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FAO JECFA Monographs 14

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

77th meeting 2013

World Health Organization

Food and Agriculture Organization of the United Nations

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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Rome, 4 – 13 June, 2013

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 77th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome on 4 - 13 June 2013. The specifications monographs are one of the outputs of JECFA's risk assessment of food additives, and should be read in conjunction with the safety evaluation, reference to which is made in the section at the head of each specifications monograph. Further information on the meeting discussions can be found in the summary report of the meeting (see Annex 1), and in the full report which will be published in the WHO Technical Report series. Toxicological monographs of the substances considered at the meeting will be published in the WHO Food Additive Series.

Specifications monographs prepared by JECFA up to the 65th meeting, other than specifications for flavouring agents, have been published in consolidated form in the Combined Compendium of Food Additive Specifications which is the first publication in the series FAO JECFA Monographs. This publication consists of four volumes, the first three of which contain the specifications monographs on the identity and purity of the food additives and the fourth volume contains the analytical methods, test procedures and laboratory solutions required and referenced in the specifications monographs. FAO maintains an on-line searchable database of all JECFA specifications monographs from the FAO JECFA Monographs, which is available at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/. The specifications for flavourings evaluated by JECFA, and previously published in FAO Food and Nutrition Paper 52 and subsequent Addenda, are included in a database for flavourings (flavouring agent) specifications which has been updated and modernized. All specifications for flavourings that have been evaluated by JECFA since its 44th meeting, including the 76th meeting, are available in the new format online searchable database at the JECFA website at FAO: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/en/. The databases have query pages and background information in English, French, Spanish, Arabic and Chinese. Information about analytical methods referred to in the specifications is available in the Combined Compendium of Food Additive Specifications (Volume 4), which can be accessed from the query pages.

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

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

Contact and Feedback

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

jecfa@fao.org

SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

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

Advantame (N, T)
Aluminium silicate (R, T)
Calcium aluminium silicate (R, T)
Calcium silicate (R, T)
Glucoamylase from *Trichoderma reesei* expressed in *Trichoderma reesei* (N)
Glycerol ester of gum rosin (R, T)
Glycerol ester of wood rosin (R)
Mineral oil (medium viscosity) (R)
Modified starches (R)
Nisin (R)
Octenyl succinic acid modified gum Arabic (R, T)
Paprika extract (R)
Phytase from *Aspergillus niger* expressed in *A. niger* (R)
Potassium aluminium silicate (R)
Potassium aluminium silicate–based pearlescent pigments, Type I (N)
Potassium aluminium silicate–based pearlescent pigments, Type II (N)
Potassium aluminium silicate–based pearlescent pigments, Type III (N)
Silicon dioxide, amorphous (R, T)
Sodium aluminosilicate (R, T)

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

The tentative specifications for glycerol ester of tail oil rosin (GETOR) (INS 445(ii)) were withdrawn since no data was submitted and the JECFA Secretariat was informed that the compound was no longer supported by the previous data sponsor

Editorial changes to specifications

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

Specifications monographs	***INS***	***Description of changes***
Monosodium L-glutamate	621	The test for pH should read *(1 in 20 soln)*
Sodium percarbonate		Method of assay: error in the formula corrected

ADVANTAME
(TENTATIVE)

New tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI of 0-5 mg/kg body weight was established at the 77th JECFA (2013).

Information required on:

- *Suitability of the head space GC method (using appropriate dissolution solvent) for determination of residual solvents published in the "Combined Compendium of Food Additives Specifications, Vol. 4" and data, in a minimum of 5 batches, using the method,*
- *An alternative/improved HPLC method for the assay of advantame and acid of advantame using a standard curve,*
- *Additional data and analytical methods for determination of palladium and platinum,*
- *Information on the purity and availability of the commercial reference standards used in the assay of advantame and acid of advantame*

SYNONYMS INS No. 969

DEFINITION Advantame is manufactured by *N*-alkylation of aspartic acid portion of aspartame (L-α-aspartyl-L-phenylalanine methylester) with 3-(3-hydroxy-4-methoxyphenyl) propionaldehyde produced by selective catalytic hydrogenation from 3-hydroxy-4-methoxycinnamaldehyde. The product is purified through re-crystallisation and dried.

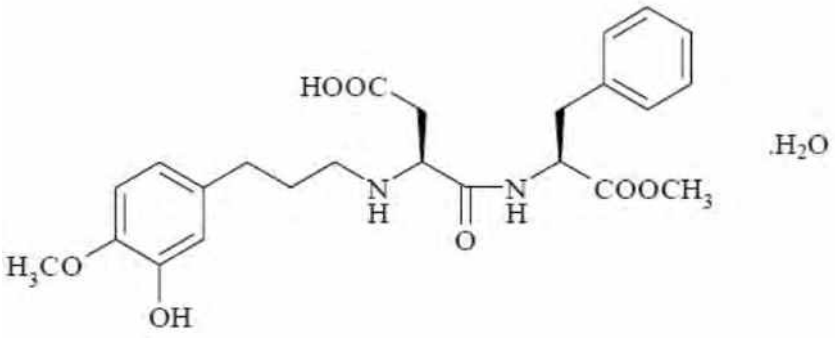
Only the following solvents may be used for the production: methanol and ethyl acetate.

Chemical names (3S)-3-[3-(3-hydroxy-4-methoxyphenyl)propylamino]-4-[[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-4-oxobutanoic acid hydrate, *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-L-α-aspartyl]-L-phenylalanine 1-methyl ester, monohydrate

C.A.S. number 714229-20-6

Chemical formula $C_{24}H_{30}N_2O_7 \cdot H_2O$

Structural formula



Formula weight 476.52

Assay	Not less than 97.0% and not more than 102.0% on the anhydrous basis
DESCRIPTION	White to yellow powder
FUNCTIONAL USES	Sweetener, flavour enhancer
CHARACTERISTICS	
IDENTIFICATION	
Solubility (Vol. 4)	Very slightly soluble in water, sparingly soluble in ethanol
Infrared spectrum	The infrared spectrum of a potassium bromide dispersion of the sample corresponds to the standard infrared spectrum in Appendix A.
PURITY	
Water (Vol. 4)	Not more than 5% (Karl Fischer)
Residue on ignition (Vol. 4)	Not more than 0.2% (use 5 g of the sample)
N-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenyl-alanine (acid of advantame)	Not more than 1% See description under TESTS
Other related substances	Not more than 1.5% (expressed as acid of advantame) See description under TESTS
Specific rotation (Vol. 4)	$[\alpha]_D^{20}$: Between -45° and -38°(0.2% solution in ethanol)
Residual solvents	Methanol: Not more than 500 mg/kg Ethyl acetate: Not more than 500 mg/kg See description under TESTS
Palladium	Information required
Platinum	Information required
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

N-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenyl-alanine (acid of advantame)

Determination of *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine by HPLC (Tentative)

Mobile phase:
Mobile phase A: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 100 ml of acetonitrile to 900 ml of this solution, mix well, and sonicate for about 5 min.
Mobile phase B: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 600 ml of acetonitrile to 400 ml of this solution, mix well, and sonicate for about 5 min.

Preparation of Standard Solution: To prepare the standard stock solution of acid of advantame, accurately weigh about 100 mg of acid of advantame standard (ANS9801-acid Standard Reagent, available from Ajinomoto Co., Inc., Japan) and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 100 ml, in order to prepare acid of advantame standard solution, pipet 2 ml of standard stock solution and add a mixture of water and acetonitrile (7:3 v/v) to make an exact volume of 20 ml.

Preparation of Sample Solution: Accurately weigh about 100 mg of advantame and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 100 ml.

HPLC conditions:

Column:	Inertsil ODS-2 (25 cm x 4.6 mm i.d., 5 µm), GL Sciences, or equiv.
Column temperature:	50°
Mobile phase:	
Mobile phase A:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (9:1 v/v)
Mobile phase B:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (2:3 v/v)
Flow rate:	1.0 ml/min
Injection volume:	20 µl
Detector:	UV detector at 210 nm
Run Time:	80 min

Gradient program:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	85	15
30.0	85	15
55.0	75	25
75.0	0	100
80.0	0	100
80.1	85	15
90.0	85	15

Calculate the content (%) of acid of advantame using the following formula:

Acid of advantame (%) = $[(W_S \times C_S)/W_T] \times [A_{T1}/A_S] \times [1/100]$

where
A_{T1} is peak area of acid of advantame from the sample solution;
A_s is peak area of acid of advantame from the standard solution;
W_T is weight (g) of advantame;
W_S is weight (g) of acid of advantame standard reagent; and
C_S is purity (%) of acid of advantame standard reagent.

Other related substances Calculate from the results of the Test for acid of advantame using the following formula:

Total content of other related substances (%) = $[(W_S \times C_S)/W_T] \times [A_{T2}/A_S] \times 1/100$

where
A_{T2} is total peak area other than advantame and other than acid of advantame from the sample solution. The peak areas below the quantitation limit (i.e., 0.02%) are not used in the calculation.
A_S is peak area of acid of advantame from the standard solution;
C_S is purity (%) of acid of advantame Standard Reagent;
W_T is weight (g) of advantame; and
W_S is weight (g) of acid of advantame Standard Reagent.

Residual solvents Determine by GC using the following conditions:
(Tentative)
Preparation of Sample Solution: Accurately weigh about 0.1 g of advantame, and add 1-butanol to make exactly 10 ml.

Preparation of Standard Solution: Accurately weigh 0.1 g methanol, and add 1-butanol to make exactly 50 ml (stock solution 1). Accurately weigh 0.1 g ethyl acetate, and add 1-butanol to make exactly 50 ml (stock solution 2). Pipet and transfer 2.5 ml each of stock solution 1 and stock solution 2 into a 50-ml volumetric flask, and add 1-butanol to make exactly 50 ml (mixture stock solution). Pipet and transfer 2 ml of mixture stock solution into a 20 ml volumetric flask, and add 1-butanol to make exactly 20 ml.

GC conditions:

Column:	DB-WAX (30 m x 0.53 mm i.d., 1.0 µm), J & W Scientific, or equiv.
Column temperature:	40° – 7 min - 80°/min - 220° – 5 min
Detector:	Flame-ionization detector
Carrier gas:	Helium
Carrier gas flow rate:	36 cm/sec (2.8 ml/min)
Injection volume:	1 µl
Detector temperature:	220°
Injector temperature:	120°

Calculation: Calculate the content (µg/g) of each residual solvent using the following formula:

Content of methanol or ethyl acetate (µg/g) = $[W_S \times C_S/W_T] \times [A_T/A_S] \times 10$
where
A_T is a peak area of methanol or ethyl acetate from the sample solution;

A_S is a peak area of methanol or ethyl acetate from the standard solution;
W_S is weight (mg) of methanol or ethyl acetate in the standard solution;
W_T is weight (mg) of advantame sampled;
C_S is purity (%) of methanol or ethyl acetate; and
10 is correction factor for dilution.

METHOD OF ASSAY (Tentative)

Determine by HPLC using the following conditions:

Mobile phase:
Mobile phase A: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 250 ml of acetonitrile to 750 ml of this solution, mix well, and sonicate for about 5 min.

Mobile phase B: Dissolve 13.61 g of potassium dihydrogen phosphate in 1000 ml of water, and adjust the pH to 2.8 with phosphoric acid. Add 500 ml of acetonitrile to 500 ml of this solution, mix well, and sonicate for about 5 min.

Preparation of Internal Standard: Accurately weigh about 40 mg of benzoic acid and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

Preparation of Standard Solution: Accurately weigh about 40 mg of advantame reference standard (available from Ajinomoto Co., Inc., Japan), dissolve in a mixture of water and acetonitrile (7:3 v/v) to make 50 ml. Pipet 10 ml of this solution, add 5 ml of the internal standard solution, and add a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

Preparation of Sample Solution: Accurately weigh about 40 mg of advantame and dissolve in a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml. Pipet 10 ml of this solution, add 5 ml of the internal standard solution, and add a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

HPLC conditions:

Column:	Inertsil ODS-2 (25 cm x 4.6 mm i.d., 5 µm) GL Sciences, or equiv.
Column temperature:	40°
Mobile phase:	
Mobile phase A:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (75:25 v/v)
Mobile phase B:	Mixture of phosphate buffer solution (pH 2.8) and acetonitrile (50:50 v/v)
Flow rate:	1.0 ml/min
Injection volume:	20 µl
Detector:	UV detector at 280 nm
Run Time:	55 min

Gradient program:

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
20	100	0
50	0	100
55	0	100

Calculate the content (%) of advantame using the following formula:

Advantame (%) = $[W_S/W_T] \times [Q_T/Q_S] \times [(100 - W_{std} - S_{std})/(100 - W_{smp} - S_{smp})] \times 100$

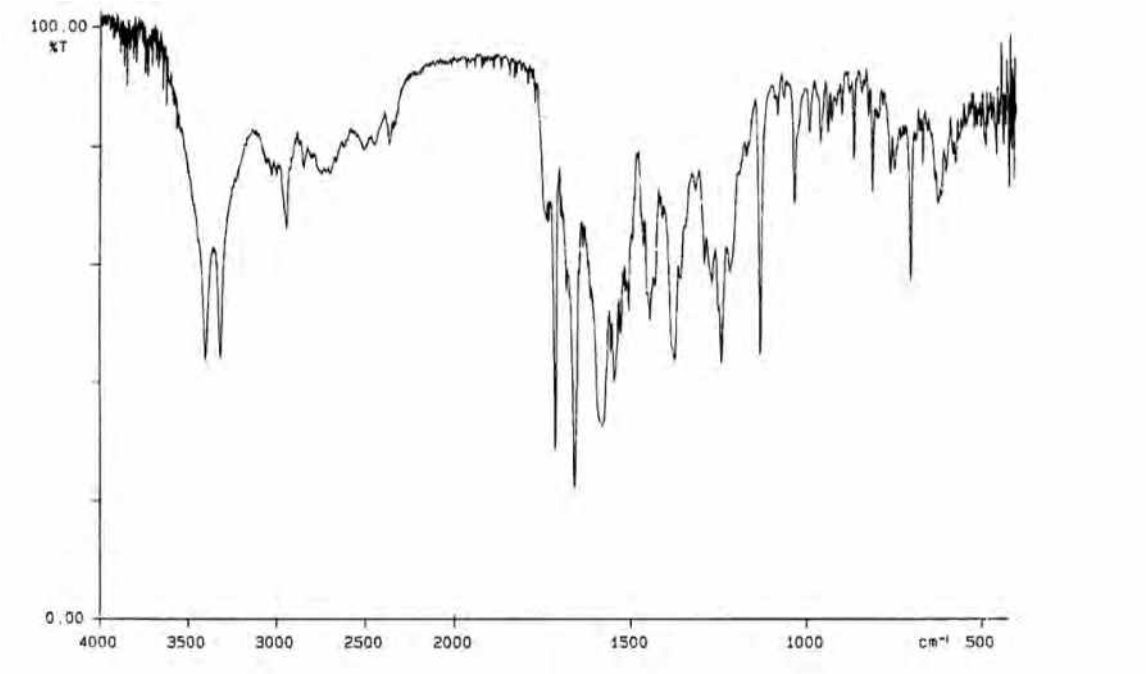
where

Q_T is ratio of the peak area of advantame to that of the internal standard from the sample solution;
Q_S is ratio of the peak area of advantame to that of the internal standard from the standard solution;
S_{std} is residual solvent content (%) of advantame reference standard;
S_{smp} is residual solvent content (%) of advantame sample;
W_S is weight (g) of advantame reference standard sampled;
W_T is weight (g) of advantame sample sampled;
W_{std} is water content (%) of advantame reference standard sample determined by Karl Fischer (Vol.4);
W_{smp} is water content (%) of advantame sample determined by Karl Fischer (Vol.4); and
100 is correction factor

See Appendix B for example of chromatogram obtained using the method.

