensis has not been assessed for this use.
Glycerol ester of gum rosin (GEGR)	R, T	**The Committee withdrew the group ADI for GEGR and GEWR and established a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg body weight**, pending the submission of the full reports of the 90-day toxicity studies on GEGR as well as additional compositional information on the GEWR from *Pinus elliottii*. The Committee noted that the temporary group ADI will be withdrawn if the requested information is not submitted by the end of 2012.
Glycerol ester of tall oil rosin (GETOR)	R, T	**The Committee was unable to complete the evaluation of GETOR** because additional data are required to characterize the GETOR in commerce. Validated methods for the determination of the substances considered in the specifications are also required. The above information should be submitted by the end of 2012.
Glycerol ester of wood rosin (GEWR)	R, T	**The Committee withdrew the group ADI for GEGR and GEWR and established a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg body weight**, applying an additional safety factor of 2**,** because new information raises questions about the identity and composition of the product in commerce. Additional compositional information on the GEWR from *Pinus elliottii* to assess similarity with the GEWR from *Pinus palustris* is required. The Committee noted that the temporary group ADI will be withdrawn if the requested information is not submitted by the end of 2012.
Octenyl succinic acid (OSA) modified gum arabic	R	**The Committee deferred further evaluation of OSA modified gum arabic** pending the submission of data on its stability in food and on the extent to which it is hydrolysed in the gastrointestinal tract, to be provided by the end of 2013. **The existing temporary ADI "not specified"[c] was retained.**
Polydimethyl siloxane	M	**The Committee withdrew the temporary ADI of 0–0.8 mg/kg body weight per day and re-established the ADI of 0–1.5 mg/kg body weight**, originally established at the eighteenth meeting.
Ponceau 4R	R	**The Committee concluded that new data do not indicate a need to revise the existing ADI of 0–4 mg/kg body weight** and that dietary exposure to Ponceau 4R does not present a health concern.
Pullulan	R	Dietary exposure to pullulan as a dietary fibre could reach 1g/kg body weight per day for children (2–5 years old) and 0.4 g/kg body weight per day for the general population (2 years of age and older). These estimates are 8 and 20 times lower, respectively, than the no-observed-effect level (NOEL) observed in the 90-day rat study evaluated previously. Gastrointestinal effects observed in humans should be taken into account when considering appropriate use levels. The Committee stressed that it assessed the safety of use and not the efficacy of pullulan used as a dietary fibre. The Committee maintained the previously established ADI

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
		"not specified"[c] for the previously evaluated food additive uses.
Pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis*	N	**The Committee established an ADI "not specified"[c] for pullulanase from *B. deramificans* expressed in *B. licheniformis*** when used in the applications specified and in accordance with good manufacturing practice.
Quinoline Yellow	R, T	**The Committee established a temporary ADI of 0–5 mg/kg body weight**, incorporating an additional 2-fold safety factor, pending submission of requested toxicological studies by the end of 2013. **The previously established ADI of 0–10 mg/kg body weight was withdrawn.** The conservative exposure estimates were within the range of the temporary ADI. Additional information on the composition of the product in commerce is required, in particular relating to the identity and purity of the unmethylated form of Quinoline Yellow.
Sunset Yellow FCF	M	**The Committee established an ADI of 0–4 mg/kg body weight and withdrew the previous ADI of 0–2.5 mg/kg body weight.** The Committee concluded that dietary exposure to Sunset Yellow FCF does not present a health concern.

[a] M, existing specifications maintained; N, new specifications prepared; R, existing specifications revised; T, tentative specifications.

[b] For potassium aluminium silicate and pearlescent pigments containing potassium aluminium silicate.

[c] 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.

