



COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

71st meeting 2009



**World Health
Organization**



**Food and Agriculture
Organization of
the United Nations**

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Geneva, Switzerland, 16 – 24 June, 2009****Members**

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 71st meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Geneva, Switzerland, on 16-24 June 2009. In addition, a revised analytical method for measuring softening point for glycerol esters of rosins was prepared and included in this publication. The specifications monographs are one of the outputs of JECFA's risk assessment of food additives, and should be read in conjunction with the safety evaluation, reference to which is made in the section at the head of each specifications monograph. Further information on the meeting discussions can be found in the summary report of the meeting (see Annex 1), and in the full report which will be published in the WHO Technical Report series. Toxicological monographs of the substances considered at the meeting will be published in the WHO Food Additive Series.

Specifications monographs prepared by JECFA up to the 65th meeting, other than specifications for flavouring agents, have been published in consolidated form in the Combined Compendium of Food Additive Specifications which is the first publication in the series FAO JECFA Monographs. This publication consist of four volumes, the first three of which contain the specifications monographs on the identity and purity of the food additives and the fourth volume contains the analytical methods, test procedures and laboratory solutions required and referenced in the specifications monographs. FAO maintains an on-line searchable database of all JECFA specifications monographs from the FAO JECFA Monographs, which is available at: <http://www.fao.org/ag/agn/jecfa-additives/search.html> . The specifications for flavourings evaluated by JECFA, and previously published in FAO Food and Nutrition Paper 52 and subsequent Addenda, are included in a database for flavourings (flavouring agent) specifications which has been updated and modernized. All specifications for flavourings that have been evaluated by JECFA since its 44th meeting, including the 69th meeting, are available in the new format online searchable database at the JECFA website at FAO: <http://www.fao.org/ag/agn/jecfa-flav/search.html>. The databases have query pages and background information in English, French, Spanish, Arabic and Chinese. Information about analytical methods referred to in the specifications is available in the Combined Compendium of Food Additive Specifications (Volume 4), which can be accessed from the query pages.

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

Chemical and Technical Assessments (CTAs) for some of the food additives have been prepared as background documentation for the meeting. These documents are available online at: http://www.fao.org/ag/agn/agns/jecfa_archive_cta_en.asp .

Contact and Feedback

More information on the work of the Committee is available from the FAO homepage of JECFA at: http://www.fao.org/ag/agn/agns/jecfa_index_en.asp . Readers are invited to address comments and questions on this publication and other topics related to the work of JECFA to:

jecfa@fao.org

SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

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

Branching glycosyltransferase from *Rhodothermus obamensis* expressed in *Bacillus subtilis* (N)
 Cassia gum (N) Tentative
 Cyclotetraglucose syrup (R)
 Diacetyltartaric and fatty acid esters of glycerol (R)
 Ethyl lauroyl arginate (R)
 Ferrous ammonium phosphate (N)
 Glycerol ester of gum rosin (N) Tentative
 Glycerol ester of tall oil rosin (N) Tentative
 Glycerol ester of wood rosin (R) Tentative
 Lycopene extract from tomato (N)
 Nisin (R) (previously named Nisin preparation)
 Nitrous oxide (R) Tentative
 Octenyl succinic acid modified gum Arabic (N)
 Pectins (R)
 Sodium hydrogen sulfate (R)
 Modified starches - Starch sodium octenyl succinate (R)
 Sucrose oligoesters Type I (N)
 Sucrose oligoesters Type II (N)
 Tannic acid (R)
 Titanium dioxide (R)

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

In addition to these specifications monographs, minor revisions were made to the specifications monographs for the food additive Triethyl citrate, including correction of the INS number to 1505. The Committee decided that republication in the FAO JECFA Monographs of this specifications monograph was not necessary.

Furthermore, changes have been introduced in the JECFA food additive specifications monographs in the on-line version of the Combined Compendium of Food Additive Specifications for Benzyl alcohol, Calcium lignosulfonate (40-65), Cyclotetraglucose, Glycerol diacetate, Lycopene (synthetic), Lycopene from *Blakeslea trispora*, Microcrystalline cellulose, Potassium sulfate, Sodium sulfate, Sodium L(+)-tartrate, Sucroglycerides and Sucrose esters of fatty acids. The modifications result from changes related to the ADI established for cyclotetraglucose, the group ADIs established for lycopenes (Lycopene (synthetic) and Lycopene from *Blakeslea trispora*) and sucrose esters (Sucroglycerides and Sucrose esters of fatty acids) and the changes in INS numbers and functional uses (except for deletion of functions or addition of functions that may have an impact on the use of the substance and dietary exposure) assigned to food additives by the Codex Alimentarius Commission at its 32nd session in 2009, (ALINORM 09/32/12, Appendix VII). These updated specifications monographs have also been edited to include the currently used reference to the test method for lead, where applicable. The specifications monographs for these food additives are not reproduced in this publication.

BRANCHING GLYCOSYLTRANSFERASE FROM *RHODOTHERMUS OBAMENSIS* EXPRESSED IN *BACILLUS SUBTILIS*

New specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs7 (2009). An ADI "not specified" was established at the 71st JECFA (2009).