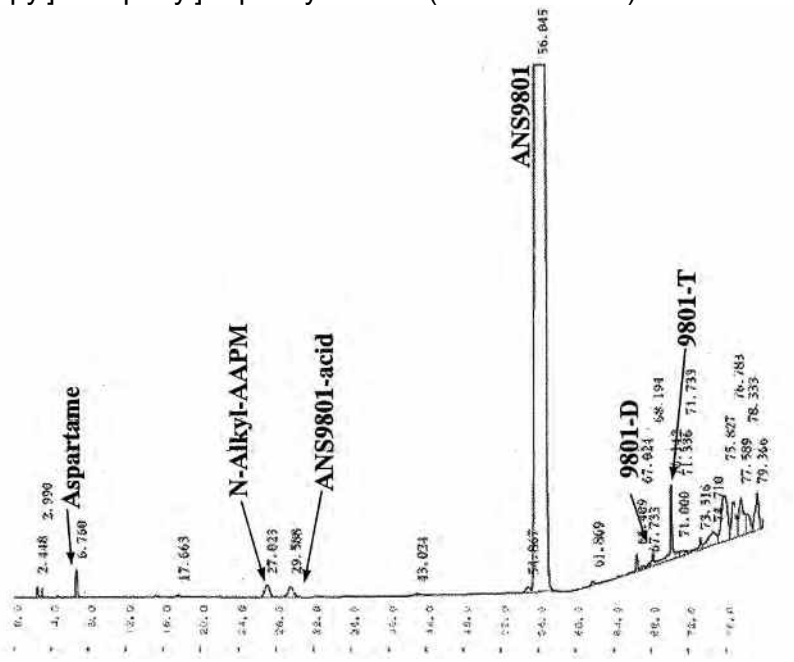
Appendix A

IR spectrum of advantame standard (Ajinomoto Co., Inc.)



Appendix B

Representative chromatogram for advantame (ANS9801) and N-[N-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine (ANS9801-acid) at 210 nm.



Other identified compounds:

- L-α-aspartyl-L-phenylalanine methylester (Aspartame)
- *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine (ANS9801-acid);
- *N*-[*N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-α-L-aspartyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (N-Alkyl-AAPM);
- N-[*N*-[3-(3-hydroxy-4-methoxyphenyl)pentyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (9801-D); and
- *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)heptyl]-α-L-aspartyl]-L-phenylalanine 1-methyl ester (9801-T);

ALUMINIUM SILICATE
(TENTATIVE)

Tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013), superseding specifications prepared at the 57th JECFA (2001) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives.

Information required on:

- *the use as food additive:*
- *Composition and methods of manufacture*
  C.A.S. number and chemical formula
- *Functional uses other than anticaking agent, if used*
- *Data on loss on drying, loss on ignition and pH of a slurry in water*
- *Data, on a minimum of five batches, on the content of calcium, aluminium and silicon using the proposed "Method of assay"*
- *Data on lead, arsenic and mercury content, in a minimum of five batches, carried out in the impurities soluble in 0.5 M hydrochloric acid using the proposed methods.*

SYNONYMS Kaolin, light or heavy; INS No. 559

DEFINITION A native hydrated aluminium silicate, free from most of its impurities carried out by elutriation and drying.

Chemical names — Aluminium silicate

C.A.S. number — Information required

Chemical formula — Information required

Assay — Information required
Not less than XX% and not more than XX% of Al, and not less than XX% and not more than XX% of Si on the dried basis.

DESCRIPTION A soft, whitish powder free from gritty particles; odourless

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) — Insoluble in water, ethanol and mineral acids

Plasticity — To 8 g of the sample add 5 ml of water and mix well. The mixture is plastic

Test for aluminium — Passes test
See description under TESTS

Test for silicon	Passes test See description under TESTS

PURITY

pH	Information required
Loss on drying (Vol. 4)	Information required
Loss on ignition (Vol. 4)	Information required
Water soluble substances	Not more than 0.3% See description under TESTS
Acid soluble substances	Not more than 2% See description under TESTS
Impurities soluble in 0.5 M hydrochloric acid	Lead : Information required Arsenic: Information required Mercury: Information required See description under TESTS

TESTS

IDENTIFICATION TESTS

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

PURITY TESTS

Water soluble substances	Weigh 5 g of the sample to the nearest mg, and boil with 50 ml of water for 30 min, adding water from time to time to maintain approximately the original volume. Filter, evaporate the filtrate to dryness, dry at 105° for 1 h, and weigh. % Water soluble substances = m/[10 x W] where m is the weight of the residue, in mg; and W is the weight of the sample, in g.

Acid soluble substances

Weigh 2 g of the sample to the nearest mg, and boil with 100 ml of dilute hydrochloric acid TS under a reflux condenser for 15 min, cool, and filter. Evaporate 50 ml of the filtrate to dryness, then ignite gently to constant weight.

% Acid soluble substances = m/[5 x W]

where
m is the weight of the residue, in mg; and
W is the weight of the sample, in g.

Impurities soluble in 0.5 M hydrochloric acid

Extract 20 g of finely ground sample under reflux conditions (to prevent loss of mercury) with 100 ml of 0.5 M hydrochloric acid (spectroscopic grade) for 30 min. Let solution cool, then filter through a 0.1 µm membrane filter. Wash the filter twice with hot 0.5 M hydrochloric acid. Combine the filtrate and wash solution in a 200 ml volumetric flask and make up to volume with 0.5 M hydrochloric acid.

Determine arsenic using an AAS (Hydride generation) technique; lead using an AAS (Electrothermal atomization) technique; and mercury using an AAS (Cold vapour generation) technique. See “Metallic impurities” in the Combined Compendium of Food Additive Specifications (Volume 4).

METHOD OF ASSAY

Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, Mix and melt completely using a torch burner and allow to stand at room temperature. Place the reaction product along with crucible into 150 ml hot deionized water in a 250-ml PTFE beaker 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 polypropylene volumetric flask. Wash the beaker three times with hot deionized water, transfer the washings to the volumetric flask and make up to volume. Dilute with 2% hydrochloric acid and prepare the test solution. Analyse aluminium and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer. Use analytical lines for Al (396.152 nm) and Si (251.611 nm) and construct standard curve using standard solutions 0.2 – 5.0 µg/ml each. Read the concentration of Al and Si in sample solution (as µg/ml) and calculate the aluminium and silicon content of the sample using the formula:

$$\text{Al or Si (\%)} = \frac{C \times 250 \times DF}{W \times 10^6} \times 100$$

Where: C is concentration of Al or Si in the test solution, µg/ml
W is weight of sample, g
DF is dilution factor

CALCIUM ALUMINIUM SILICATE
(TENTATIVE)

Tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013), superseding specifications prepared at the 57th JECFA (2001) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives.

Information required on the use as food additive:

- *Composition and methods of manufacture*
- *C.A.S. number and chemical formula*
- *Functional uses other than anticaking agent, if used*
- *Data on pH of a slurry in water*
- *Data, on a minimum of five batches, on the content of calcium, aluminium and silicon using the proposed "Method of assay"*
- *Data on lead, arsenic and mercury content, in a minimum of five batches, carried out in the impurities soluble in 0.5 M hydrochloric acid using the proposed methods.*

SYNONYMS Aluminium calcium silicate; calcium aluminosilicate; calcium silicoaluminate; aluminosilicic acid calcium salt; silicic acid aluminum calcium salt; INS No. 556

DEFINITION Information required

Chemical names Aluminium calcium silicate

C.A.S. number Information required

Chemical formula Information required

Assay Information required
Not less than XX% and not more than XX% of silicon (Si)
Not less than XX% and not more than XX% of aluminium (Al)
Not less than XX% and not more than XX% of calcium (Ca)

DESCRIPTION Fine, white, free-flowing powder

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Test for calcium Passes test
See description under TESTS

Test for aluminium Passes test
See description under TESTS

Test for silicon Passes test
See description under TESTS

PURITY

pH	Information required
Loss on drying (Vol. 4)	Not more than 10% (105°, 2 h)
Loss on ignition (Vol. 4)	Not less than 14% and not more than 18% (ignition at 1000° to constant weight)
Fluoride (Vol. 4)	Not more than 50 mg/kg Weigh 1 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method III).
Impurities soluble in 0.5 M hydrochloric acid	Lead : Information required Arsenic: Information required Mercury: Information required See description under TESTS

TESTS

IDENTIFICATION TESTS

Test for calcium, aluminium and silicon	Prepare the test solution as shown under method of assay. Analyze aluminum and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical lines for Ca (393.366 nm), Al (396.15 nm) and Si (251.611 nm).

PURITY TESTS

Impurities soluble in 0.5 M hydrochloric acid	Extract 20 g of finely ground sample under reflux conditions (to prevent loss of mercury) with 100 ml of 0.5 M hydrochloric acid (spectroscopic grade) for 30 min. Let solution cool, then filter through a 0.1 µm membrane filter. Wash the filter twice with hot 0.5 M hydrochloric acid. Combine the filtrate and wash solution in a 200 ml volumetric flask and make up to volume with 0.5 M hydrochloric acid. Determine arsenic using an AAS (Hydride generation) technique; lead using an AAS (Electrothermal atomization) technique; and mercury using an AAS (Cold vapour generation) technique. See “Metallic impurities” in the Combined Compendium of Food Additive Specifications (Volume 4).
METHOD OF ASSAY	Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, mix and melt completely using a torch burner and allow to stand at room temperature. Place the reaction product along with crucible into 150 ml hot deionized water in a 250-ml PTFE beaker 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 polypropylene 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, to get the readings within the standard curve range. Analyze silica, aluminium and calcium in the

test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical lines for Si (251.611 nm), Al (396.152 nm) and Ca (393.366 nm) and construct standard curve using standard solutions 0.1 – 5.0 µg/ml each. Read the concentration of Si, Al and Ca in test solution (as µg/ml) and calculate the silicon, aluminium and calcium content of the sample using the formula:

$$\text{Si, Al or Ca (\%)} = \frac{C \times 250 \times DF}{W \times 10^6} \times 100$$

Where

C is concentration of Si, Al or Ca in the test solution, µg/ml;
DF is dilution factor for the dilution of Solution A to test solution
W is weight of sample, g

CALCIUM SILICATE
(TENTATIVE)

Tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013), superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). An ADI 'not specified' for silicon dioxide and certain silicates including calcium silicate was established at the 29th JECFA (1985)

Information required on the:

- *Use as food additive:*
- *Composition and methods of manufacture*
- *Functional uses other than anticaking agent, if used*
- *Data, on a minimum of five batches, on the content of calcium and silicon using the proposed "Method of assay"*
- *Data on loss on drying, loss on ignition, and pH of a 10% water slurry*
- *Data on lead, arsenic and mercury content in a minimum of five batches, carried out in the impurities soluble in 0.5 M hydrochloric acid using the proposed methods.*

SYNONYMS INS No. 552

DEFINITION A synthetic hydrous calcium silicate or polysilicate prepared by various reactions between siliceous material (e.g. diatomaceous earth) and natural calcium compounds (e.g. lime with varying proportions of other elements, such as magnesium, etc).

Chemical names Calcium silicate

C.A.S. number 1344-95-2

Chemical formula Information required

Assay Information required
Not less than XX% and not more than XX% of calcium and not less than XX%, and not more than XX% of silicon on the dried basis.

DESCRIPTION A very fine, white or off-white powder with low bulk density and high physical water adsorption

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ethanol

Test for calcium	Passes test See description under TESTS
Test for silicon	Passes test See description under TESTS

PURITY

pH	Information required
Loss on drying (Vol. 4)	Information required
Loss on ignition (Vol. 4)	Information required
Fluoride (Vol. 4)	Not more than 50 mg/kg Weigh 1 g of the sample to the nearest mg, and proceed as directed in the Limit Test (Method II).
Impurities soluble in 0.5 M hydrochloric acid	Lead : Information required Arsenic: Information required Mercury: Information required See description under TESTS

TESTS

IDENTIFICATION TESTS

Test for calcium and silicon	Prepare the test solution as shown under method of assay. Analyze aluminum and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical lines for Ca (393.366 nm) and Si (251.611 nm).

PURITY TESTS

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

METHOD OF ASSAY Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, Mix and melt completely using a torch burner and allow to stand at room temperature. Place the reaction product along with crucible into 150 ml hot deionized water in a 250-ml PTFE beaker 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 polypropylene volumetric flask. Wash the beaker three times with hot deionized water, transfer the washings to the volumetric flask and make up to volume. Dilute with 2% hydrochloric acid and prepare the test solution. Analyse aluminium and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer. Use analytical lines for Ca (393.366 nm) and Si (251.611 nm) and construct standard curve using standard solutions 0.2 – 5.0 µg/ml each. Read the concentration of Ca and Si in sample solution (as µg/ml) and calculate the calcium and silicon content of the sample using the formula:

$$\text{Ca or Si (\%)} = \frac{C \times 250 \times DF}{W \times 10^6} \times 100$$

Where: C is concentration of Ca or Si in the test solution, µg/ml
W is weight of sample, g
DF is dilution factor

GLUCOAMYLASE FROM *TRICHODERMA REESEI* EXPRESSED IN *TRICHODERMA REESEI*

New specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI "not specified" was established at the 77th JECFA (2013).