Food additives considered for specifications only

Food additive	Specifications[a]
β-Apo-8′-carotenal	R
β-Apo-8′-carotenoic acid ethyl ester	R
β-Carotene, synthetic	R
Hydroxypropyl methyl cellulose	R[b]
Magnesium silicate, synthetic	R
Modified starches	R
Nitrous oxide	R
Sodium carboxymethyl cellulose	R
Sucrose monoesters of lauric, palmitic or stearic acid	R

[a] R, existing specifications revised; T, tentative specifications.

[b] The Committee concluded that levels of propylene chlorohydrins up to the new limit of not more than 1 mg/kg for the sum of both isomers in hydroxypropyl methyl cellulose were not of toxicological concern.

Analytical methods for food additives in the Combined Compendium of Food Additive Specifications, Volume 4 (FAO JECFA Monographs 1, 2006)

Food additive	Method[a]
Colouring matters content by spectrophotometry	R,T

[a] R, existing method revised; T, tentative method.

Contaminants evaluated toxicologically

Cyanogenic glycosides

The Third Session of the Codex Committee on Contaminants in Food (CCCF) in 2009 requested that JECFA reconsider the available data on cyanogenic glycosides, advise on the public health implications of cyanogenic glycosides and their derivatives in food and decide whether risk assessment is feasible and appropriate.

Reports of acute human poisoning associated with the consumption of foods containing cyanogenic glycosides were reviewed. The Committee therefore considered it appropriate to establish an acute reference dose (ARfD) for cyanogenic glycosides, expressed as cyanide equivalents. In addition, as there are a number of human diseases, specifically konzo, tropical ataxic neuropathy and iodine deficiency disorders, associated with the chronic consumption of underprocessed cassava as a staple food, it was recognized that the derivation of a chronic health-based guidance value would also be relevant.

Derivation of the ARfD

Following review of a developmental toxicity study with linamarin, the Committee considered this study as suitable for establishing an ARfD. Benchmark dose (BMD) modelling of the data from this study provided a lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) for linamarin of 85 mg/kg body weight for increased skeletal defects in developing hamster fetuses following acute exposure of maternal animals. While the study did not use dietary exposure, gavage dosing was considered relevant to establishing the ARfD.

Following application of a 100-fold uncertainty factor, the Committee established an ARfD for linamarin of 0.9 mg/kg body weight (equivalent to 0.09 mg/kg body weight as cyanide). This value was considered, when compared on a cyanide molar basis, to also be applicable to other cyanogenic glycosides. Therefore, the Committee recommended conversion of the ARfD for linamarin to a cyanide-equivalent dose of 0.09 mg/kg body weight. This cyanide-equivalent ARfD applies only to foods containing cyanogenic glycosides as the main source of cyanide.

Derivation of the provisional maximum tolerable daily intake (PMTDI)

In a 13-week United States National Toxicology Program study not previously evaluated by the Committee, in which exposure to sodium cyanide was continuous via drinking-water, a variety of effects related to male reproductive organs were observed—namely, decreased cauda epididymis weights, decreased testis weights and decreased testicular spermatid concentration. Dose–response analysis of continuous data on absolute cauda epididymis weights generated the lowest BMDL for a one standard deviation response ($BMDL_{1SD}$) of 1.9 mg/kg body weight per day. On the basis of this $BMDL_{1SD}$, the Committee established a PMTDI of 0.02 mg/kg body weight by applying a 100-fold uncertainty factor. The Committee decided that it was not necessary to apply an additional uncertainty factor to account for the absence of a long-term study, considering the generally acute nature of cyanide toxicity and the sensitivity of the effect (i.e. the reduction of absolute cauda epididymis weight).

Comparison of estimated dietary exposures with health-based guidance values and the impact of maximum limits (MLs) on dietary exposure

Estimated dietary exposures to total available hydrocyanic acid (HCN) were converted to cyanide equivalents and compared with the health-based guidance values established by the Committee at this meeting.