SYNONYMS	1,4- α -glucan branching enzyme; amylo-(1,4 \rightarrow 1,6)-transglycosylase; α -glucan-branching glycosyltransferase; branching enzyme; Q-enzyme
SOURCES	Branching glycosyltransferase is produced by submerged fed-batch fermentation of a genetically modified strain of <i>Bacillus subtilis</i> which contains a gene coding for branching glycosyltransferase from <i>Rhodothermus obamensis</i> . The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cellular biomass, concentration by ultrafiltration and/or evaporation, and germ filtration. The final product is formulated using food-grade stabilizing and preserving agents and is standardized to the desired activity.
Active principles	Branching glycosyltransferase
Systematic names and numbers	1,4- α -D-glucan:1,4- α -D-glucan 6- α -D-(1,4- α -D-glucano)-transferase; EC 2.4.1.18; CAS No. 9001-97-2
Reactions catalysed	Transfers a segment of a 1,4- α -D-glucan chain to a primary hydroxy group in a similar glucan chain to create 1,6- α -linkages
Secondary enzyme activities	No significant levels of secondary enzyme activities
DESCRIPTION	Light brown liquid
FUNCTIONAL USES	Enzyme preparation. Used in starch processing to obtain modified starch with improved functional properties
GENERAL SPECIFICATIONS	Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.
CHARACTERISTICS	
IDENTIFICATION	
<u>Branching glycosyltransferase activity</u>	The sample shows branching glycosyltransferase activity. See description under TESTS.

TESTS

Branching glycosyltransferase activity

Principle

Branching glycosyltransferase catalyses the transfer of a segment of a 1,4- α -D-glucan chain to a primary hydroxy group in a similar glucan chain to create 1,6- α -linkages. The activity of branching glycosyltransferase is determined by measuring the rate of introduction of 1,6- α -linkages into the substrate amylose.

The enzyme activity is expressed in branching enzyme units (BEUs). One BEU is defined as the quantity of the enzyme that causes a decrease in absorbance at 660 nm of an amylose-iodine complex of 1% per minute under standard conditions (pH 7.2; 60°).

Apparatus

Spectrophotometer (660 nm)
Microtitre plates (Nunc F96 MicroWell Plates Cat. No. 467320 (MediSorp) or equivalent)
Water bath with thermostatic control or Eppendorf Thermomixer (60°)
pH meter

Reagents and solutions

0.1 M Tris-HCl buffer (pH 7.2)
(Note: Use distilled water)

Substrate solution: Accurately weigh (to ± 0.05 mg) 10 mg of amylose type III (Sigma A0512 or equivalent) into a 10-ml volumetric flask. Gently add 0.2 ml of 96% ethanol, make sure that all the amylose is wet, and leave for 3-4 min. Add 0.5 ml of 2 N sodium hydroxide (80 g of NaOH per liter), then 1 ml of water and stir for 10 min to dissolve amylose. Add 0.5 ml of 2 N hydrochloric acid and then 7.7 ml of Tris-HCl buffer. Check the pH of the solution and adjust to 7.2 if necessary. Add Tris-HCl buffer to volume.

The solution should be freshly prepared on the day of use.

Lugols solution: Weigh 0.26 g of iodine and 2.6 g of potassium iodide into a 10-ml volumetric flask. Add water to volume and mix.

The solution should be prepared at least three days before use to ensure that all the iodine has dissolved. The solution is stable for up to 6 months when stored in darkness at room temperature.

Stop reagent: Mix 100 μ l Lugols solution, 50 μ l of 2 N hydrochloric acid, and 26 ml of water in a measuring cylinder. The reagent should be freshly prepared on the day of use.

Sample solution: Accurately weigh (to ± 0.5 mg) approximately 1 g (W) of the enzyme preparation into a beaker and add about 80 ml of Tris-HCl buffer. Stir the solution slowly for 30 min with the magnetic stirrer to make sure that the sample is completely dissolved. Transfer the solution quantitatively to a 100-ml

volumetric flask and add Tris-HCl buffer to volume (V). Dilute this solution again with Tris-HCl buffer, if necessary, to obtain an activity between 30 and 50 BEU/ml. Calculate the dilution factor (D) and use it in the calculation formula. (NOTE: Typically, the dilution factor ranges from 10 to 40). The solution should be freshly prepared on the day of use.

Procedure

1. Prepare the following in 2-ml Eppendorf tubes:
Sample: Mix 50 µl of the sample solution ($V_s = 0.050$ ml) and 50 µl of the substrate solution (4 replicates)
Reference: Mix 50 µl of water and 50 µl of the substrate solution (4 replicates)
Blank: Add 100 µl of water (4 replicates)
2. Mix well and incubate all the tubes at 60° for 30 min (t)
3. After 30 min, add to each tube 2 ml of the stop reagent and mix well
4. Leave for 20 min at room temperature for colour stabilisation
5. Transfer 200 µl of each solution to microtitre plate wells and measure the absorbance at 660 nm
6. Calculate the mean absorbance values based on four replicates for the sample (A_s), reference (A_R), and blank (A_B)

NOTE: The absorbance of the sample should be within 0.15 - 0.3. If the absorbance falls outside this range, dilute the sample again with 0.1 M Tris-HCl (pH 7.2) and repeat the procedure.

Calculation

Use the following formula to calculate the activity of the branching enzyme:

$$\text{Activity (BEU/g)} = \frac{(A_R - A_s) \times V \times D \times 100}{(A_R - A_B) \times t \times V_s \times W}$$

where

$A_R - A_s$ is the difference in absorbance between the reference and sample;

$A_R - A_B$ is the difference in absorbance between the reference and blank;

V is the initial volume of the sample solution (ml);

D is the dilution factor;

100 is the conversion factor to express the enzyme activity in BEU/g;

t is the incubation time (min);

V_s is the volume of the sample solution used in the procedure (ml); and

W is the sample weight (g).

CASSIA GUM (TENTATIVE)

New tentative specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). An ADI "not specified" was established at the 71st JECFA (2009).