SYNONYMS Amyloglucosidase; γ-amylase; lysosomal α-glucosidase; acid maltase; exo-1,4-α-glucosidase; glucose amylase; γ-1,4-glucan glucohydrolase; acid maltase; and 1,4-α-D-glucan

SOURCES Produced by submerged straight-batch or fed-batch fermentation of a genetically modified non-pathogenic, non-toxigenic strain of *Trichoderma reesei* which contains a gene coding for glucoamylase from *T. reesei.* The enzyme is secreted to the fermentation broth. The cell mass along with the solid waste slurry carrying the residual microorganism is separated from the enzyme by centrifugation and/or filtration. The liquid enzyme filtrate is concentrated by ultrafiltration followed by diafiltration to remove colour. The product is further polish-filtered and formulated using food-grade stabilizing agents and standardized to the desired activity.

Active principles: Glucoamylase

Systematic names and numbers: Glucan 1,4-α-glucosidase; EC 3.2.1.3; CAS No. 9032-08-0

Reactions catalysed: Hydrolysis of terminal (1→4)-linked α-D-glucose residues successively from non-reducing ends of the chains with release of β-D-glucose.

Secondary enzyme activities: No significant levels of secondary enzyme activities

DESCRIPTION Amber liquid

FUNCTIONAL USES Enzyme preparation.
Used in the manufacture of corn sweeteners such as high fructose corn syrup, baking, brewing and potable alcohol manufacture

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

CHARACTERISTICS

IDENTIFICATION

Glucoamylase activity: The sample shows glucoamylase activity
See description under TESTS.

TESTS

Glucoamylase activity

Principle

Glucoamylase hydrolyses the substrate *p*-nitrophenyl-alpha-D-glucopyranoside (PNPG) to glucose and *p*-nitrophenol (PNP) at alkaline pH. The released PNP is proportional to enzyme activity and measured at 400 nm. Enzyme activity is expressed in GlucoAmylase Units (GAU). One GAU is defined as the amount of glucoamylase that releases one gram of glucose per hour (= 5.6 mmol of glucose per hour) from soluble starch substrate at pH 4.3 and temperature of 30 º.

Apparatus

Spectrophotometer (400 nm)
Water bath with thermostatic control (40 º ± 1)
Water bath with thermostatic control (30 º ± 1)
Vortex mixer
Magnetic stir plate and stir bars
Positive displacement and repeater pipettes

Reagents and solutions

Preparation of Sodium Acetate buffer (0.1 M, pH 4.3):
Weigh 4.4 g of sodium acetate trihydrate ($NaC_2H_3O_2.3H_2O$) and transfer to a 1-litre volumetric flask. Add 800 ml of deionised water and mix until dissolved. Adjust the pH to 4.30 ± 0.05 with glacial acetic acid. Make up the volume to 1000 ml with water and mix. This solution can be stored at 4° for two weeks.

Preparation of Borax Solution (0.1 M, pH 9.2):
Weigh and transfer 19.04 g of sodium borate decahydrate to a 500 ml volumetric flask. Add 400 ml of water and mix until dissolved. Measure pH. Adjust pH, if needed, to 9.20 $\pm$ 0.05. Bring the volume to 500 ml with water and mix. The solution can be stored at room temperature for six weeks.

Preparation of PNPG substrate (1.1 mg/ml) (light sensitive):
Weigh 55.0$\pm$0.5 mg of PNPG substrate (Sigma) in a beaker. Add 40 ml of Sodium Acetate buffer (0.1 M, pH 4.3) and stir on a magnetic stirrer until dissolved (may require heating in a water bath at 30 º). Transfer contents into a 50 ml volumetric flask. Make up the volume to 50 ml. Transfer the solution to an amber bottle for storage. This solution can be stored at 4 º for two weeks.

Preparation of Standard and Sample Solutions:

Preparation of Stock Standard Solution:
Heat a 1 ml aliquot of glucoamylase standard solution with the activity expressed in GAU/ml as reported on the Certificate of Analysis, in a water bath at 40 º for 10 min. Label as Stock Standard Solution.

Preparation of Working Standard Solutions:
Dilute different aliquots of the stock standard with the 0.1M Sodium Acetate Buffer to obtain at least three working standards with concentrations that fall within the linear range of the assay (the linear range of the assay is ~0.5 - 2.4 GAU/ml) after subtracting blank. The

prepared solutions should be kept at room temperature and must be used within 2 h of their preparation.

Preparation of sample:
Heat a 1 ml aliquot of glucoamylase sample in a water bath at 40 º for 10 min. Dilute the glucoamylase sample with the Sodium Acetate buffer. The solution should be kept at room temperature and used within 2 h of its preparation.

Procedure

Preheat water bath to 30 º. Prepare duplicate tubes in a rack and label as Working Standard, Sample and Blank. Using a positive displacement pipette, dispense 250 µl of 0.1M Sodium Acetate buffer, pH 4.3 to each labeled tube.

Using a positive displacement pipette, add 200 µl of each Working Standard and Sample to the respective labeled tubes. Add an additional 200 µl of Sodium Acetate buffer to the blank tube. Place the tubes in the 30 º water bath for 5 min. Add to each set of duplicate tubes, 500 µl of 1.1 mg/ml PNPG substrate solution with a repeater pipette at timed intervals and vortex for 3 sec each. Incubate all tubes in a water bath for 10± 0.1 min from the time of addition of the substrate. Remove from the water bath and immediately add 1.0 ml of 0.1M Borax solution at the same time interval used for the substrate addition using a repeater pipette to each tube. Vortex each tube for 3 sec and place on a second rack. Transfer the standards, samples and blanks to 1.5 ml cuvettes. Measure the absorbances at 400 nm, after appropriately zeroing with blank. Prepare the standard curve using linear regression. The correlation coefficient must be ≥0.99. Determine the glucoamylase concentration of each sample from the standard curve.

Weigh sample. Record the value as density in g/ml, up to two significant figures.

Calculate the glucoamylase activity for each sample of the glucoamylase preparation in GAU/g as follows:

$$\text{Glucoamylase activity, GAU/g} = \frac{C \times D}{\text{Sample density}}$$

Where:

C is the glucoamylase concentration of the sample determined from the standard curve (GAU/ml), and

D is the dilution factor of the sample divided by the sample density (g/ml)

GLYCEROL ESTER OF GUM ROSIN
(TENTATIVE)

Prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013), superseding tentative specifications prepared at the 74th JECFA (2011), published in FAO JECFA Monographs 11 (2011). A temporary group ADI of 0-12.5 mg/kg bw for glycerol ester of gum rosin and glycerol ester of wood rosin was withdrawn at the 77th JECFA (2013).

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 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 esters of resin acids with regard to the levels (%) of:*
  - *a) glycerol monoesters;*
  - *b) Sum of glycerol di- and tri- esters (assay)*

NOTE:
Detailed data and information using the validated methods including sample preparation 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 glycerol di- and tri-esters of resin acids from gum rosin, with a residual fraction of glycerol monoesters. Besides these esters, up to x % free 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 glycerol di- and tri- 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

Glycerol monoesters:	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

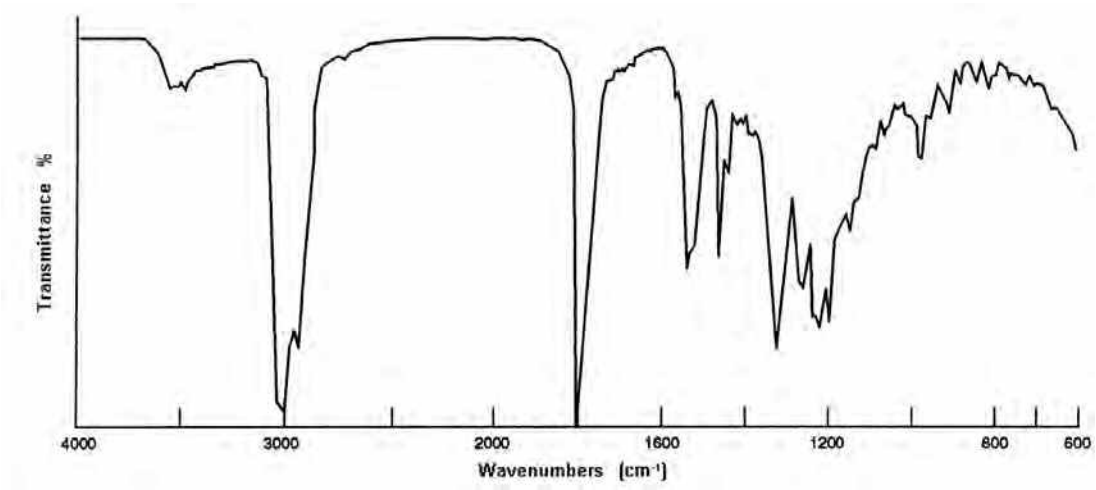
PURITY TESTS

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

METHOD OF ASSAY

Sum of glycerol di- and tri- esters: Information required

Infrared spectrum



NOTE: The IR spectrum for glycerol ester of gum rosin is referenced from the Food Chemicals Codex, 8th Edition, 2012, p. 501. Reprinted with permission from the US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

GLYCEROL ESTER OF WOOD ROSIN

Prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013), superseding tentative specifications prepared at the 74th JECFA (2011), published in FAO JECFA Monographs 11 (2011). An ADI of 0-25 mg/kg bw for glycerol ester of wood rosin was established at the 77th JECFA (2013).

SYNONYMS INS No. 445(iii)

DEFINITION Glycerol ester of wood rosin is a complex mixture of glycerol di- and tri-esters of resin acids from wood rosin, with a residual fraction of glycerol monoesters. Besides these esters, neutrals (non-acidic saponifiable and unsaponifiable substances) and residual free resin acids 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 approximately 90% resin acids and approximately 10% neutrals. The resin acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, 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

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)

Gas chromatography of resin alcohols and glycerol	Passes test See description under TESTS

PURITY

Specific gravity (Vol. 4)	d (20, 25): Not less than 0.935 (50% solution in d-limonene)
Ring and ball softening point (Vol. 4)	Not less than 82° (see "Specific Methods, Glycerol Esters of Rosins")
Acid value (Vol. 4)	Between 3 and 9 (see "Specific Methods, Fats, Oils, and Hydrocarbons")
Lead (Vol. 4)	Not more than 1 mg/kg Determine using 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

IDENTIFICATION TESTS

Gas chromatography of resin alcohols and glycerol

The complex ester groups in the glycerol esters of wood rosin are reduced by reaction with a metal hydride (sodium bis(2-methoxy-ethoxy) aluminium dihydride) in toluene solution to form a mixture of resin alcohols and glycerol. Excess reagent is then hydrolyzed with aqueous acid forming two phases. Gas chromatography of the toluene phase produces a characteristic chromatogram of the resin alcohols that abietyl and dehydroabietyl alcohols are predominates. Confirm the presence of two predominate peaks of both resin alcohols with the typical gas chromatogram below. Chromatography of the neutralized aqueous phase on a different column verifies the presence of glycerol.

Apparatus

Gas Chromatograph equipped with a flame ionization detector.
Recorder: 0 to 1 V
Analytical Balance: capable of weighing to the nearest 0.001 g
Centrifuge: table top, capable of achieving 3200 rpm

Reagents

Toluene, reagent grade.
Sodium Vitride™ Reagent [Sodium bis(2-methoxyethoxy) aluminium dihydride, pract. ~ 70% in toluene (~ 3.5 mol/l)] (Fluka Chemical Corp., Hauppage, NY, USA, or equivalent). Pipet 10.0 ml into a 100 ml volumetric flask. Dilute to volume with toluene and mix thoroughly.
Hydrolysis Solution: Slowly add 50 ml of concentrated sulfuric acid, reagent grade, to 200 ml distilled water while stirring in an ice bath. Cool to room temperature.
Phenolphthalein Solution: 1% in ethanol.
Sodium Hydroxide Solution: Dissolve 16 g of reagent grade NaOH in 70-80 ml of distilled water and cool to room temperature. Dilute to 100 ml with distilled water and mix thoroughly. Store in a polyethylene bottle.
1,4-Butanediol: 99+%

Glycerol: 99+%
Internal Standard Solution: weigh 0.1 g of 1,4-butanediol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly.
Glycerol Solution: weigh 0.1 g of 1,4-butanediol and 0.1 g glycerol into a 100 ml volumetric flask. Dilute to volume with distilled water and mix thoroughly.

Procedure I (Resin alcohols)
Weigh 250-300 mg sample into a 25 ml Erlenmeyer flask containing a stirring bar (Teflon coated, 1 inch). Pipet 5.0 ml toluene into the flask and stir magnetically until sample is dissolved. Pipet 5.0 ml of Sodium Vitride™ Reagent into the flask, cap, and stir for 30 min. Uncap and, while stirring, pipet 3.0 ml of Hydrolysis Solution into the flask. Continue stirring for 3 min. Transfer contents of flask to centrifuge tube (15 mL), stopper, and shake vigorously. Vent and centrifuge at 2800-3200 rpm for 5 min. Inject 0.5 µl of the upper layer into the gas chromatograph operating under the specified conditions and record the chromatogram. Compare with the chromatograms shown below to verify the approximate retention order of the resin alcohols.