From the national acute dietary exposure estimates available to the Committee for review, the ARfD of 0.09 mg/kg body weight as cyanide equivalents was exceeded 3-fold for cassava for adults (based on raw samples), less than 2-fold for apple juice for children, between 2- and 5-fold for bitter apricot kernels and up to 10-fold for ready-to-eat cassava chips/crisps, depending on the population group. If ready-to-eat cassava chips contained a level equivalent to the recently established ML in Australia and New Zealand of 10 mg/kg as HCN, there was only a marginal exceedance of the ARfD for children. These results are based on dietary exposure to total HCN, which represents the maximum possible exposure for foods containing cyanogenic glycosides.

Based on national estimates of chronic dietary exposure to total HCN, there is also the potential to exceed the PMTDI of 0.02 mg/kg body weight as cyanide for populations reliant on cassava as a staple food: between 1- and 3-fold for children and between 1- and 2-fold for adults. There is also a potential for those populations not reliant on cassava to exceed the PMTDI: between 1- and 5-fold for children and between 1- and 3-fold for adults. For Australia and New Zealand, ready-to-eat cassava chips were the major contributor to dietary exposure to HCN (84–93%). When the cassava chips contain a level equivalent to the ML of 10 mg/kg as HCN, all mean dietary exposures were below the PMTDI. High-percentile exposures for children were between 1- and 2-fold above the PMTDI. All chronic dietary exposure estimates based on exposures from flavouring agents did not exceed the PMTDI. These results are based on dietary exposure to total HCN, which is a worst-case scenario.

Application of the ML of 50 mg/kg as HCN for sweet cassava could result in dietary exposures that exceed the ARfD by less than 2-fold for the general population and up to 4-fold for children and exceed the PMTDI by between 2- and 10-fold, depending on the population group assessed. These estimates do not take into consideration any reduction in concentration of total HCN as a result of food preparation or processing. For the ML of 10 mg/kg as HCN for cassava flour, there are no estimates of dietary exposure available that exceed the ARfD or PMTDI. This is supported by the maximum amount of food that can be consumed based on existing Codex MLs before the health-based guidance values would be exceeded, which is as low as 25 g/day for cassava for chronic exposure. More detailed estimates of cassava and cassava flour consumption and concentrations in food for cassava-eating communities would help in supporting the conclusion that dietary exposures to total HCN could exceed health-based guidance values.

The ML for sweet cassava is for the raw product. If the starting level of HCN in the raw sweet cassava were 50 mg/kg as HCN, the minimum effective processing would result in a concentration of 15 mg/kg as HCN, and the most effective processing would give a HCN concentration of 2 mg/kg.

ARfD: **0.09 mg/kg body weight as cyanide (applies only to foods containing cyanogenic glycosides as the main source of cyanide)**

PMTDI: **0.02 mg/kg body weight as cyanide**

Fumonisins

For the current evaluation of fumonisins, the Committee reviewed all relevant studies performed on fumonisins since 2001.

Exposure to fumonisins has been associated with a wide range of effects, which are often species and sex specific. Laboratory studies have identified the liver as the most sensitive organ in mice and the kidney as the most sensitive organ in rats.

Studies suitable for dose–response analysis have been conducted with rodents either employing purified fumonisin B_1 (FB_1) or using *Fusarium verticillioides* culture material containing FB_1. The latter studies typically use FB_1 as a marker for dietary exposure to the fumonisins and other metabolites of *Fusarium*. The studies employing purified FB_1 are generally better in experimental design for dose–response analysis. However, the Committee concluded that the studies with culture material were of sufficient quality to clearly indicate that other toxins produced by *F. verticillioides* either add to or potentiate the toxicity of FB_1. Although naturally contaminated corn would probably be more representative of actual human dietary exposure than either purified FB_1 or culture material, no suitable studies were identified that used naturally contaminated corn as a test material. As the implications are somewhat different, the Committee evaluated studies with purified FB_1 and *F. verticillioides* culture material separately.

For pure FB_1, the lowest identified $BMDL_{10}$ was 165 µg/kg body weight per day for megalocytic hepatocytes in male mice. Using a safety factor of 100 for intraspecies and interspecies variation, the Committee derived a PMTDI of 2 µg/kg body weight per day. As this was the same value as the previously established group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, this group PMTDI was retained.