Information required:

- Suitable method for the determination of anthraquinones at a level of less than 0.5 mg/kg

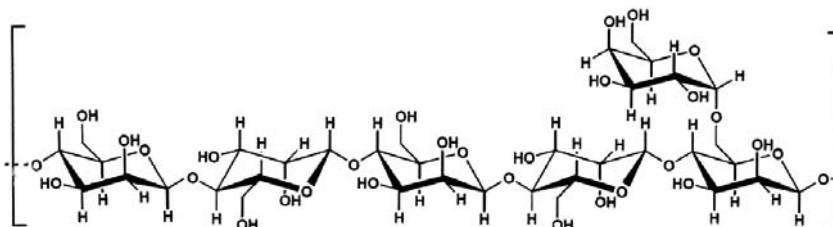
SYNONYMS

INS 427

DEFINITION

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

Structural formula



Assay

Not less than 75% of galactomannan

DESCRIPTION

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

FUNCTIONAL USES

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

CHARACTERISTICS

IDENTIFICATION

Solubility

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

Gel formation with borate

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

Gel formation with xanthan gum

Passes test
See description under TESTS

<u>Gum constituents</u> (Vol. 4)	Proceed as directed under Gum Constituents Identification (Vol. 4) using 100 mg of sample instead of 200mg and 1-10 µl of the hydrolysate instead of 1-5 µl. Use galactose and mannose as reference standards. These constituents should be present.
<u>Viscosity</u>	Less than 500 mPas (25°, 2h) (1% solution) See description under tests
<u>pH</u> (Vol. 4)	5.5-8.0 (1% solution)
PURITY	
<u>Loss on drying</u> (Vol. 4)	Not more than 12% (105°, 5 h)
<u>Total ash</u> (Vol. 4)	Not more than 1.2%
<u>Acid-insoluble matter</u> (Vol. 4)	Not more than 2.0%
<u>Protein</u> (Vol. 4)	Not more than 7.0% Proceed as directed under Nitrogen Determination (Kjeldahl Method; Vol. 4). The percent of nitrogen in the sample multiplied by 6.25 gives the percent of protein in the sample.
<u>Crude fat</u>	Not more than 1% See description under TESTS
<u>Starch</u>	To a 1 in 10 dispersion of the sample add a few drops of iodine TS; no blue colour is produced.
<u>Anthraquinones</u>	Not more than 0.5 mg/kg <i>A Suitable method for determination at this level is requested</i>
<u>Residual solvents</u>	Isopropanol: Not more than 1.0% See description under TESTS
<u>Lead</u> (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").
<u>Microbiological criteria</u> (Vol. 4)	Total plate count: Not more than 5,000 cfu/g Yeast and mould: Not more than 100 cfu/g <i>E. coli</i> : Negative in 1 g <i>Salmonella</i> : Negative in 25 g

TESTS

IDENTIFICATION TESTS

Gel formation with xanthan gum

Weigh 1.5 g of the sample and 1.5 g of xanthan gum and blend them.

Add this blend with (rapid stirring) into 300 ml water at 80° in a 400 ml beaker. Stir until the mixture is dissolved and continue stirring for an extra 30 min after dissolution (maintain the temperature above 60° during the stirring process). Discontinue stirring and allow the mixture to cool at room temperature for at least 2 h.

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

Viscosity

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

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

Crude fat

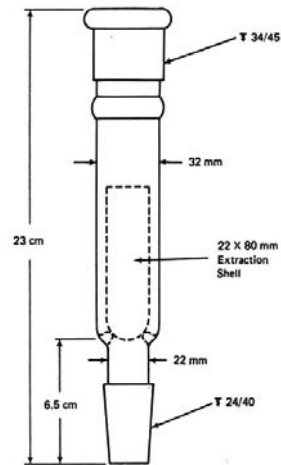
Apparatus

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

Procedure

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

Butt-Type Extractor for fat determination.



NOTE: The method for crude fat is referenced from the Food Chemicals Codex, 6th Edition, 2008, p. 1163.

METHOD OF ASSAY

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

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CYCLOTETRAGLUKOSE SYRUP

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding tentative specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI “not specified” was established at the 71st JECFA (2009).

SYNONYMS	Cyclotetraglucose syrup; Cyclic nigerosyl-(1→6)-nigerose syrup; cycloalternan syrup; cycloalternanotetraose syrup
DEFINITION	A mixture consisting of mono-, di- and oligosaccharides, of which cyclotetraglucose is the major component. It is produced from hydrolyzed food-grade starch by the actions of a mixture of 6- α -glucosyltransferase α -isomaltosyltransferase derived from <i>Sporosarcina globispora</i> , and cyclodextrin glucosyltransferase derived from <i>Bacillus stearothermophilus</i> . The final product is a syrup or a spray-dried solid.
Assay	Not less than 97.0 % of total saccharides and 30 – 40% of cyclotetraglucose on the anhydrous basis
DESCRIPTION	Colourless and odourless, clear viscous liquid or dry white crystalline mass.
FUNCTIONAL USES	Carrier
CHARACTERISTICS	
IDENTIFICATION	
<u>Chromatography</u>	The retention time of cyclotetraglucose is approx. 62 min using the conditions described under the Method of Assay.
PURITY	
<u>Water</u> (Vol. 4)	Not more than 30% for the syrup and not more than 10% for the syrup solids (Karl Fischer Method).
<u>Total ash</u> (Vol. 4)	Not more than 0.05% on the anhydrous basis (500°, 5h)
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).
<u>Microbiological criteria</u> (Vol.4)	Total (Aerobic) plate count: Not more than 300 CFU/g Coliforms: Negative in 10 g Yeast and moulds: Not more than 100 CFU/g

METHOD OF ASSAY 1. Total saccharides

Determine by the Anthrone-sulfuric acid method described below.

Anthrone solution

Add 0.2 g of Anthrone to 100 ml of diluted sulfuric acid (prepare by cautiously adding 75 ml of sulfuric acid to 20 ml of water and make up 100 ml with water). Mix and dissolve completely by ultrasonic treatment.