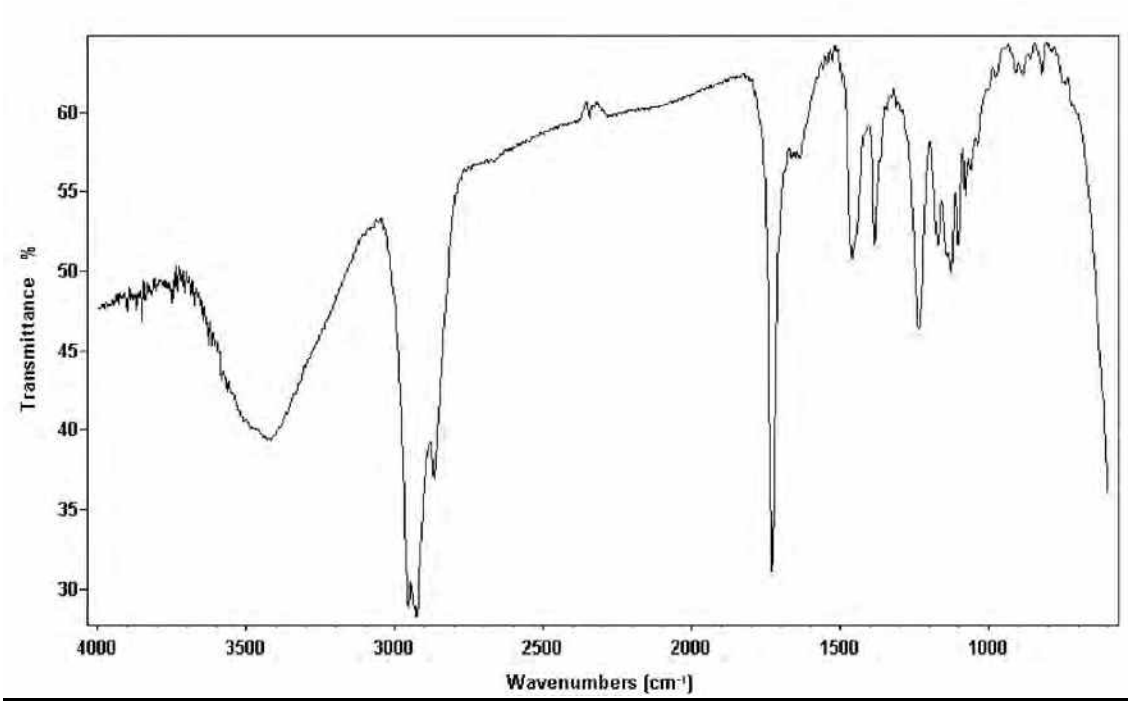
Chromatography conditions
Column: DB-1 methyl silicone (bonded and crosslinked), 15 m, wide-bore capillary (0.53 mm i.d.), film thickness 1.5 µm, temperature range 60º to 300/320º (e.g., J & W Scientific Inc,. Cat. No. 125-1012). A direct flash vaporization injection port liner is recommended.
Flow Rates
Carrier Gas (He): 30 ml/min at 63 psi
Hydrogen: 30 ml/min
Air: 240 ml/min
Temperatures
Column: Isothermal, 190º
Inlet: 250º
Detector: 250º

Procedure II (Glycerol)
Using a pipet or hypodermic syringe, remove the toluene layer and part of the aqueous layer leaving approximately 2 ml of the aqueous layer in the centrifuge tube. Add 1 drop of phenolphthalein solution and neutralize with the Sodium Hydroxide Solution. Aluminium salts will precipitate. Pipet 5 ml of the Internal Standard Solution into the tube, dilute to 15 ml with distilled water, stopper, shake, and then centrifuge at 2800-3200 rpm for 5 min. Inject 1 µl of the clear supernatant liquid into the gas chromatograph operating under the specified conditions and record the chromatogram.
Inject 1 µl of the Glycerol Solution and record the chromatogram. Measure the retention times of any observed peaks relative to 1,4-butanediol. Compare retention times to that of glycerol.

Chromatography conditions
Column: DB-WAX polyethyleneglycol (bonded and cross-linked), 15 m, wide bore capillary (0.53 mm i.d.), film thickness 1.0 µm, temperature range 20º to 230º (e.g., J & W Scientific Inc., Cat, No. 125-7012).
Flow Rates
Carrier Gas (He): 30 ml/min at 60 psi
Hydrogen: 30 ml/min
Air: 240 ml/min

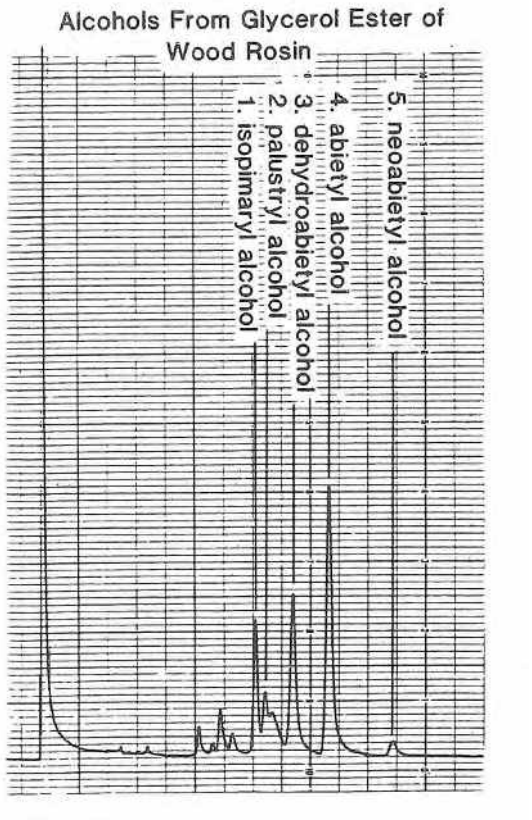
Temperatures
Column: Programmed, 120 to 200º at 6º /min
Inlet: 250º
Detector: 250º

Infrared spectrum



NOTE: The IR spectrum for glycerol ester of wood rosin is referenced from the Food Chemicals Codex, 8th Edition, 2012, p. 506. Reprinted with permission from the US Pharmacopeial Convention, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

Gas chromatography of resin alcohols



MINERAL OIL (MEDIUM VISCOSITY)

Prepared at the 77th JECFA (2013), published in FAO JECFA Monographs 14 (2013), superseding specifications prepared at the 76th JECFA and published in FAO JECFA Monographs 13 (2012). An ADI of 0-10 mg/kg bw was established at the 59th JECFA for mineral oil (medium viscosity).

SYNONYMS Liquid paraffin, liquid petrolatum, food grade mineral oil, white mineral oil, INS No. 905e

DEFINITION A mixture of highly refined paraffinic and naphthenic liquid hydrocarbons with boiling point above 200°; obtained from mineral crude oils through various refining steps (eg. distillation, extraction and crystallisation) and subsequent purification by acid and/or catalytic hydrotreatment; may contain antioxidants approved for food use.

DESCRIPTION Colourless, transparent and odourless oily liquid, without fluorescence in daylight.

FUNCTIONAL USES Release agent, glazing agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, sparingly soluble in ethanol, soluble in ether

Burning Burns with bright flame and with paraffin-like characteristic smell

PURITY

Viscosity, 100° (Vol. 4) 8.5-11 mm^2/s

Carbon number at 5% distillation point (Vol. 4) Not less than 5% of molecules with a carbon number less than 25 Boiling point at the 5% distillation point higher than 391°.

Average molecular weight (Vol. 4) 480-500

Acidity or alkalinity To 10 ml of the sample add 20 ml of boiling water and shake vigorously for 1 min. Separate the aqueous layer and filter. To 10 ml of the filtrate, add 0.1 ml of phenolphthalein solution TS. Not more than 0.1 ml of 0.1N sodium hydroxide or 0.1N HCl is required to change the colour.

Readily carbonizable substances Place 5 ml of the sample in a glass-stoppered test tube that has previously been rinsed with hot water, acetone, heptane and finally acetone. Add 5 ml of sulfuric acid TS, and heat in a boiling water bath for 10 min. After the test tube has been in the bath for 30 sec, remove it quickly, and while holding the stopper in place, give three vigorous vertical shakes over an amplitude of about 10 cm. Repeat every 30 sec. Do not keep the test tube out of the bath longer than 3 sec for each shaking period. At the end of 10 min from the time when first placed in the water bath, remove the test tube. The sample remains unchanged in colour, and the acid does not become darker than standard colour

produced by mixing in a similar test tube 3 ml of ferric chloride TSC, 1.5 ml of cobaltous chloride TSC, and 0.5 ml of cupric sulfate TSC, this mixture being overlaid with 5 ml of mineral oil.

Polycyclic aromatic hydrocarbons

Use hexane, dimethyl sulfoxide and trimethylpentane in quality specified for ultraviolet spectrometry.
Transfer 25.0 ml of sample to a 125 ml separating funnel with unlubricated ground-glass parts (stopper, stopcock). Add 25 ml of hexane which has been previously shaken twice with one-fifth its volume of dimethyl sulfoxide. Mix and add 5.0 ml of dimethyl sulfoxide. Shake vigorously for 1 min and allow to stand until two clear layers are formed. Transfer the lower layer to a second separating funnel, add 2 ml of hexane and shake the mixture vigorously. Allow to stand until two clear layers are formed. Separate the lower layer and measure its absorbance between 260 nm and 420 nm, against a blank solution of dimethyl sulfoxide obtained by vigorously shaking 5.0 ml of dimethyl sulfoxide with 25 ml of hexane for 1 min. Prepare a reference solution in trimethylpentane containing 7.0 mg of naphthalene per litre and measure the absorbance of the solution at the maximum at 275 nm, using trimethylpentane as the compensation liquid. The absorbance of the test solution, in the wavelength region between 260 nm and 420 nm, shall not exceed one-third that of the reference solution at 275 nm.

Solid paraffins

Dry a suitable quantity of the substance to be examined by heating at 100° for 2 h and cool in a desiccator over concentrated sulfuric acid. Place in a glass tube with an internal diameter of about 25 mm, close the tube and immerse in a bath of iced water. After 4 h the liquid is sufficiently clear for a black line, 0.5 mm wide against a white background held vertically behind the tube, to be easily seen.

Lead (Vol. 4)

Not more than 1 mg/kg
Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

MODIFIED STARCHES

Prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013), superseding the specifications prepared at the 76th JECFA (2012) and published in FAO JECFA Monographs 13 (2012). 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).

This is a partial revision of the modified starches specifications. Degree of substitution in starch sodium octenyl succinate under "methods for additional purity specifications" is replaced as below. Other sections of specifications remain unchanged.

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

Octenylsuccinyl groups in starch sodium octenyl succinate

Procedure

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

Calculation

$$\text{Octenyl succinyl groups (\%)} = \frac{21.1 \times V \times N}{W}$$

where
V is the volume of sodium hydroxide, ml
N is the normality of sodium hydroxide
W is the weight of sample, g

NISIN

Prepared at the 77th JECFA (2013), and published in FAO JECFA Monographs 14 (2013), superseding specifications for Nisin prepared at the 71st JECFA (2009). An ADI of 0–2 mg/kg bw was established at the 77th JECFA.

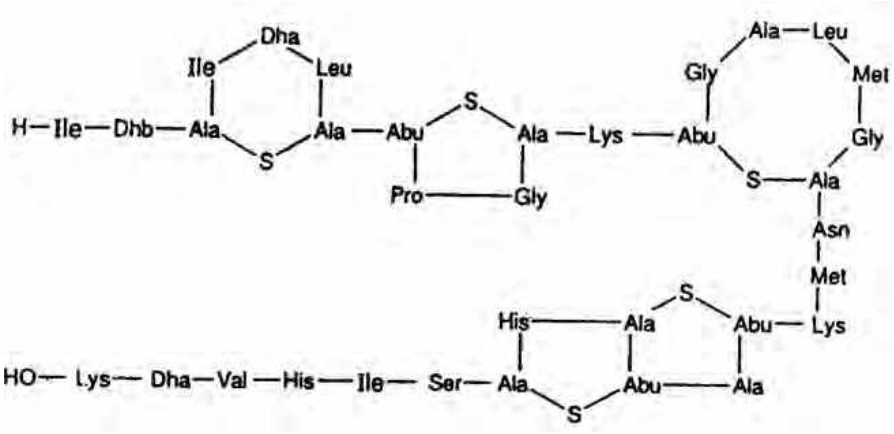
SYNONYMS INS No. 234

DEFINITION Nisin is a mixture of closely related antimicrobial polypeptides produced by strains of *Lactococcus lactis* subsp. *lactis* under appropriate fermentation conditions. The major polypeptide from the fermentation is Nisin A.
Nisin is produced in a sterilized medium of non-fat milk solids or non-milk-based fermentation source, such as yeast extract and carbohydrate solids. The fermentation process is controlled for time and pH, until optimum nisin production has been achieved. The nisin is then concentrated, recovered and purified from the fermentation medium by various methods, such as sterile injection, membrane filtration, acidification, salting out, ultrafiltration or spray-drying. The purified nisin is then standardized with sodium chloride to achieve desired activity levels of nisin preparation.
Nisin is stable at ambient temperatures and when heated under acidic conditions (up to pH 3). Nisin is commercially available as nisin preparation, which contains 2.5% w/w nisin, >50% sodium chloride; the remaining components of the preparation are milk solids and products of fermentation that include proteins and carbohydrates.
The activity of nisin is measured in International Units (IU). 1 IU of nisin is equivalent to 0.025 µg.

C.A.S. Number 1414-45-5

Chemical formula $C_{143}H_{230}O_{37}N_{42}S_7$ (Nisin A)

Structural formula



Abu=alpha-aminobutyric acid, Dha=dehydroalanine, Dhb=dehydrobutyrine (Nisin A)

Formula weight 3354.12 (Nisin A)

Assay Not less than 900 IU of nisin per milligram (or 22.5 microgram/milligram)

DESCRIPTION White to light brown micronized powder

FUNCTIONAL USES Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Soluble in water and insoluble in non-polar solvents
Differentiation from other antimicrobial substances	Passes tests See description under TESTS
Nisin activity	The sample shows nisin activity See description under METHOD OF ASSAY

PURITY

Loss on drying (Vol. 4)	Not more than 3.0% (105°, 2 h)
Sodium Chloride	Not less than 50% See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS (Electrothermal Atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).
Microbiological criteria (Vol. 4)	*Salmonella* species: Absent in 25 g of sample Total coliforms: Not more than 30 per gram *Escherichia coli*: Absent in 25 g of sample

TESTS

IDENTIFICATION TESTS

Differentiation from other antimicrobial substances

Stability to acid

Sample stock solution: Suspend 1 g of sample in 1 L of 0.02 N hydrochloric acid to give a solution containing 1000 IU/mL.

Sample Preparation: Make a dilution of the Sample stock solution with 0.02 N hydrochloric acid to arrive at a concentration of 50 IU/ml. Boil this solution for 5 min and measure the nisin activity as directed under ‘Determination of Nisin Activity’, in METHOD OF ASSAY.
The calculated nisin concentration of the boiled sample should be 100% (+/- 5%) of the assay value indicating no significant loss of activity following this heat treatment.

Instability to Alkali

Adjust the pH of the unused portion of the boiled nisin solution from ‘Stability to acid’ to 11.0 by adding 5 N sodium hydroxide. Heat the

solution at 65° for 30 min, and then cool. Adjust the pH to 2.0 by adding hydrochloric acid dropwise. Measure the nisin activity as directed under 'Determination of Nisin Activity' in METHOD OF ASSAY. Record loss of the antimicrobial activity of nisin following this treatment. Complete loss of the antimicrobial activity should be observed following the treatment described.