For culture material, the lowest identified $BMDL_{10}$ using FB_1 as a marker was 17 µg/kg body weight per day for renal toxicity in male rats. The Committee chose not to establish a health-based guidance value for culture material, because its composition was not well characterized and may not be representative of natural contamination.

The Committee concluded that, based on the national and international estimates, dietary exposure to FB_1 for the general population ranges from 0.12×10^{-3} to 7.6 µg/kg body weight per day at the mean, whereas the 95th percentile exposure was estimated to be up to 33.3 µg/kg body weight per day. Dietary exposure to total fumonisins for the general population would range, for a consumer with average consumption, from 0.087×10^{-3} to 10.6 µg/kg body weight per day, whereas for consumers with high consumption, exposure would be up to 44.8 µg/kg body weight per day. Maize is still the predominant source of exposure to FB_1 and total fumonisins.

Comparison of these estimates with the group PMTDI indicates that the group PMTDI is exceeded at the population level in some regions within some countries. The Committee concluded that adverse effects from fumonisin exposure may occur and that reduction of exposure to fumonisin and other toxins produced by *F. verticillioides* is highly desirable, particularly in areas of the world where maize is a major dietary staple food and where high contamination can occur.

As fumonisins do not carry over from feed to animal products in significant amounts, the occurrence of fumonisins in feed was considered not to be a human health concern.

The Committee concluded that implementation of the MLs proposed by CCCF could significantly reduce exposure (by more than 20%) to total fumonisins in six GEMS/Food consumption clusters (A, D, G, B, K, F). The main contribution to reduction was due to the proposed Codex ML for the category "Corn/maize grain, unprocessed". The Committee noted that implementation of the proposed MLs would result in rejection of 2–88% of "Corn/maize grain, unprocessed" and 4–57% of "Corn/maize flour/meal" across the clusters. The Committee also noted that the national estimates of exposure to fumonisins show that the exceedance of the PMTDI occurs only in limited regions presenting high maize consumption levels and highly contaminated maize.

The Committee concluded that no or little effect was noticed on the international exposure estimates resulting from the implementation of MLs higher than those proposed by CCCF.

Group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, of 2 µg/kg body weight was retained.

ANNEX 2: RECOMMENDATIONS AND FURTHER INFORMATION REQUIRED

Further information required or desired

Aluminium-containing food additives

There is a need for convincing data to demonstrate that aluminium is not bioavailable from potassium aluminium silicate–based pearlescent pigments.

No data were available to identify the forms of aluminium present in soya-based formula and their bioavailability. Such studies were requested at the sixty-seventh meeting and are still required.

In the case of potassium aluminium silicate, information is required on preparation and purification methods, particle size distribution, methods of identification for silicate and aluminium, data on the levels of the inorganic impurities, the suitability of an inductively coupled plasma atomic emission spectrometry (ICP-AES) method for the determination of inorganic impurities, and the suitability of a proposed method based on alkali fusion followed by ICP-AES for the assay for potassium aluminium silicate based on the determination of aluminium.

In the case of potassium aluminium silicate–based pearlescent pigments, information is required on their manufacture, stability in food, particle size distribution, pH range, methods for the identification of iron, titanium and aluminium, data on the levels of the inorganic impurities, a filtration method appropriate for the small particle sizes associated with the pigments, and the suitability of a proposed method based on alkali fusion followed by ICP-AES for the assay for titanium, iron and aluminium.

The requested information should be made available by the end of 2012.

Benzoe tonkinensis

The Committee requested additional information regarding the complete composition of the ethanolic extract, data on microbiological contaminants and data on inorganic contaminants (lead, arsenic, antimony, chromium, mercury and cadmium). The Committee also requested an analytical method to distinguish between Benzoe tonkinensis and Benzoe sumatranus.