Sample solution

Weigh accurately about 1.0 g of the anhydrous basis sample into a 100-ml volumetric flask, and dissolve with water to make 100 ml. Dilute 1 ml of this solution with water to make 100-ml solution accurately.

Standard solution

Weigh accurately about 1.0 g of glucose (analytical reagent grade, dry solid basis) into a 100-ml volumetric flask, and dissolve with water to make 100 ml. Accurately measure 1 ml of this solution and make 100-ml solution with water.

Procedure

Add 5 ml of Anthrone solution into each of three test tube (18 mm i.d. × 180 mm) and cool in ice-cold water. Add 0.5 ml of water, standard solution or sample solution into separate tube slowly. After mixing gradually without generating heat, put a glass ball on the tube and heat in boiling water for 10 minutes accurately. After cooling with running water, measure the absorbances (A_B , A_S and A_T for water blank, standard solution and sample solution, respectively) with the wave length of 620 nm using a 10-mm length cuvette. Calculate the concentration of total saccharides in the sample as follows:

$$\text{Total saccharides (\%)} = (A_T - A_B) / (A_S - A_B) \times W_S / W_T \times 0.908 \times 100$$

where

W_S is the weight of glucose (g, dry solid basis);

W_T is the weight of sample (g, anhydrous basis); and

0.908 is the anthrone correction factor.

2. Cyclotetraglucose

Determine by HPLC (Vol. 4) using the following conditions:

Sample solution

Weigh accurately about 1.0 g of the anhydrous basis sample into a 50-ml volumetric flask and add about 40 ml of water. Dissolve the sample completely and dilute to 50 ml with water.

Standard solution

Dissolve accurately weighed cyclotetraglucose standard (available under the name of cyclotetraose from Hayashibara Co., Ltd, 2-3 Shimoishii 1-chome, Okayama 700, Japan) in water to obtain a solution having known concentration of about 10 mg of cyclotetraglucose per ml.

Chromatography

Liquid chromatograph equipped with a column oven and a refractive index detector.

Column and packing: strong acidic cation exchange resin

- length: 200–400 mm

- diameter: 8–10 mm

- temperature: 80°

Mobile phase: water

Flow rate: Adjust to obtain a retention time of 55–65 min

Injection volume: 20 µl

Procedure

Inject standard and sample solutions, and measure the areas of the cyclotetraglucose peak. The retention time of cyclotetraglucose is approx. 62 min.

Calculate the percentage of cyclotetraglucose in the test sample as follows:

$$\% \text{ cyclotetraglucose (anhydrous basis)} = 100 \times (A_S/A_R)(W_R/W_S)$$

where

A_S and A_R are the areas of the peaks due to cyclotetraglucose for the sample solution and standard solution, respectively; and

W_S and W_R are the weights of the test sample and standard cyclotetraglucose, respectively (mg, anhydrous basis).

DIACETYLTARTARIC and FATTY ACID ESTERS of GLYCEROL

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009) superseding specifications prepared at the 57th JECFA (2001) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-50 mg/kg bw was established at the 61st JECFA (2003).

SYNONYMS

Diacetyltartaric acid esters of mono- and diglycerides; DATEM; Tartaric, acetic and fatty acid esters of glycerol, mixed; Mixed acetic and tartaric acid esters of mono and diglycerides of fatty acids; INS No. 472e

DEFINITION

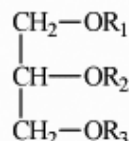
The product consists of mixed glycerol esters of mono- and diacetyltartaric acid and fatty acids from edible fats and oils. It is made by the interaction of diacetyltartaric anhydride and mono- and diglycerides of fatty acids in the presence of acetic acid, or by interaction of acetic anhydride and mono- and diglycerides of fatty acids in the presence of tartaric acid.

Because of inter- and intramolecular acyl-group exchange, both methods of production lead to the same essential components, the distribution of which depends on the relative proportions of the basic raw materials, on temperature, and on reaction time. The product may contain small amounts of free glycerol, free fatty acids, and free tartaric and acetic acids.

C.A.S. numbers

308068-42-0
100085-39-0

Structural formula



in which one or two of the R groups is a fatty acid moiety and the other R groups are either:

- diacetylated tartaric acid moiety
- monoacetylated tartaric acid moiety
- tartaric acid moiety
- acetic acid moiety
- hydrogen

DESCRIPTION

Liquid, paste, or wax-like solid

FUNCTIONAL USES

Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Dispersible in cold and hot water; soluble in methanol, ethanol, acetone, and ethyl acetate.

<u>1,2-diols</u>	To a solution of 500 mg in 10 ml methanol, add dropwise, lead acetate TS. A white, flocculent, insoluble precipitate is formed.
<u>Fatty acids</u> (Vol. 4)	Passes test (See under "Specific methods, Fats, Oils, and Hydrocarbons; Identification Tests for Functional Groups; Test A: Methyl Esters of Fatty Acids")
<u>Acetic acid</u> (Vol. 4)	Passes test (See under "Specific methods, Fats, Oils, and Hydrocarbons; Identification Tests for Functional Groups")
<u>Tartaric acid</u> (Vol.4)	Passes test (See under "Specific methods, Fats, Oils, and Hydrocarbons; Identification Tests for Functional Groups")
<u>Glycerol</u> (Vol. 4)	Passes test (See under "Specific methods, Fats, Oils, and Hydrocarbons; Identification Tests for Functional Groups")
PURITY	
<u>Acids</u> (Vol. 4)	Acids other than acetic, tartaric and fatty acids, shall not be detectable (See under "Specific methods, Fats, Oils and Hydrocarbons; Identification Tests for Functional Groups")
<u>Sulfated ash</u> (Vol. 4)	Not more than 0.5% determined at 800±25° Test 5 g of sample (Method I for solids; Method II for liquids) (See under, "General methods, Inorganic Components; Ash")
<u>Acid value</u> (Vol. 4)	Not less than 40 and not more than 130 (See under, "Specific methods, Fats, Oils and Hydrocarbons")
<u>Total acetic acid</u>	Not less than 8% and not more than 32% after hydrolysis See description under TESTS
<u>Total tartaric acid</u>	Not less than 10% and not more than 40% after saponification See description under TESTS
<u>Total glycerol</u>	Not less than 11% and not more than 28 % after saponification See description under TESTS
<u>Free glycerol</u> (Vol. 4)	Not more than 2.0% Prepare the aqueous extracts as directed under the "Procedure for 1-Monoglyceride (see under, "Specific methods, Fats, Oils and Hydrocarbons; 1-Monoglyceride and Free Glycerol Content") and proceed as directed under the "Procedure for Glycerol".
<u>Lead</u> (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method