Tolerance of *Lactococcus lactis* to high concentrations of Nisin
Prepare cultures of *L. lactis* (ATCC 11454, NCIMB 8586) in sterile skim (<1% fat) milk by incubating for 18 h at 30°. Prepare one or more flasks containing 100 ml of litmus milk, and sterilize at 121° for 15 min. Suspend 0.1 g of sample in the sterilized litmus milk, and allow to stand at room temperature for 2 h. Add 0.1 ml of the *L. lactis* culture, and incubate at 30° for 24 h. Record *L. lactis* growth. *L. lactis* will grow at this concentration of sample (about 1000 IU/ml); however, it will not grow in similar concentrations of other antimicrobial substances. (NOTE: This test will not differentiate nisin from subtilin.)

PURITY TESTS

Determination of sodium chloride
Transfer about 200 mg of the sample, accurately weighed, into a glass-stoppered flask containing 50 ml of water. Agitate the flask to dissolve the sample while adding 3 ml of nitric acid, 5 ml of nitrobenzene, 50.0 ml of standardized 0.1 N silver nitrate, and 2 ml of ferric ammonium sulfate TS. Shake the solution well, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate. The titration endpoint is indicated by the appearance of a red colour. Calculate the percentage of sodium chloride in the sample taken by the equation:

$$\text{Sodium chloride, \%} = \frac{100 \times 58.44 \times [(50 \times A) - (V \times B)]}{W}$$

where
A is the concentration of the silver nitrate solution;
B is the concentration of the ammonium thiocyanate solution;
V is the volume of the ammonium thiocyanate in ml; and
W is the weight of the sample in mg

METHOD OF ASSAY

Principle
Nisin activity, expressed in International Units (IU), refers to the amount of nisin required to inhibit growth of 1 bacterial cell in 1 millilitre of broth. 1 IU of nisin is equivalent to 0.025 µg. Commercial nisin preparations consist typically of 2.5% w/w of nisin along with sodium chloride and milk-fat solids.

Determination of Nisin Activity

Preparation of the test organism
*Lactococcus lactis subsp. cremoris (*ATCC 14365, NCDO 495) is subcultured daily in sterile separated milk by transferring one loopful to a McCartney bottle of litmus milk and incubating at 30°. Prepare inoculated milk for the assay by inoculating a suitable quantity of sterile skim milk with 2 percent of a 24 h culture, and place it in a water-bath at 30° for 90 min. Use immediately.

Standard stock solution
Dissolve an accurately weighed quantity of standard nisin in 0.02 N hydrochloric acid to give a solution containing 5,000 IU/ml. Immediately before use, dilute the solution further with 0.02 N hydrochloric acid to give

50 units/ml. (NOTE: Nisin containing 2.5% w/w nisin, at a minimum potency of 10^6 IU nisin per gram (IU/g) is obtainable from Sigma, St. Louis, USA or Fluka, Buchs, Switzerland. A preparation under the name of Nisaplin, containing at a minimum potency of $3x10^6$ IU/g, of nisin available from DuPont Nutrition Biosciences, Copenhagen, Denmark, may also be used for the Standard stock solution).

Sample solution

Weigh an amount of sample sufficient to ensure that corresponding tubes of the sample and standard series match, i.e., within close limits, so that the nisin content in the sample and standard are similar. Dilute the sample solution in 0.02 N hydrochloric acid to obtain an approximate concentration of 50 IU per ml (IU/ml).

Resazurin solution

Prepare a 0.0125% w/v solution of resazurin in water immediately prior to use.

Procedure

Pipet graded volumes (0.60, 0.55, 0.50, 0.45, 0.41, 0.38, 0.34, 0.31, 0.28, 0.26 ml) of the 50 IU/ml sample and standard solutions into two rows of 10 dry 6-inches x 5/8-inch bacteriological test tubes. Add 4.6 ml of the inoculated milk to each by means of an automatic pipetting device. (NOTE: The addition of inoculated milk should be made in turn across each duplicate row of tubes containing the same nominal concentration, and not along each row of ten tubes). Place the tubes in a water-bath at 30° for 15 min, then cool in an ice-water bath while adding 1 ml resazurin solution to each. Add the resazurin solution in the same order as the addition of inoculated milk, using an automatic pipetting device. Thoroughly mix the contents of the tubes by shaking. Continue incubation at 30° in a water bath for a further 3-5 min.

Examine the standard and sample tubes under fluorescent light in a black matte-finish cabinet. Compare the sample tube of the highest concentration that shows the first clear difference in colour (i.e., has changed from blue to mauve) with tubes of the standard to find the nearest match in colour. Make further matches at the next two lower concentrations of the sample with the standard. Interpolation of matches may be made at half dilution steps. Obtain three readings of the sample solution and average them. Calculate the activity of nisin in the sample from the standard nisin activities.

Convert nisin activity from IU to µg nisin, using the conversion factor 1 IU = 0.025µg

OCTENYL SUCCINIC ACID MODIFIED GUM ARABIC
(TENTATIVE)

Tentative specifications prepared at the 77th JECFA (2013) and published in the FAO Monographs 14 (2013), superseding specifications prepared at the 74th JECFA (2011) and published in the FAO Monographs 11 (2011). A temporary ADI "not specified" was established at the 71st JECFA (2009).

Information required:

- *Updated analytical method for the determination of the degree of substitution (DS) and results of at least five different batches of commercially available product.*

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 substitution Information required

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

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

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

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

Calculation

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

where

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

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

PAPRIKA EXTRACT

Prepared at the 77th JECFA, published in FAO JECFA Monographs 14 (2013), superseding tentative specifications prepared at the 69th JECFA (2008). No ADI was allocated at the 69th JECFA (2008).

SYNONYMS INS No. 160c(ii), Capsanthin

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

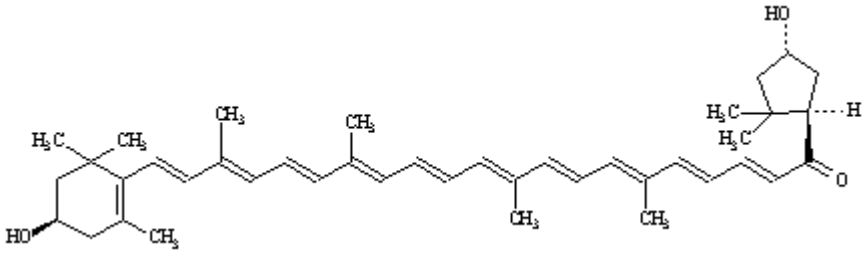
Only methanol, ethanol, isopropanol, acetone, hexane, ethyl acetate and supercritical carbon dioxide may be used as solvents in the extraction.

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

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

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

Structural formula



Capsanthin

Formula weight Capsanthin: 584.85

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

DESCRIPTION Dark-red viscous liquid

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility Practically insoluble in water, soluble in acetone

Spectrophotometry Maximum absorption in acetone at about 462 nm and in hexane at about 470 nm.

Colour reaction — To one drop of sample add 2-3 drops of chloroform and one drop of sulfuric acid. A deep blue colour is produced.

High performance liquid chromatography (HPLC) — Passes test.
See Method of assay, Capsanthin

PURITY

Residual solvents — Acetone, Ethanol, Ethyl acetate, Hexane, Isopropanol, Methanol: Not more than 50 mg/kg, singly or in combination
See description under TESTS

Capsaicinoids — Not more than 200 mg/kg
See description under TESTS

Arsenic (Vol. 4) — Not more than 1 mg/kg
Determine using an AAS (Hydride generation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

Lead (Vol. 4) — Not more than 1 mg/kg
Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

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

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

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

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

Apparatus

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

Column: LC-18 (150 mm x 4.6 mm id, 5 µm)

Detector: Fluorescence - Excitation 280 nm and emission 325 nm
UV Detector - 280 nm

Mobile phase: 40% acetonitrile and 60% deionised water containing 1% Acetic acid (v/v).

Flow rate: 1.5 ml/min

Procedure

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

Calculations

Calculate individual capsaicinoids (µg/ml) as follows:

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

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

where

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

Response factors:

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

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

Residual solvents — Water and methanol are not suitable for the head-space gas

chromatographic analysis of solvent-extracted paprika extracts as given in Method I and Method II for the method for residual solvent determination by head-space gas chromatography in Vol.4. A refined vegetable oil (e.g. soybean oil) is the preferred solvent for sample dissolution. Weigh accurately 1.0 g sample into a headspace vial and add 10 ml soybean oil. Cap and seal immediately. Prepare blanks, standard solutions and calibration samples in a similar fashion. Use the same soybean oil to determine residual solvents in the blank. Determine residual solvents following the Procedure given in Vol. 4.

METHOD OF ASSAY

Total carotenoids

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

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

where

A is the absorbance of sample

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

W is the weight of sample (g)

Capsanthin

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

Sample preparation

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

Chromatography

Filter acetone (HPLC grade) and deionised water and de-gas before use.
Column: Reversed-phase C-18 (250 x 4 mm i.d.)
Precolumn: Reversed-phase C-18 (50 x 4 mm i.d.)
Mobile phase: Program a gradient acetone/water as follows:

Time (min)	Acetone (%)	Water (%)
-10 (pre-injection)	75	25
0	75	25
5	75	25

10	95	5
17	95	5
22	100	0
27	75	25

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

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

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

PHYTASE FROM *ASPERGILLUS NIGER* EXPRESSED IN *A. NIGER*

Prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013) superseding specifications prepared at the 76th JECFA (2012) and published in FAO JECFA Monographs 13 (2012). An ADI "not specified" was established at the 76th JECFA (2012).

SYNONYMS Phytase, 3-phytase

SOURCES Phytase is produced by submerged fed-batch fermentation of a non-pathogenic and non-toxicogenic genetically modified strain of *Aspergillus niger* which contains the phytase encoding gene derived from *A. niger*. The enzyme is secreted and isolated from the fermentation broth by filtration to remove the biomass and concentrated by ultrafiltration. The enzyme concentrate is subjected to germ filtration and is subsequently formulated and standardized to the desired activity using food-grade compounds.

Active principles: 3-phytase

Systematic names and numbers: Myo-Inositol hexakisphosphate 3-phosphohydrolase, EC 3.1.3.8, CAS 37288-11-2

Reactions catalysed: Hydrolysis of myo-inositol hexakisphosphate (phytate) to inositol pentaphosphate (IP5), and further to give a mixture of myo-inositol di-phosphate (IP2), myo-inositol mono-phosphate (IP1) and free orthophosphate

Secondary enzyme activities: No significant levels of secondary enzyme activities

DESCRIPTION Brownish liquid or yellow to light brown powder.

FUNCTIONAL USES Enzyme preparation.
Used to degrade phytate found in plant derived foods, particularly cereal grains and legumes, in order to improve mineral bioavailability.

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

CHARACTERISTICS

IDENTIFICATION

Phytase activity: The sample shows phytase activity
See description under TESTS

TESTS

Phytase activity

Principle
This procedure is used to determine the activity of enzymes releasing phosphate from phytate. The assay is based on enzymatic hydrolysis of sodium phytate under controlled conditions by measurement of the amount of inorganic phosphate released. The phytase activity is expressed in phytase units (FTU). One phytase unit (FTU) is defined as

the amount of enzyme that liberates 1 micromole of inorganic phosphorus per minute from 0.0051 mol/l sodium phytate at 37° and pH 5.50 under the conditions of the test.

Reagents and Solutions
NOTE - Ensure the absence of phosphate in all glassware.

Acetate buffer pH 5.50: Dissolve 1.76 g (1.68 ml) glacial acetic acid (C2H4O2), 30.02 g of sodium acetate trihydrate ($C_2H_3O_2Na•3H_2O$), and 0.147 g of calcium chloride dihydrate ($CaCl_2.2H_2O$) in about 900ml of water. Transfer the solution into a 1000 ml volumetric flask, dilute to volume with water, and mix. Adjust the pH to 5.50 ± 0.05.

Substrate solution: Dissolve 8.40 g of sodium phytate decahydrate ($C_6H_6O_{24}P_6Na_{12}•10H_2O$) in 900 ml of acetate buffer. Adjust the pH to 5.50 ± 0.05 at 37 ° by adding 4 M acetic acid. Cool to ambient temperature. Quantitatively transfer the mixture to a 1000 ml volumetric flask, dilute to volume with acetate buffer, and mix. Prepare fresh daily.

Nitric acid solution (27%): While stirring, slowly add 70 ml of 65% nitric acid to 130 ml of water.

Ammonium heptamolybdate solution: Dissolve 100 g of ammonium heptamolybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}•4H_2O$] in 900 ml of water in a 1000 ml volumetric flask. Add 10 ml of 25% ammonia solution, dilute to volume with water, and mix. This solution is stable for 4 weeks when stored at ambient temperature and shielded from light.

Ammonium vanadate solution: Dissolve 2.35 g of ammonium monovanadate (NH_4VO_3) in 400 ml of warm (60°) water. While stirring, slowly add 20ml of nitric acid solution (27%). Cool to ambient temperature. Quantitatively transfer the mixture to a 1000 ml volumetric flask, dilute to volume with water, and mix. This solution is stable for 4 weeks when stored at ambient temperature and shielded from light.

Colour/stop solution: While stirring, add 25 ml of ammonium vanadate solution to 25 ml of ammonium heptamolybdate solution. Continue stirring and slowly add 16.5 ml of 65% nitric acid. Cool to ambient temperature; quantitatively transfer the mixture to a 100 ml volumetric flask, dilute to volume with water, and mix. Prepare fresh daily.

Potassium dihydrogen phosphate solution: Dry a sufficient amount of >99% purity potassium dihydrogen phosphate (KH_2PO_4) in an oven at 105° for 4 h. Cool to ambient temperature in a desiccator over dried silica gel. In two separate 1 L volumetric flasks, weigh accurately 0.245 g of dried potassium dihydrogen phosphate and dilute with acetate buffer to 1 L to obtain potassium dihydrogen phosphate solutions A and B, each containing 1.80 mmol/L of potassium dihydrogen phosphate.

Phytase standard: obtained from DSM, Delft, The Netherlands, with an assigned activity or equivalent.

Phytase standard solution: In duplicate, accurately weigh an adequate amount of phytase standard and dissolve and dilute in acetate buffer to obtain a solution containing 0.06 ± 0.01 phytase units per 2.0 ml. Quantify the activity of the phytase standard according to procedure described below.