Cyanogenic glycosides

Further research is needed to more accurately quantify how nutritional factors ultimately contribute to the human diseases observed in populations whose diets consist mainly of improperly processed cassava, which involves high cyanide exposure.

There is a need for more extensive occurrence data for cyanogenic glycosides. These include data showing the ratio of cyanogenic glycosides to cyanohydrins to HCN in raw and processed versions of a range of foods containing cyanogenic glycosides. More occurrence data for foods other than cassava are needed, as are occurrence data for all foods from a broader range of countries around the world. Concentrations in foods as ready to consume would enable more accurate estimates of dietary exposure to be undertaken. Individual data points from analytical surveys would be of use to evaluate distributions of cyanogenic glycosides in foods and to define adequate sampling protocols. Distributions of occurrence data could then be used for probabilistic dietary exposure assessments.

More consumption data for cassava and cassava products from a broader range of countries would enable more detailed estimates of dietary exposure to be conducted or refined. More estimates of acute and chronic dietary exposures from a broader range of countries, particularly African countries, would enable a better estimation of the global risk of dietary exposure to cyanogenic glycosides.

Fumonisins

To be able to fully assess the toxic potential of culture material or naturally contaminated food, characterization and quantification of its mycotoxin content are necessary

To obtain a realistic representation of the effects of “real life” exposure and in order to compare the toxic potential of naturally contaminated feed with the findings in the studies used for the final evaluation, naturally contaminated feed should be tested in dose–response studies in animals.

As hidden and bound fumonisins have been detected in corn and corn products, further studies should be performed to elaborate more appropriate analytical methods in order to obtain additional occurrence data and information on the effects of processing.

As dietary exposure to fumonisins may occur with other mycotoxins, such as aflatoxins, well-designed laboratory and epidemiological studies are needed to assess interactions.

For the evaluation of co-occurrence, in food and feed, of fumonisins with other mycotoxins, concentrations of fumonisins and other mycotoxins must be provided at the level of the individual analytical sample.

Additional data on fumonisin distribution in corn food products should be collected in order to establish appropriate sampling procedures.

To validate urinary FB_1 as a potential candidate for a human biomarker of short-term exposure, large-scale human studies that indicate a well-characterized dose–response relationship between urinary FB_1 and dietary fumonisin exposures are needed. A biomarker for long-term exposure is also needed.

To investigate the association of fumonisin exposure with oesophageal cancer risk, child growth impairment and neural tube defects in humans, studies on fumonisin exposure and the incidence of these conditions in individuals (such as a cohort or case–control study) are needed. These studies should use a validated fumonisin exposure biomarker and control for confounders and for known risk factors.

Glycerol ester of gum rosin (GEGR)

The requested full reports of the unpublished 90-day oral toxicity studies were not provided, and the validity of evaluating GEGR on the basis of toxicological data on glycerol ester of wood rosin (GEWR) still requires confirmation. To complete the evaluation of GEGR, the unpublished studies are required as well as additional data to characterize GEGR in commerce in relation to the composition of 1) the refined gum rosin currently used as the source rosin for the production of GEGR, 2) the glycerol ester of gum rosin, 3) the total glycerol esters of resin acids and 4) the neutrals. Validated methods for the determination of the substances considered in the specifications are also required. The information is required by the end of 2012.

Glycerol ester of tall oil rosin (GETOR)

To complete the evaluation of GETOR, additional data are required to characterize the GETOR in commerce in relation to the composition of 1) the refined tall oil rosin used as the source rosin, 2) the glycerol ester of tall oil rosin, 3) the total glycerol esters of resin acids and 4) the neutrals. Validated methods for the determination of the substances considered in the specifications are also required. The above data are required by the end of 2012.

Glycerol ester of wood rosin (GEWR)

To complete the evaluation of GEWR, additional data are required to characterize the GEWR in commerce in relation to the composition of 1) the refined wood rosin used as the source rosin for the production of GEWR, 2) the glycerol ester of wood rosin, 3) the total glycerol esters of resin acids and 4) the neutrals. Validated methods for the determination of the substances considered in the specifications are also required.