described in Volume 4 (under "General Methods, Metallic Impurities").

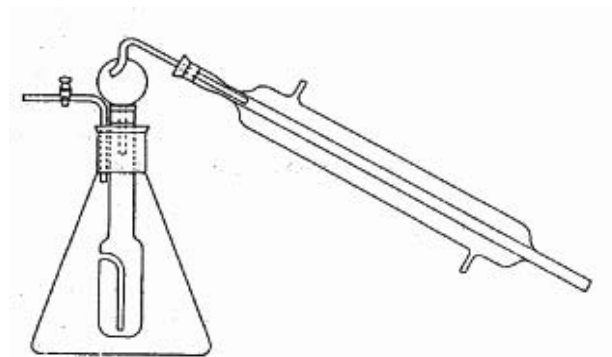
TESTS

PURITY TESTS

Total acetic acid

Apparatus

Assemble a modified Hortvet-Sellier distillation apparatus as shown in the figure, using a sufficiently large (approximately 38- x 203-mm) inner Sellier tube and large distillation trap.



Modified Hortvet-Sellier Distillation Apparatus

Procedure

Transfer 4 g of sample, accurately weighed, into the inner tube of the assembly, and insert the tube in the outer flask containing about 300 ml of recently boiled hot water. To the sample cautiously add 10 ml of approximately 4N perchloric acid [35 ml (60 g) of 70% perchloric acid in 100 ml of water], and connect the inner tube to a water-cooled condenser through the distillation trap. Distil by heating the outer flask so that 100 ml of distillate is collected within 20 to 25 min. Collect the distillate in 100-ml portions, add phenolphthalein TS to each portion, and titrate with 0.5N sodium hydroxide. Continue the distillation until a 100-ml portion of the distillate requires no more than 0.5 ml of 0.5N sodium hydroxide for neutralization. (CAUTION: Do not distil to dryness.) Calculate the weight, in mg, of volatile acids in the sample taken by the formula $V \times e$, in which V is the total volume, in ml, of 0.5N sodium hydroxide consumed in the series of titrations and e is the equivalence factor 30.03.

Total tartaric acid

Sample Preparation

Transfer about 4 g of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, and add 80 ml of approximately 0.5N potassium hydroxide and 0.5 ml of phenolphthalein TS. Connect an air condenser at least 65 cm in length to the flask, and heat the mixture on a hot plate for about 2.5 h. Add to the hot mixture

approximately 10% phosphoric acid until it is definitely acid to congo red test paper. Reconnect the air condenser, and heat until the fatty acids are liquefied and clear. Cool and then transfer the mixture into a 250-ml separator with the aid of small portions of water and hexane. Extract the liberated fatty acids with three successive 25-ml portions of hexane and collect the extracts in a second separatory funnel. Wash the combined hexane extracts with two 25-ml portions of water and add the washings to the first separatory funnel containing the water layer. Transfer the contents of the first funnel to a 250-ml beaker, heat on a steam bath to remove traces of hexane, filter through acid-washed, fine-texture filter paper into a 500-ml volumetric flask, and finally dilute to volume with water (Solution I). Pipet 25.0 ml of this solution into a 100-ml volumetric flask, and dilute to volume with water (Solution II). Retain the rest of Solution I for the determination of Total glycerol.

Standard solutions and blank

Transfer 100 mg of reagent-grade tartaric acid, accurately weighed, into a 100-ml volumetric flask, dissolve it in about 90 ml of water, add water to volume, and mix well. Transfer 3.0-, 4.0-, 5.0-, and 6.0-ml portions into separate 19- x 150-mm matched cuvettes, and add sufficient water to make 10.0 ml. To each cuvette add 4.0 ml of a freshly prepared 1 in 20 solution of sodium metavanadate and 1.0 ml of acetic acid. (NOTE: Use these solutions within 10 min after colour development.) Prepare a blank in the same manner, using 10 ml of water in place of the tartaric acid solutions.

Sample solution

Transfer 10.0 ml of Solution II into a 19- x 150-mm cuvette and add 4.0 ml of a freshly prepared 1 in 20 solution of sodium metavanadate and 1.0 ml of acetic acid. (NOTE: Use this solution within 10 min after colour development.)

Analysis

Set the suitable spectrophotometer at zero with the blank. Then determine the absorbance of the four Standard solutions of tartaric acid and the Sample solution at 520 nm. From the data thus obtained, prepare a standard curve by plotting the absorbances on the ordinate against the corresponding quantities, in mg, of the tartaric acid on the abscissa. Then from the curve, determine the weight, in mg, of tartaric acid in the final dilution, multiply this by 20, and divide the result by the weight of the original sample to give the percentage of tartaric acid.