Procedure for quantifying the activity of the phytase standard
Using 6 individual 20×150 mm glass test tubes add to one tube 2.00 ml of the phytase standard solution, add to 3 tubes 2.00 ml of potassium dihydrogen phosphate solution A, and to the remaining 2 tubes 2.00 ml of potassium dihydrogen phosphate solution B. Place the tubes into a 37.0 ± 0.1° water bath, at regular time intervals and allow their contents to equilibrate for 5 min.
At time equals 5 min, in the same order and within the same time intervals as the tubes were added, add 4.0 ml of substrate solution (previously equilibrated to 37.0±0.1°), to each of the tubes. Mix the tubes, and replace in the 37.0 ± 0.1° water bath.
At time equals 35 min, in the same order and within the same time intervals, terminate the incubation by adding 4.0 ml of colour/stop solution to each of the tubes. Mix, and cool to ambient temperature.
Prepare an enzyme blank by adding 2.00 ml of phytase standard solution into one 20 ×150 mm glass test tube. Prepare reagent blanks by adding 2.00 ml of water into a series of five separate 20 ×150 mm glass test tubes. Add 4.0 ml of colour/stop solution to all blank tubes and mix. Next add 4.0 ml of substrate solution, and mix.
Determine the absorbance of all solutions at 415 nm in a 1 cm path-length cell with a spectrophotometer, using water to zero the instrument.

Calculation of the activity of the phytase standard
Calculate the corrected absorbances (AR) for each sample preparation (absorbance of the standard phytase solution minus the corresponding absorbance of the blank) and for each potassium dihydrogen phosphate solution, Ap (absorbance of the potassium dihydrogen phosphate solution minus average absorbance of the reagent blanks). Calculate C, the phosphate concentration of each potassium dihydrogen phosphate solution:

$$C\ (\text{mmol}/2\ \text{ml}) = (W \times 1000 \times 2)/MW.$$

Calculate the absorbances (D) for each potassium dihydrogen phosphate solution after correction for the amount of potassium dihydrogen phosphate weighed:

$$Ap/C = D\ (\text{absorbance units/mmol of phosphate per 2 ml})$$

Calculate the average of results D, giving E (maximum allowable difference, 5%).

Calculate the activity for the phytase standard:

$$FTU/g = (AR \times f)/(30 \times R \times E)$$

where,
AR is the corrected absorbance of the phytase standard solution;
f is the total dilution factor of the standard preparation;
30 is the incubation time, in min;
R equals sample weight, in g;
E is average of D factors;
W is the weight of potassium dihydrogen phosphate, in g;
MW is the molecular weight of potassium dihydrogen phosphate, 136.09 (g/mol).

Determination of phytase activity in samples

Sample preparation: Suspend or dissolve and dilute accurately weighed amounts of sample in acetate buffer so that 2.0 ml of the final solution will contain between 0.01 and 0.08 phytase units.

Preparation of phytase standard curve: Weigh, in duplicate, with an accuracy of ± 1 mg, an amount of phytase standard based on the activity which corresponds to about 20,000 phytase units in 200 ml volumetric flasks. Dissolve in and dilute to volume with acetate buffer, and mix. Use this stock solution and dilute with acetate buffer to obtain standard solutions containing approximately 0.01, 0.02, 0.04, 0.06, and 0.08 phytase units per 2.0 ml.

Add 2.00 ml of each phytase standard solution and 2.00 ml of the sample solution into separate 20-×150-mm glass test tubes. Place the tubes into a 37.0±0.1° water bath, at regular time intervals and allow their contents to equilibrate for 5 min. At time equals 5 min, in the same order as the tubes were added, add 4.0 ml of substrate solution (previously equilibrated to 37.0 ± 0.1°) to the each of the test tubes. Mix, and replace in the 37.0 ± 0.1°water bath. At time equals 35 min, in the same order and within the same time intervals, terminate the incubation by adding 4.0 ml of colour/stop solution to each of the tubes. Mix, and cool to ambient temperature.

Prepare enzymes blanks as described for quantifying the activity of the phytase standard. Centrifuge all test tubes for 5 min at 3000 ×g. Determine the absorbance of each solution at 415 nm in a 1-cm path-length cell with a spectrophotometer, using water to zero the instrument.

Calculation of phytase activity in samples

Calculate the corrected absorbance (sample minus blank) for each sample preparation and phytase standard solution. Plot the calculated phytase activity (FTU per 2 ml) of each phytase solution against the corresponding absorbance. From the curve, determine the phytase activity in each sample preparation (FTU per 2 ml):

$$\text{Activity (FTU/g)} = (\text{FTU per 2 ml} \times \text{dilution})/\text{sample weight}$$

POTASSIUM ALUMINIUM SILICATE

Prepared at the 77th JECFA (2013), published *in FAO Monographs 14 (2013), superseding the specifications prepared at the 74th JECFA (2011), published in FAO Monographs 11 (2011). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives.*

SYNONYMS Mica, Muscovite, INS No. 555

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

Chemical name Potassium aluminium silicate

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

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

Formula weight 398.31

Assay Not less than 98%

DESCRIPTION Light grey to white crystalline platelets or powder.

FUNCTIONAL USES Anticaking agent, Carrier (used as a carrier substrate in pearlescent pigments made with titanium dioxide and/or iron oxide)

CHARACTERISTICS

IDENTIFICATION

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

Test for aluminium and silicon Passes test
See description under TESTS

PURITY

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

Impurities soluble in 0.5 M hydrochloric acid

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

Zinc: Not more than 25 mg/kg
See description under TESTS

TESTS

IDENTIFICATION TESTS

Test for aluminium and silicon

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

PURITY TESTS

Impurities soluble in 0.5 M hydrochloric acid

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

METHOD OF ASSAY

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

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

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

POTASSIUM ALUMINIUM SILICATE-BASED PEARLESCENT PIGMENTS, Type I

Prepared at the 77th JECFA (2013), published in FAO Monographs 14 (2013), replacing the tentative specifications prepared for potassium aluminium silicate-based pearlescent pigments prepared at the 74th JECFA (2011), published in FAO Monographs 11 (2011). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives. An ADI of 'not limited' was established for titanium dioxide at the 13th JECFA (1969).

SYNONYMS Mica-based pearlescent pigments, Type I

DEFINITION Potassium aluminium silicate-based pearlescent pigments, Type I, are produced by the deposition of titanium salts on potassium aluminium silicate followed by calcination at high temperatures. The resulting pigment consists of potassium aluminium silicate coated with titanium dioxide. The pigments can be produced with a variety of different pearlescent colour effects depending upon particle size and the thickness of titanium dioxide deposited on the potassium aluminium silicate. Particles below a size of 100 nm shall not be present.

While values will vary for each individual pearlescent pigment in regards to the amounts of titanium dioxide and potassium aluminium silicate, particle size and pH of an aqueous slurry, general information can be provided for the pigments as a class. When considered together as a class, Type I pigments typically show ranges for titanium dioxide and potassium aluminium silicate in the pigments of 10-61%, and 39-90%, respectively. Similarly, when taken as a class, median particle size typically ranges from 3-82 µm.

Assay	Titanium dioxide (TiO_2): Potassium aluminium silicate:	As labeled.

DESCRIPTION Powder with distinctive sheen.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble or insoluble in water.

Test for Titanium Passes test
See description under TESTS

PURITY

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

Impurities soluble in 0.5 M hydrochloric acid	Antimony:	Not more than 3 mg/kg
	Arsenic:	Not more than 3 mg/kg
	Barium:	Not more than 25 mg/kg
	Cadmium:	Not more than 1 mg/kg
	Chromium:	Not more than 100 mg/kg
	Copper:	Not more than 25 mg/kg
	Lead:	Not more than 4 mg/kg
	Mercury:	Not more than 1 mg/kg
	Nickel:	Not more than 50 mg/kg
	Zinc:	Not more than 25 mg/kg
	See description under TESTS	

TESTS

IDENTIFICATION TESTS

Test for Titanium — Use the test solution as shown under method of assay. Analyze titanium in the test solution by ICP-AES technique (Volume 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical line for Ti (334.941 nm).

PURITY TESTS

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

METHOD OF ASSAY

Determination of percent titanium 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 titanium and aluminium 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), and aluminium (396.152 nm). Determine the concentration (as µg/ml) of titanium and aluminium from the respective standard curves. Calculate the percentage of titanium dioxide and potassium aluminium silicate using the formulas below:

$$\%\ TiO_2\ (w/w)\ \text{in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{1.668 \times C_{Ti} \times 250 \times DF}{W \times 10^6}$$

$$\%\ \text{Potassium aluminium silicate (w/w) in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{4.92 \times C_{Al} \times 250 \times DF}{W \times 10^6}$$

Where:

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

POTASSIUM ALUMINIUM SILICATE-BASED PEARLESCENT PIGMENTS, Type II

Prepared at the 77th JECFA (2013), published *in FAO Monographs 14 (2013) replacing the tentative specifications prepared for potassium aluminium silicate-based pearlescent pigments prepared at the 74th JECFA (2011), published in FAO Monographs 11 (2011). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives. An ADI of 0-0.5 mg/kg bw for iron oxides was established at the 53rd JECFA (1999).*

SYNONYMS Mica-based pearlescent pigments, Type II

DEFINITION Potassium aluminium silicate-based pearlescent pigments, Type II, are produced by the deposition of iron salts on potassium aluminium silicate followed by calcination at high temperatures. The resulting pigment consists of potassium aluminium silicate coated with iron oxide. The pigments can be produced with a variety of different pearlescent colour effects depending upon particle size and the amount of iron oxide deposited on the potassium aluminium silicate. Particles below a size of 100 nm shall not be present.

While values will vary for each individual pearlescent pigment in regards to the amounts of iron oxide and potassium aluminium silicate, particle size and pH of an aqueous slurry, general information can be provided for the pigments as a class. When considered together as a class, Type II pigments typically show ranges for iron oxide and potassium aluminium silicate in the pigments of 32-55%, and 45-68%, respectively. Similarly, when taken as a class, median particle size typically ranges from 18-25 µm.

Assay	Iron oxide (Fe_2O_3): Potassium aluminium silicate:	As labeled.

DESCRIPTION Powder with distinctive sheen.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Practically insoluble or insoluble in water.
Test for Iron	Passes test See description under TESTS

PURITY

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

Impurities soluble in 0.5 M hydrochloric acid	Antimony:	Not more than 3 mg/kg
	Arsenic:	Not more than 3 mg/kg
	Barium:	Not more than 25 mg/kg
	Cadmium:	Not more than 1 mg/kg
	Chromium:	Not more than 100 mg/kg
	Copper:	Not more than 25 mg/kg
	Lead:	Not more than 4 mg/kg
	Mercury:	Not more than 1 mg/kg
	Nickel:	Not more than 50 mg/kg
	Zinc:	Not more than 25 mg/kg
	See description under TESTS	

TESTS

IDENTIFICATION TESTS

Test for Iron

Use the test solution as shown under method of assay. Analyze iron in the test solution by ICP-AES technique (Volume 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical line for Fe (259.940 nm).

PURITY TESTS

Impurities soluble in 0.5 M hydrochloric acid

Extract 20 g of finely ground sample under reflux conditions (to prevent loss of mercury) with 100 ml of 0.5 M hydrochloric acid (spectroscopic grade) for 30 min. Let solution cool, then filter through a 0.1 µm membrane filter. Wash the filter twice with hot 0.5 M hydrochloric acid. Combine the filtrate and wash solution in a 200 ml volumetric flask and make up to volume with 0.5 M hydrochloric acid.

Determine arsenic using an AAS (Hydride generation) technique; antimony, barium, chromium, copper, nickel and zinc by an ICP-AES technique; lead and cadmium using an AAS (Electrothermal atomization) technique; and mercury using an AAS (Cold vapour generation) technique. See “Metallic impurities” in the Combined Compendium of Food Additive Specifications (Volume 4).

METHOD OF ASSAY

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

$$\%\ Fe_2O_3\ (w/w)\ \text{in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{1.43 \times C_{Fe} \times 250 \times DF}{W \times 10^6}$$

$$\%\ \text{Potassium aluminium silicate (w/w) in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{4.92 \times C_{Al} \times 250 \times DF}{W \times 10^6}$$

Where:

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

POTASSIUM ALUMINIUM SILICATE-BASED PEARLESCENT PIGMENTS, Type III

Prepared at the 77th JECFA (2013), published in FAO Monographs 14 (2013), replacing the tentative specifications prepared for potassium aluminium silicate-based pearlescent pigments prepared at the 74th JECFA (2011), published in FAO Monographs 11 (2011). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives. ADIs of 'not limited' for titanium dioxide and 0-0.5 mg/kg bw for iron oxides were established at the 13th JECFA (1969) and 53rd JECFA (1999), respectively.

SYNONYMS Mica-based pearlescent pigments, Type III

DEFINITION Potassium aluminium silicate-based pearlescent pigments, Type III, are produced by the deposition of titanium and iron salts on potassium aluminium silicate followed by calcination at high temperatures. The resulting pigment consists of potassium aluminium silicate coated with titanium dioxide, iron oxide and, potentially, mixed oxides. The pigments can be produced with a variety of different pearlescent colour effects depending upon particle size and the combination of titanium dioxide and iron oxide deposited on the potassium aluminium silicate. Particles below a size of 100 nm shall not be present.
While values will vary for each individual pearlescent pigment in regards to the amounts of iron oxide, titanium dioxide and potassium aluminium silicate, particle size and pH of an aqueous slurry, general information can be provided for the pigments as a class. When considered together as a class, Type III pigments typically show ranges for iron oxide, titanium dioxide and potassium aluminium silicate in the pigments of 2-12%, 33-52%, and 36-65%, respectively. Similarly, when taken as a class, median particle size typically ranges from 7-25 µm.

Assay

Titanium dioxide (TiO_2):
Iron oxide (Fe_2O_3): As labeled
Potassium aluminium silicate:

DESCRIPTION Powder with distinctive sheen.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble or insoluble in water.

Test for Titanium and Iron
Passes test
See description under TESTS

PURITY

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

Impurities soluble in 0.5 M hydrochloric acid	Antimony:	Not more than 3 mg/kg
	Arsenic:	Not more than 3 mg/kg
	Barium:	Not more than 25 mg/kg
	Cadmium:	Not more than 1 mg/kg
	Chromium:	Not more than 100 mg/kg
	Copper:	Not more than 25 mg/kg
	Lead:	Not more than 4 mg/kg
	Mercury:	Not more than 1 mg/kg
	Nickel:	Not more than 50 mg/kg
	Zinc:	Not more than 25 mg/kg
	See description under TESTS	

TESTS

IDENTIFICATION TESTS

Tests for Titanium and Iron

Use the test solution as shown under method of assay. Analyze titanium and iron in the test solution by ICP-AES technique (Volume 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical lines for Ti (334.941 nm) and Fe (259.940 nm).