Method for colouring matter content by spectroscopy (Volume 4)

Data on the wavelength of maximum absorbance, absorptivity and/or specific absorbance are requested for the following colours: Allura Red AC, Amaranth, Azorubine, Brilliant Black PN, Brilliant Blue FCF, Brown HT, Erythrosine, Fast Green FCF, Fast Red E, Green S, Indigotine, Patent Blue V, Ponceau 4R, Quinoline Yellow, Red 2G, Sunset Yellow FCF and Tartrazine. The data to be provided should also indicate the solvents used as well as any standardization for pH in order to allow for the establishment of consensus values for the wavelength of maximum absorbance, absorptivity and/or specific absorbance.

Octenyl succinic acid (OSA) modified gum arabic

The Committee requested that data resolving the concern about the stability of OSA modified gum arabic in food as well as data on the extent to which OSA modified gum arabic is hydrolysed in the gastrointestinal tract be provided by the end of 2013.

Quinoline Yellow

The Committee is aware of unpublished long-term studies in mice and rats with in utero exposure to Quinoline Yellow that had been completed by Biodynamics Laboratories in 1980–1981 but had not been submitted for evaluation and which might affect the ADI. These studies are requested by the end of 2013. The specifications are tentative pending submission of information regarding the principal components, maximum wavelengths for absorption, organic impurities, the level of zinc and a method of assay.

CORRIGENDA

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS
FAO JECFA MONOGRAPHS 10, ROME, 2010.

Page 29, in the method of assay in revised specifications for sucrose esters of fatty acids, the last line is amended to read: T is the sum of all peak areas eluting within 43 min.

FAO TECHNICAL PAPERS

FAO JECFA MONOGRAPHS

1 Combined compendium of food additive specifications – JECFA specifications monographs from the 1st to the 65th meeting. (E)
Vol. 1 Food additives A – D
Vol. 2 Food additives E – O
Vol. 3 Food additives P – Z
Vol. 4 Analytical methods, test procedures and laboratory solutions

2 Residue evaluation of certain veterinary drugs - Joint FAO/WHO Expert Committee on Food Additives 66th meeting 2006 (E)

3 Compendium of food additive specifications - Joint FAO/WHO Expert Committee on Food Additives 67th meeting 2006 (E)

4 Compendium of food additive specifications - Joint FAO/WHO Expert Committee on Food Additives 68th meeting 2007 (E)

5 Compendium of food additive specifications - Joint FAO/WHO Expert Committee on Food Additives 69th meeting 2008 (E)

6 Residue evaluation of certain veterinary drugs - Joint FAO/WHO Expert Committee on Food Additives 70th meeting 2008 (E)

7 Compendium of food additive specifications - Joint FAO/WHO Expert Committee on Food Additives 71st meeting 2009 (E)

8 Safety evaluation of certain contaminants in food - Joint FAO/WHO Expert Committee on Food Additives 72nd meeting 2010 (E)
Joint FAO/WHO publication WHO Food Additives Series No. 63/ FAO JECFA Monographs 8.

9 Residue evaluation of certain vweterinary drugs - Joint FAO/WHO Expert Committee on Food Additives Meeting 2010 – Evaluation of data on ractopamine residues in pig tissues (E)

10 Compendium of food additive specifications - Joint FAO/WHO Expert Committee on Food Additives 73rd meeting 2010 (E)

Availability: 2010

Ar – Arabic
C – Chinese
E – English
F – French
P – Portuguese
S – Spanish

Multil – Multilingual
* Out of print
** In preparation

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COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives
74th Meeting 2011

This document contains food additive specifications monographs, analytical methods and other information, prepared at the seventy-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome, Italy, from 14 to 23 June 2011. The specifications 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 additive is 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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