Total glycerol

Transfer 5.0 ml of Solution I prepared in the test for Total tartaric acid into a 250-ml glass-stoppered Erlenmeyer or iodine flask. Add to the flask 15 ml of glacial acetic acid and 25.0 ml of periodic acid solution, prepared by dissolving 2.7 g of periodic acid (H_5IO_6) in 50 ml of water, adding 950 ml of glacial acetic acid, and mixing thoroughly; protect this solution from light. Shake the mixture for 1 or 2 min, allow it to stand for 15 min, add 15 ml of potassium iodide solution (150 mg/ml) and 15 ml of water, swirl, and let stand 1 min. Titrate the liberated iodine with 0.1N sodium thiosulfate, using

starch TS as the indicator. Perform a residual blank titration using water in place of the sample. The corrected volume is the number of ml of 0.1N sodium thiosulfate required for the glycerol and the tartaric acid in the sample represented by the 5 ml of Solution I. From the percentage of the tartaric acid determined in the test for Total tartaric acid, calculate the volume of 0.1N sodium thiosulfate required for the tartaric acid in the titration. The difference between the corrected volume and the calculated volume required for the tartaric acid is the number of ml of 0.1N sodium thiosulfate consumed due to the glycerol in the sample. One ml of 0.1N sodium thiosulfate is equivalent to 2.303 mg of glycerol and to 7.505 mg of tartaric acid.

ETHYL LAUROYL ARGINATE

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 69th JECFA (2008) and published in FAO JECFA Monographs 5 (2008). An ADI of 0-4 mg/kg bw per day for ethyl-N^α-lauroyl-L-arginate was established at the 69th JECFA (2008).

SYNONYMS

Lauric arginate ethyl ester; lauramide arginine ethyl ester; ethyl-N^α-lauroyl-L-arginate·HCl; LAE; INS No. 243

DEFINITION

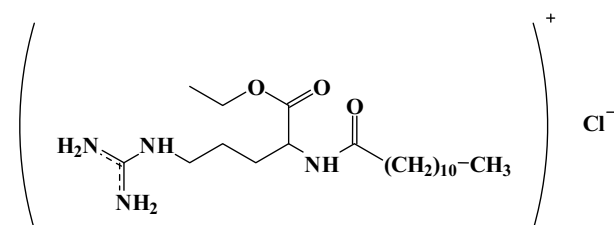
Ethyl lauroyl arginate is synthesized by esterifying arginine with ethanol, followed by reacting the ester with lauroyl chloride. The resultant ethyl lauroyl arginate is recovered as the hydrochloride salt, which is filtered and dried.

Chemical name Ethyl-N^α-dodecanoyl-L-arginate·HCl

C.A.S. number 60372-77-2

Chemical formula C₂₀H₄₁N₄O₃Cl

Structural formula



Formula weight 421.02

Assay Not less than 85% and not more than 95%

DESCRIPTION White powder

FUNCTIONAL USES Preservative

CHARACTERISTICS

IDENTIFICATION

pH (Vol.4) 3.0-5.0 (1% solution)

Solubility (Vol. 4) Freely soluble in water, ethanol, propylene glycol and glycerol

Chromatography The retention time for the major peak in a HPLC chromatogram of the sample is approx. 4.3 min using the conditions described under the Method of Assay.

PURITY

<u>Total ash</u> (Vol. 4)	Not more than 2% (700°)
<u>Water</u> (Vol. 4)	Not more than 5%. Determine by the methods described in Volume 4 under "General Methods, Water Determination (Karl Fischer Method)".
<u>N^α-Lauroyl-L-arginine</u>	Not more than 3% See description under TESTS
<u>Lauric acid</u>	Not more than 5% See description under TESTS
<u>Ethyl laurate</u>	Not more than 3% See description under TESTS
<u>L-Arginine·HCl</u>	Not more than 1% See description under TESTS
<u>Ethyl arginate·2HCl</u>	Not more than 1% See description under TESTS
<u>Lead</u> (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

<u>N^α-Lauroyl-L-arginine</u>	Determine by HPLC in Volume 4 (under "Analytical Techniques, Chromatography") using the conditions described in the Method of Assay. NOTE: The retention time of N ^α -lauroyl-L-arginine is approx. 2.2 min. Calculate the percentage of N ^α -lauroyl-L-arginine in the test sample as follows:
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$$\% \text{ N}^{\alpha}\text{-Lauroyl-L-arginine} = \frac{C \times 50}{W \times 1000} \times 100$$

where

C is the N^α-lauroyl-L-arginate·HCl concentration determined (μg/ml);
W is the weight of sample (mg); and
50 is the volume of sample solution (ml).

Lauric acid and ethyl laurate

Determine by HPLC in Volume 4 (under “Analytical Techniques, Chromatography”) using the following conditions.

Chromatography

Liquid chromatograph equipped with a spectrophotometric detector.
 Column: Symmetry C18, 150 x 3.9 mm, 5µm (Waters) or equivalent
 Column temperature: room temperature
 Mobile phase: acetonitrile/water (85:15) containing 0.1% trifluoroacetic acid
 Flow rate: 1 ml/min
 Wavelength: 212 nm
 Injection volume: 10 µl

Standard solution

Weigh accurately about 125 mg of lauric acid standard and 75 mg ethyl laurate standard into a 50-ml volumetric flask. Dissolve and dilute with the mobile phase to obtain a solution of about 2500 µg/ml of lauric acid and 1500µg/ml of ethyl laurate. Take 5, 10 and 15 ml of the solution and dilute to 50 ml with mobile phase for the standard curves.

Sample solution

Weigh accurately about 500 mg of test sample into a 50-ml volumetric flask. Dissolve and dilute to 50 ml with mobile phase.

Procedure

Inject the standard and sample solutions into the chromatograph and measure their concentration (C µg/ml) from their peak area and their standard curves.