PURITY TESTS

Impurities soluble in 0.5 M hydrochloric acid

Extract 20 g of finely ground sample under reflux conditions (to prevent loss of mercury) with 100 ml of 0.5 M hydrochloric acid (spectroscopic grade) for 30 min. Let solution cool, then filter through a 0.1 µm membrane filter. Wash the filter twice with hot 0.5 M hydrochloric acid. Combine the filtrate and wash solution in a 200 ml volumetric flask and make up to volume with 0.5 M hydrochloric acid. Particles below a size of 100 nm shall not be present.

Determine arsenic using an AAS (Hydride generation) technique; antimony, barium, chromium, copper, nickel and zinc by an ICP-AES technique; lead and cadmium using an AAS (Electrothermal atomization) technique; and mercury using an AAS (Cold vapour generation) technique. See “Metallic impurities” in the Combined Compendium of Food Additive Specifications (Volume 4).

METHOD OF ASSAY

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 dioxide, iron oxide and potassium aluminium silicate using the formulas below:

$$\%\ TiO_2\ (w/w)\ \text{in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{1.668 \times C_{Ti} \times 250 \times DF}{W \times 10^6}$$

$$\%\ Fe_2O_3\ (w/w)\ \text{in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{1.43 \times C_{Fe} \times 250 \times DF}{W \times 10^6}$$

$$\%\ \text{Potassium aluminium silicate (w/w) in Potassium Aluminium Silicate-Based Pearlescent Pigments} = \frac{4.92 \times C_{Al} \times 250 \times DF}{W \times 10^6}$$

Where:

C is Concentration of Ti, Fe or Al in the test solution, µg/ml
DF is Dilution factor (dilution of Solution A to get test solution)
W is Weight of sample, g

SILICON DIOXIDE, AMORPHOUS
(TENTATIVE)

Tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013), superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978). An ADI 'not specified' for silicon dioxide and certain silicates was established at the 29th JECFA (1985).

Information required on different types of silicon dioxide, used as food additive:

- *Composition and methods of manufacture*
- *Functional use other than anticaking agent, if used*
- *Data on pH and loss on drying*
- *Description, assay and loss of ignition for silicic acid and dehydrated silica gel*
- *Data on a minimum of five batches, on the content of silicon dioxide using the proposed "Method of assay"*
- *Data on lead, arsenic and mercury content, in a minimum of five batches, carried out in the impurities soluble in 0.5 M hydrochloric acid using the proposed methods.*

SYNONYMS Silica; INS No. 551

DEFINITION The products included under this specification are: silica aerogel (precipitated silicon dioxide), hydrated silica, "silicic acid" and dehydrated silica gel.

Chemical names Silicon dioxide

C.A.S. number 7631-86-9

Chemical formula $(SiO_2)_x$

Formula weight 60.09 (SiO_2)

Assay

Silica aerogel:	not less than 90% of SiO_2 on the ignited basis.
Hydrated silica:	not less than 89% of SiO_2 on the ignited basis.
Silicic acid and dehydrated silica gel:	information required

DESCRIPTION

Silica aerogel:	a microcellular silica occurring as a fluffy powder or granules
Hydrated silica:	a precipitated, hydrated silicon dioxide occurring as a fine, white, amorphous powder, or as beads or granules
Silicic acid and dehydrated silica gel:	information required

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Insoluble in water and ethanol$
Test for silicon	Passes test See description under TESTS

PURITY

pH	Information required
Loss on drying (Vol. 4)	Information required
Loss on ignition (Vol. 4)	Not more than 6% on the dried basis (105° to constant weight), after igniting at 600o (for silica aerogel) or at 900o (for hydrated silica) to constant weight. Store the ignited sample in a desiccator for use in the method of assay. Information required for Silicic acid and dehydrated silica gel
Impurities soluble in 0.5 M hydrochloric acid	Lead : Information required Arsenic: Information required Mercury: Information required See description under TESTS

TESTS

IDENTIFICATION TESTS

Test for silicon	Prepare the test solution as shown under method of assay. Analyze aluminum and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical lines for Si (251.611 nm).

PURITY TESTS

Impurities soluble in 0.5 M hydrochloric acid	Extract 20 g of finely ground sample under reflux conditions (to prevent loss of mercury) with 100 ml of 0.5 M hydrochloric acid (spectroscopic grade) for 30 min. Let solution cool, then filter through a 0.1 µm membrane filter. Wash the filter twice with hot 0.5 M hydrochloric acid. Combine the filtrate and wash solution in a 200 ml volumetric flask and make up to volume with 0.5 M hydrochloric acid. Determine arsenic using an AAS (Hydride generation) technique; lead using an AAS (Electrothermal atomization) technique; and mercury using an AAS (Cold vapour generation) technique. See “Metallic impurities” in the Combined Compendium of Food Additive Specifications (Volume 4).
METHOD OF ASSAY	Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, mix and melt completely using a torch burner and allow to stand at room temperature. Place the reaction product along with crucible into 150 ml hot deionized water in a 250-ml PTFE beaker 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 polypropylene 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, to get the readings within the standard curve range. Analyze silica, aluminium and calcium in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical line for Si (251.611 nm) and construct standard curve using standard solutions 0.1 – 5.0 µg/ml. Read the concentration of Si in test solution (as µg/ml) and calculate the silicon dioxide content of the sample using the formula:

$$\%SiO2 = \frac{2.139 \times C \times 250 \times DF}{W \times 106} \times 100$$

Where

C is concentration of Si in the test solution, µg/ml;
DF is dilution factor (dilution of Solution A to get test solution);
W is weight of the ignited sample, g.

SODIUM ALUMINOSILICATE
(TENTATIVE)

Tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013), superseding specifications prepared at the 17th JECFA (1973), published in FNP 4 (1978) and in FNP 52 (1992). Metals and arsenic specifications revised at the 57th JECFA (2001). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives.

Information required:

- *Composition and methods of manufacture*
- *C.A.S. number and chemical formula*
- *Functional uses other than anticaking agent, if used*
- *Data on loss on drying, loss on ignition and pH of a slurry in water*
- *Data, on a minimum of five batches, on the content of aluminium and silicon using the proposed "Method of assay"*
- *Data on lead, arsenic and mercury content, in a minimum of five batches, carried out in the impurities soluble in 0.5 M hydrochloric acid using the proposed methods.*

SYNONYMS Sodium silicoaluminate; aluminium sodium silicate; silicic acid, aluminium sodium salt; INS No. 554

DEFINITION A series of hydrated sodium aluminium silicates.

Chemical names Aluminium sodium silicate

C.A.S. number Information required

Chemical formula Information required

Assay Information required
Not less than XX% and not more than XX% of Al, and not less than XX% and not more than XX% of Si on the dried basis.

DESCRIPTION Odourless, fine, white amorphous powder, or as beads.

FUNCTIONAL USES Anticaking agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ethanol, partially soluble in strong acids and alkali hydroxides

Test for sodium (Vol. 4) Passes test

Test for aluminium Passes test

	See description under TESTS
Test for silicon	Passes test See description under TESTS

PURITY

pH	Information required
Loss on drying (Vol. 4)	Information required
Loss on ignition (Vol. 4)	Information required
Impurities soluble in 0.5 M hydrochloric acid	Lead : Information required Arsenic: Information required Mercury: Information required See description under TESTS

TESTS

IDENTIFICATION TESTS

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

PURITY TESTS

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

METHOD OF ASSAY Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, Mix and melt completely using a torch burner and allow to stand at room temperature. Place the reaction product along with crucible into 150 ml hot deionized water in a 250-ml PTFE beaker 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 polypropylene volumetric flask. Wash the beaker three times with hot deionized water, transfer the washings to the volumetric flask and make up to volume. Dilute with 2% hydrochloric acid and prepare the test solution. Analyse aluminium and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer. Use analytical lines for Al (396.152 nm) and Si (251.611 nm) and construct standard curve using standard solutions 0.2 – 5.0 µg/ml each. Read the concentration of Al and Si in sample solution (as µg/ml) and calculate the aluminium and

silicon content of the sample using the formula:

$$\text{Al or Si (\%)} = \frac{C \times 250 \times DF}{W \times 10^6} \times 100$$

Where:
C is concentration of Al or Si in the test solution, µg/ml
W is weight of sample, g
DF is dilution factor

ANALYTICAL METHODS

The following analytical methods were prepared by the Committee at the 77th meeting.

The method for determination of residual solvents in certain annatto extracts is tentative and will be revised as appropriate at a forthcoming meeting of the JECFA (see page 93).

The two methods that use *Inductively Coupled Plasma-Atomic Emission Spectrophotometry* (ICP-AES) for the determination of inorganic components were adopted and will be made available in the online edition of Volume 4 of the Combined Compendium of Food Additive Specifications.

The gas chromatographic method using a packed column for the determination of carbon number at 5% distillation (based on ASTM D 2887) in Volume 4 of the Combined Compendium of Food Additive Specifications .is replaced by the proposed gas chromatographic method using a wide-bore column (based on ASTM D 6352-04). The replacement method was introduced into the specifications of the monograph mineral oil, medium viscosity. The method will be added to the online version of Volume 4 of the Combined Compendium of Food Additive Specifications.

Determination of residual solvents in annatto extracts (solvent-extracted bixin and norbixin)

(Tentative)

The Committee noted at the 77th meeting that the method for determination of residual solvent in the Combined Compendium of Food Additive Specifications, Volume 4 was not suitable for the determination of residual solvents in solvent-extracted bixin and norbixin. The Committee agreed that more results were needed to ensure that the method proposed below is a suitable substitute for the head-space gas chromatography method in the Specification and listed in Compendium of food additive specifications, Volume 4.

Chromatographic system

Detector:	Flame ionization detector (FID)
Column:	25% diphenyl-75% dimethylpolysiloxane (60 m x 0.25 mm I.D., 1.4 µm-film) [Aquatic-2 (GL-Sciences Inc.) or equivalent]
Carrier gas:	Helium
Flow rate:	205 kPa, 1.8 ml/min
Injector temperature:	260°
Detector temperature:	250°
Oven temperature:	Hold for 5min at 40°; then 40°to 92° at 4°/min; then Hold for 2 min at 92°, then 92° to 230° at 40°C/min

Head space sampler

Sample heating temperature:	60°
Sample heating period:	20 min
Syringe temperature:	100°
Transfer line temperature:	120°
Sample gas injection:	3.0 ml in split mode (25:1)

Stock standard solutions: Add 10 ml dimethylformamide to six 20 ml volumetric flasks. Accurately weigh, to within 0.01 mg, each flask. Pipet 250 µl each of chromatography grade methanol, ethanol, isopropanol, and ethyl acetate, and 150 µl each of acetone and hexane into each of the flask. Reweigh accurately and then fill the flask with dimethylformamide. Mix well.

Mixed standard solution A: Pipet each 3.0 ml of stock standard solution into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Mixed standard solution B: Pipet 4.0 ml solution A into a 10 ml volumetric flask and fill the flask with dimethylformamide.

Mixed standard solution C: Pipet 2.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Mixed standard solution D: Pipet 1.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Samples: Weigh accurately 0.2 g sample into a 20 ml head-space vial. Add 2.5 ml dimethylformamide and seal.

Standard solutions: Introduce 0.1 ml of the each standard mixture solution (A, B, C and D) into each 20 ml injection vial. Add 2.4 ml dimethylformamide and seal.

Standard curves

Place the four standard solutions in the sample tray on head-space gas chromatography. Heat vials at 60° for 20 min with continuous agitation. Analyze using the analytical condition as described above. Measure the peak area for each solvent. Construct the standard curves by plotting the peak areas of each solvent against the concentrations of each solvent (mg/ml) in the standards solutions.

Procedure

Place the sample solution in the sample tray on head-space gas chromatograph. Heat vials at 60° for 20 min with continuous agitation. Analyze using the analytical condition as described above. Measure the peak area for each solvent and obtain the concentration of each solvent from the standard curves. Calculate the concentration of each solvent as follows:

$$\text{Solvent (mg/kg)} = C \times 2.5/W \times 1000$$

Where:
C is the concentration of solvent (mg/ml).
W is weight of sample (g).

Phosphorous, calcium, magnesium and aluminium determination by Inductively Coupled Plasma-Atomic Emission Spectrophotometry (ICP-AES) [1]

Accurately weigh about 0.500 g of finely ground sample into a 100-ml PTFE beaker. Add about 10 ml of deionized water, 5 ml aquaregia (HNO_3: HCl, 3:1) and leave it in a fume hood for about 15 min. Place the beaker, if required, on a hot plate maintained at low heat for about 30 min. Control the temperature in a way that the sample do not over boil or get dried up. Ensure that the solution does not spurt. Quantitatively transfer into a 100-ml volumetric flask and make up to volume with deionized water. Dilute sample solution to bring the solution within the working range of the standard curve. Prepare a reagent blank omitting sample and dilute the blank as required.

Note: *The selection of sample size and method of sample preparation may be based on principles of methods described in the Combined Compendium of Food Additives Specifications, Volume 4. Sample weight may vary according to the concentration of element under the assay. Purity of acids and other reagents used shall be of atomic spectroscopy grade.*

Determine the elements phosphorous, magnesium, calcium and aluminium using ICP-AES technique appropriate to the specified level. Set instrument parameters as specified by the instrument manufacturer. Use analytical line for P (213.618 nm), Mg (279.078 nm), Ca (318.127), Al (396.152 nm) and deduce the concentration of element (µg/ml) in the sample. Calculate percentage as follows:

$$\% \text{ element} = (A-B) \times V \times DF / 10000 \times W$$

Where:

A is the concentration of element in the sample solution, µg/ml
B is the concentration of element in the corresponding reagent blank solution, µg/ml
V is the volume of sample made up, ml
DF is the dilution factor
W is the weight of sample, g

Convert the mineral to the corresponding oxides using the formulae:

$\%w/w\ P_2O_5 = \%\ P \times 4.583$
$\%w/w\ MgO = \%\ Mg \times 1.658$
$\%w/w\ CaO = \%\ Ca \times 1.399$
$\%w/w\ Al_2O_3 = \%\ Al \times 3.779$

[1] This method is added to the section *norganic Components* of Volume 4 of the Combined Compendium of Food Additive Specifications (on page 74).

Measurement of minerals and metals by Inductively Coupled Plasma –Atomic Emission spectrophotometric (ICP-AES) Technique [2]

Preparation of standard solutions

Individual standard solutions (1000 mg/L) of Na, K, Ca, Mg, P, Al, Fe, Cu, Zn, Co, Mo, Mn, Pb, Cd, and As: Alternatively, multi-element standard solution (1000 mg/L of each standard) can be used. The linear range of the standard curve may differ based upon the instrument used and operation mode of the torch (axial or radial).

Prepare a series of mixed working standard solutions in the range of 0.1 to 50 µg/ml following serial dilution technique from the mixed standard solution (100 µg/ml). Ensure to limit dilution factor under each dilution step to less than 25.

Instrumental conditions

Select appropriate emission wavelengths to be used with each element under consideration. The recommended settings for various instrumental parameters may vary from one instrument to the other as well as model to model. Certain parameters require optimization at the time of use to obtain the best results. Instrumental conditions should therefore be optimized as described by the manufacturer. Typical emission wavelengths, curve types and calibration ranges given below are for guidance purposes and analyst needs to select the right emission wavelength and other parameters based on the instrument used and type of sample analyzed (expected interferences).

S.No	Analyte	Emission Wavelength (nm)	Curve Type	Calibration Range, µg/ml
1	Na	589.520, 588.995	Quadratic	0.50 – 20.0
2	K	766.491	Quadratic	0.50 – 20.0
3	Ca	318.127	Quadratic	0.50 – 20.0
4	Mg	279.079	Quadratic	0.50 – 20.0
5	P	213.618	Linear	0.10 – 20.0
6	Al	257.509, 308.215, 396.152	Linear	0.10 – 20.0
7	Fe	259.940	Linear	0.10 – 20.0
8	Cu	224.700, 324.754	Linear	0.10 – 10.0
9	Zn	213.857	Linear	0.10 – 20.0
10	Co	228.616, 235.341	Linear	0.10 – 10.0
11	Mo	202.032	Linear	0.10 – 10.0
12	Mn	257.610	Linear	0.10 – 10.0
13	Pb	220.353	Linear	0.10 – 10.0
14	Cd	226.502	Linear	0.10 – 5.0
15	As	188.98, 193.696	Linear	0.10 – 5.0
16	Sb	206.833	Linear	0.10 – 20.0
17	Ba	455.403	Linear	0.10 – 20.0
18	Cr	267.716	Linear	0.10 – 20.0

[2] This method is added to the section *Inorganic Components*, subsection *Metallic Impurities* of Volume 4 of the Combined Compendium of Food Additive Specifications (on page 66).

S.No	Analyte	Emission Wavelength (nm)	Curve Type	Calibration Range, µg/ml
19	Si	251.611	Linear	0.10 – 20.0
20	Ni	231.604	Linear	0.10 – 20.0
21	Ti	334.941	Linear	0.10 – 20.0

Procedure:

Set the instrumental parameters suitable for the analysis of analytes under consideration and the likely interferences. Setup the instrument, aspirate the standard blank solution and set the instrument to zero. Aspirate standards and construct standard curve for each element using the emission intensity and concentration of the element in the working standard. Check the coefficient of determination (R^2) and it shall be >0.99. Aspirate sample solution (or diluted sample solution) and deduce the concentration of the element in the solution (µg/ml).

Element (mg/kg) = (A-B) x V x DF/ x W

Where:

A is the concentration of element in the sample solution, µg/ml
B is the concentration of element in the corresponding reagent blank solution, µg/ml
V is the volume of sample made up, ml
DF is the dilution factor
W is the weight of sample, g

Carbon number at 5% distillation point

ASTM D 6352 – 04

"Carbon number" is number of carbon atoms in a molecule. Determine the boiling point distribution of the sample by gas chromatography. Individual hydrocarbons are separated on a non-polar open tubular capillary column using a linear temperature program in the order of their increasing boiling points. Detector response for each paraffin shall be close to unity.

Gas chromatography

Column:	Non-polar wall-coated open tubular column (5 m x 0.53 - 0.75 mm, i.d.) stationary phase, 100% dimethylpolysiloxane, 0.1 µm, or equivalent
Carrier gas:	Helium, at a flow rate of 18 ml/min
Detector:	FID; temperature 450˚
Oven program:	50˚ - 10˚/min - 400˚
Injector:	On-column or temperature programmable vaporizing injector.
Injection volume:	0.5 µl
Calibration mixture:	Prepare a mixture of hydrocarbons with known boiling points covering the range of the sample (e.g. from C10 to C90). Each component should be present at approximately 0.5 - 2.0%, dissolved in a suitable viscosity-reducing solvent such as carbon disulfide or cyclohexane.

Procedure:

Column resolution: Resolution is determined using C50 and C52 paraffins from a calibration mixture. Resolution shall be between 2 and 4 for satisfactory column performance.

Sample analysis: Using the schedule and temperature program used for the calibration mixture, cool the column and injector to the initial starting temperature. Inject the sample, diluted to approximately 1% in Carbon disulfide or hexane, and record the chromatogram. Inject a baseline blank, standard mixtures and samples in a predetermined order. Use the baseline blank to determine baseline drift and perform baseline subtraction from runs of samples and standards.

Calculation: Collect data, calculate the sample total area, normalise to area percent after background subtraction. Determine the initial and final boiling points by calculating 0.5% and 99.5% of the area counts, respectively. Use linear interpolation to determine the retention time associated with 5% and read the corresponding boiling temperature from the calibration curve.

ANNEX 1. SUMMARY OF RECOMMENDATIONS FROM THE 77th JECFA

Toxicological and dietary exposure information and information on specifications

Food additives considered for specifications only

Food additive	Specifications[a]
Aluminium silicate	R, T
Annatto extracts (solvent-extracted bixin)	M
Annatto extracts (solvent-extracted norbixin)	M
Benzoe tonkinensis	M, T
Calcium aluminium silicate	R, T
Calcium silicate	R, T
Food additives containing phosphates	R[b]
Mineral oil (medium viscosity)	R
Modified starches	R[c]
Paprika extract	R[d]
Phytase from *Aspergillus niger* expressed in *Aspergillus niger*	R
Potassium aluminium silicate	R[d]
Potassium aluminium silicate–based pearlescent pigments, Type I	N[e]
Potassium aluminium silicate–based pearlescent pigments, Type II	N[e]
Potassium aluminium silicate–based pearlescent pigments, Type III	N[e]
Silicon dioxide, amorphous	R, T
Sodium aluminosilicate	R, T

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

[b] The inductively coupled plasma – atomic emission spectrophotometric (ICP-AES) method for the assay of phosphate additives was added to the *Combined Compendium of Food Additive Specifications*.

[c] The method for determination of percentage of octenyl succinate groups in starch sodium octenyl succinate was revised.

[d] The tentative status of the specifications was removed.

[e] The existing combined specifications for potassium aluminium silicate–based pearlescent pigments were split into three separate specifications (Type I: coated with titanium oxide only, Type II: coated with iron oxide only and Type III: coated with both titanium dioxide and iron oxide). The tentative status of the specifications was removed.

Food additives evaluated toxicologically, assessed for dietary exposure and considered for specifications

Food additive	Specifications[a]	Acceptable daily intakes, other toxicological recommendations and dietary exposure assessment
Advantame	N, T	**The Committee established an acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw) for advantame** on the basis of a no-observed-adverse-effect level (NOAEL) of 500 mg/kg bw per day for maternal toxicity in a developmental toxicity study in rabbits and application of a 100-fold safety factor to account for interspecies and intraspecies variability. The Committee agreed that the ADI also applies to those individuals with phenylketonuria, as the formation of phenylalanine from the normal use of advantame would not be significant in relation to this condition. Using the proposed maximum use levels and conservative assumptions,

Food additive	Specifications[a]	Acceptable daily intakes, other toxicological recommendations and dietary exposure assessment
		the maximum mean dietary exposure to advantame would be 1.45 mg/kg bw per day (29% of the upper bound of the ADI), and the maximum high-percentile dietary exposure would be 2.16 mg/kg bw per day (43% of the upper bound of the ADI).
Glucoamylase from *Trichoderma reesei* expressed in *Trichoderma reesei*	N	Based on its low toxicity and because it is reasonably anticipated that dietary exposure would be very low, **the Committee established an ADI "not specified"[b] for the glucoamylase enzyme preparation from *T. reesei* expressed in *T. reesei*** used in the applications specified and in accordance with good manufacturing practice.
Glycerol ester of gum rosin (GEGR)	M, T	As the requested two unpublished 90-day oral toxicity studies on GEGR in rats and complete information on the composition of GEGR were not submitted, **the Committee withdrew the temporary group ADI of 0–12.5 mg/kg bw for GEGR and glycerol ester of wood rosin (GEWR)** (see below).
Glycerol ester of tall oil rosin (GETOR)	W	No data on GETOR were submitted, and the Secretariat was informed that this compound is no longer supported by the previous data sponsor. Therefore, **the Committee did not evaluate GETOR**.
Glycerol ester of wood rosin (GEWR)	R[c]	As the requested data on GEGR were not submitted, **the Committee withdrew the temporary group ADI of 0–12.5 mg/kg bw for GEGR and GEWR and re-established the ADI of 0–25 mg/kg bw for GEWR**.
Nisin	R	**The Committee established an ADI for nisin of 0–2 mg/kg bw** on the basis of a NOAEL of 224.7 mg of nisin per kilogram body weight per day from a 13-week study in rats and application of a safety factor of 100 to account for interspecies and intraspecies variability. The Committee did not consider it necessary to use an additional safety factor to account for the short duration of the study because the NOAEL was supported by the results of a three-generation reproductive toxicity study in rats. **The Committee withdrew the previous ADI of 0–33 000 units of nisin per kilogram body weight established at the twelfth meeting.** The highest estimated dietary exposure of 0.07 mg of nisin per kilogram body weight per day determined at the current meeting did not exceed the upper bound of the ADI.
Octenyl succinic acid (OSA) modified gum arabic	R, T	**The Committee decided to retain the temporary ADI "not specified"[b]** pending submission of additional data on the stability of OSA modified gum arabic in food by the end of 2013, which may help to explain contradictory hydrolysis data.

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

[b] 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.

[c] The tentative status of the specifications was removed.

General considerations

Analytical methods for food additives

With respect to analytical methods for food additives, the Committee recommended that:

- appropriately validated methods be used;
- in relevant cases, the detailed analytical method be provided, together with validation data, in response to specific JECFA calls for data;
- the issue of the suitability of dissolution solvents for the determination of residual solvents in food additives be investigated at a future meeting;
- the suitability of an analytical method for the determination of carbon number at 5% distillation point using a wide-bore gas chromatographic column for use in the analysis of similar substances be evaluated at a future meeting;
- the method for the determination of cyclic phosphates be reviewed at a future meeting.

Annex 2. Further information required or desired

Advantame

The specifications are tentative, pending the submission of information, by the end of 2015, on:

- the suitability of the headspace gas chromatographic method (using appropriate dissolution solvent) for determination of residual solvents, published in Volume 4 of the *Combined Compendium of Food Additive Specifications*, and data, in a minimum of five batches, using the method;
- an alternative or improved high-performance liquid chromatographic method for the assay of advantame and advantame acid using a standard curve;
- additional data and analytical methods for the determination of palladium and platinum;
- information on the purity and availability of the commercial reference standards used in the assay of advantame and advantame acid.

Annatto extracts (solvent-extracted bixin and solvent-extracted norbixin)

The Committee recommended that manufacturers supply residual solvent data from at least five batches of each of the solvent-extracted bixin and norbixin products to support the possible revision of the provision for residual solvents. To evaluate the suitability of the method for the determination of residual solvents in annatto extracts dissolved in dimethyl formamide, the Committee also recommended that manufacturers provide results from the analysis of samples of solvent-extracted bixin and norbixin products using this method as well as the general method for the determination of residual solvents published in Volume 4 of the *Combined Compendium of Food Additive Specifications*.

Benzoe tonkinensis

The tentative specifications will be withdrawn if the complete data on the composition of the ethanolic extract and microbiological contaminants are not received by the end of 2013.

Food additives containing aluminium and/or silicon

Specifications were made tentative pending the submission of information on composition; methods of manufacture; data on loss on drying and loss on ignition; impurities (lead, cadmium, arsenic and mercury) soluble in hydrochloric acid (0.5 mol/l); and suitability of the proposed ICP-AES method for assay, as well as data on the assay. Details on information required are included in the respective tentative specifications monographs. The tentative specifications will be withdrawn unless the requested information is received by the end of 2014.

Glycerol ester of gum rosin (GEGR)

The specifications were maintained as tentative pending the submission of additional information by the end of 2014. Additional data are requested to characterize GEGR in commerce in relation to the composition of 1) the refined gum rosin currently used as the source rosin with regard to the levels (%) of resin acids and neutrals, 2) the glycerol ester of gum rosin with regard to the levels (%) of a) glycerol esters, b) free resin acids and c) neutrals and 3) the total glycerol esters of resin acids with regard to the levels (%) of a) glycerol monoesters and b) the sum of glycerol di- and tri- esters (assay). Validated methods for the determination of the substances considered in the specifications are also required.

Octenyl succinic acid (OSA) modified gum arabic

The Committee noted that ongoing studies on the stability of OSA modified gum arabic in food may provide further information on its chemical state in food and aqueous solutions, which could help to explain the contradictory results of the hydrolysis study submitted to the Committee at the present meeting. The Committee decided to retain the temporary ADI “not specified” pending submission of additional data on the stability of OSA modified gum arabic in food by the end of 2013.

The Committee noted that the purity test of degree of esterification in the current specifications should be replaced by the degree of substitution and requested information for an analytical method to measure the degree of substitution and results of the analysis of at least five commercially available batches. The specifications were made tentative pending submission of these data by the end of 2013.

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

77th Meeting 2013

This document contains food additive specication monographs, analytical methods, and other information prepared at the seventy-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome, Italy from 4 – 13 June 2013. The specification monographs provide information on the identity and purity of food additives used directly in foods or in food production. The main three objectives of these specifications are to identify the food additive that has been subjected to testing for safety, to ensure that the additives are of the quality required for use in food or in processing and to reflect and encourage good manufacturing practice. This publication and other documents produced by JECFA contain information that is useful to all those who work with or are interested in food additives and their safe use in food.

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