NOTE: The retention time of lauric acid is approx. 3.65 min and that of ethyl laurate is approx. 11.2 min.

Calculate their percentage in the test sample as follows:

$$\% \text{ Lauric acid or ethyl laurate} = \frac{C \times 50}{W \times 1000} \times 100$$

where

C is the lauric acid or ethyl laurate concentration determined (µg/ml);
 W is the weight of sample (mg); and
 50 is the volume of sample solution (ml).

L-Arginine·HCl and ethyl arginate·2HCl

Determine by HPLC in Volume 4 (under “Analytical Techniques, Chromatography”) with post-column derivatization using the following conditions:

NOTE: Use deionized water

Chromatography

Liquid chromatograph equipped with a post-column derivatization and a spectrophotometric detector.
 Column and packing: µ Bondapack C18, 300 x 3.9 mm, 10µm

(Waters) or equivalent

Mobile phase: A-B-C-D (1:1:1:1.5)

A: 15 mmole/l sodium heptanesulphonate, B: 27 mmole/l phosphoric acid solution, C: 3 mmole/l sodium di-hydrogen phosphate solution, D: methanol

Flow rate: 0.8 ml/min

Flow rate of reagent solution: 0.8 ml/min

Column temperature: 65°

Wavelength: 340 nm

Injection volume: 10 µl

Standard solution

L-Arginine·HCl: Weigh accurately about 40 mg of L-arginine·HCl standard into a 100-ml volumetric flask. Dissolve and dilute to 100 ml with water to obtain a solution of about 400 µg/ml of L-arginine·HCl.

Ethyl arginate·2HCl: Weigh accurately about 200 mg of ethyl arginate·2HCl standard into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with water to obtain a solution of about 8000 µg/ml of ethyl arginate·2HCl.

Take 1, 2 and 3 ml of each solution and dilute to 20 ml with mobile phase separately for the standard curves.

Sample solution

To analyze L-arginine·HCl, weigh accurately about 100 mg of test sample into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with water.

To analyze ethyl arginate·2HCl, weigh accurately about 2 g of test sample into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with water.

Derivatizing solution

Mix 1 liter of 0.2M borate buffer solution (pH 9.4) with 0.8 g of o-phthalaldehyde dissolved in 5 ml of methanol and 2 ml of 2-mercaptoethanol. The solution is stable 48 h at room temperature and without additional preventive measure but It is advisable to keep the solution under nitrogen and to prepare it freshly every 24-48 h.

Procedure

Inject the standard and sample solutions into the chromatograph. After column, derivatization reaction is produced employing the derivatizing solution at 65° in a teflon tubular reactor (650-800 x 0.3 mm). Then, measure the area of the peak at 340 nm.

NOTE: The retention time of L-arginine·HCl is approx. 5.03 min and ethyl arginate·2HCl is approx. 6.70 min.

Calculate the percentage of L-arginine·HCl and ethyl arginate·2HCl in the test sample as follows:

$$\% \text{ L-arginine·HCl or ethyl arginate·2HCl} = \frac{C \times 25}{W \times 1000} \times 100$$

where

C is the L-arginine·HCl or ethyl arginate·2HCl concentration

determined ($\mu\text{g/ml}$);
 W is the weight of sample (mg); and
 25 is the volume of sample solution (ml).

METHOD OF ASSAY Determine by HPLC in Volume 4 (under "Analytical Techniques, Chromatography") using the following conditions:
 NOTE: Use deionized water

Standards

Ethyl- N^α -lauroyl-L-arginate·HCl standard

N^α -lauroyl-L-arginine standard

(available from Laboratorios Miret, S.A, Géminis 4, Políg. Ind. Can Parellada, 08228 Terrassa, Spain)

Chromatography

Liquid chromatograph equipped with a spectrophotometric detector.
 Column and packing: Symmetry C18, 150 x 3.9 mm, 5 μm (Waters) or equivalent

Column temperature: room temperature

Mobile phase: acetonitrile/water (50:50) containing 0.1% trifluoroacetic acid

Flow rate: 1 ml/min

Wavelength: 215 nm

Injection volume: 10 μl

Standard solution

Weigh accurately about 25 mg of N^α -lauroyl-L-arginine standard into a 25-ml volumetric flask. Dissolve and dilute to 25 ml with mobile phase (solution A). Weigh accurately about 150 mg of ethyl- N^α -lauroyl-L-arginate·HCl standard into a 50-ml volumetric flask and dissolve with some milliliters of the mobile phase. Then, add 5 ml of solution A and dilute to 50 ml with mobile phase to obtain a solution of about 3000 $\mu\text{g/ml}$ of ethyl- N^α -lauroyl-L-arginate·HCl and 100 $\mu\text{g/ml}$ of N^α -lauroyl-L-arginine (solution B). Take 2, 4, 6, 8 and 10 ml of solution B and dilute to 25 ml with mobile phase for the standard curves.

Sample solution

Weigh accurately about 50 mg of test sample into a 50-ml volumetric flask. Dissolve and dilute to 50 ml with mobile phase.

Procedure

Inject the standard and sample solutions into the chromatograph and measure the area of the peak.

Note: The retention time of ethyl- N^α -lauroyl-L-arginate·HCl is approx. 4.3 min.

Calculate the percentage of ethyl- N^α -lauroyl-L-arginate·HCl in the test sample as follows:

$$\% \text{ Ethyl-}N^\alpha\text{-lauroyl-L-arginate}\cdot\text{HCl} = \frac{C \times 50}{W \times 1000} \times 100$$

where

C is the ethyl-N^α-lauroyl-L-arginate·HCl concentration determined (μg/ml);

W is the weight of sample (mg); and

50 is the volume of sample solution (ml).

FERROUS AMMONIUM PHOSPHATE

New specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A PMTDI of 0.8 mg/kg bw for iron was established at the 35th JECFA (1989).

SYNONYMS Iron(II) ammonium phosphate; Phosphoric acid, ammonium iron (II) salt

DEFINITION Ferrous ammonium phosphate is manufactured by first combining iron powder and phosphoric acid in deionized water with stirring and heating the mixture to get ferrous hydrogen phosphate as a slurry. Ammonia is added to get ferrous ammonium phosphate. The product is then spray dried and milled. Ferrous ammonium phosphate consists primarily of the anhydrous salt with small amounts of the hydrate.

C.A.S. number 10101-60-7

Chemical formula FeNH_4PO_4

Formula weight 168.85 anhydrous

Assay Not less than 24 % and not more than 30% expressed as Iron(II)

DESCRIPTION Greyish-green powder

FUNCTIONAL USES Nutrient supplement

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, soluble in dilute mineral acids

Iron (Vol. 4) Passes test

Ammonium (Vol. 4) Passes test

Phosphate (Vol. 4) Passes test

PURITY

Water (Vol. 4) Not more than 3% (Karl Fischer method)

Fluoride (Vol. 4) Not more than 50 mg/kg (Method I or II)

Iron (III) Not more than 7%
See description under TESTS

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

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

Mercury (Vol. 4)

Not more than 1 mg/kg

Determine using cold vapour atomic absorption technique. 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").

Cadmium (Vol. 4)

Not more than 1 mg/kg

Determine using 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").

Arsenic (Vol. 4)

Not more than 3 mg/kg (Method II)

Determine using atomic absorption hydride technique. Use Method I for sample preparation.

TESTS

PURITY TESTS

Iron (III)

Transfer 1 g of sample into a 250 ml Erlenmeyer flask, add 20 ml of water, 10 ml of hydrochloric acid TS, heat to dissolve and cool to room temperature. Add 3 g of potassium iodide, stopper, swirl to mix, and allow to stand in the dark for 15 min. Remove the stopper, add approx. 100 ml of water, and titrate with 0.1 N sodium thiosulfate, adding starch TS near the end point. Each ml of 0.1 N sodium thiosulfate is equivalent to 5.585 mg of iron (III).

METHOD OF ASSAY

Weigh accurately about 0.3 g of the sample into a 250 ml conical flask, add 25 ml of dilute sulfuric acid (16% v/v) and dissolve with heating. Cool and add 75 ml of water. Add 0.1 ml of ferroin indicator solution (0.1% w/v in water). Titrate immediately with 0.1 N cerium sulfate until the colour changes from orange to light bluish-green. Each ml of 0.1 N cerium sulfate is equivalent to 5.585 mg of iron (II).

GLYCEROL ESTER OF GUM ROSIN (TENTATIVE)

New tentative specifications prepared at the 71st JECFA (2009 and, published in FAO JECFA Monographs 7 (2009). A group ADI of 0 – 25 mg/kg bw for glycerol ester of gum rosin and glycerol ester of wood rosin was established at the 71st JECFA (2009).

Information is required on batches of commercially available products:

- *representative infrared spectra, with identification of relevant peaks and conditions of analysis. Clean spectra are also required*
- *identification of the main resin acids with their relative proportions, obtained with updated chromatographic techniques*
- *other data or information useful for distinguishing among the glycerol esters of wood, gum and tall oil rosins*

DEFINITION

Glycerol ester of gum rosin is a complex mixture of tri- and diglycerol esters of resin acids from gum rosin, with a residual fraction of monoglycerol esters. It is obtained by the esterification of refined gum rosin under a nitrogen atmosphere with food-grade glycerol, and purified by counter-current steam distillation. Refined gum rosin is obtained by extracting oleoresin gum from living pine trees and refining it through washing, filtration and distillation. It is composed of approximately 90% resin acids and 10% neutrals (non-acidic compounds). 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.

DESCRIPTION

Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES

Emulsifier, density adjustment agent for 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 plate) corresponds with the typical infrared spectrum

Infrared spectra from commercially available products are requested

<u>Sulfur test</u>	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	
<u>Specific gravity</u> (Vol. 4)	d_{25}^{20} : Not less than 0.935 (50% solution in d-limonene)
<u>Ring and ball softening point</u> (Vol. 4)	Not less than 82° (see “Specific Methods, Glycerol Esters of Rosins”)
<u>Acid value</u> (Vol. 4)	Between 3 and 9 (see “Specific Methods, Fats, Oils, and Hydrocarbons”)
<u>Lead</u> (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”).

GLYCEROL ESTER OF TALL OIL ROSIN (TENTATIVE)

New tentative specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009). No ADI was allocated at the 71st JECFA (2009).

Information required on batches of commercially available products:

- *representative infrared spectra, with identification of relevant peaks and conditions of analysis. Clean spectra are also required*
- *identification of the main resin acids with their relative proportions, obtained with updated chromatographic techniques*
- *other data or information useful for distinguishing among the glycerol esters of wood, gum and tall oil rosins*

DEFINITION

Glycerol ester of tall oil rosin is a complex mixture of tri- and diglycerol esters of resin acids from tall oil rosin with a residual fraction of monoglycerol esters. It is obtained by the esterification of tall oil rosin under a nitrogen atmosphere with food-grade glycerol, and purified by steam-stripping. Tall oil rosin is obtained by distillation of crude tall oil, a by-product of Kraft (paper) pulp processing. It is composed of approximately 90% resin acids and 10% neutrals (non-acidic compounds). The resin-acid fraction is a complex mixture of isomeric diterpenoid monocarboxylic acids having the typical empirical formula $C_{20}H_{30}O_2$, of which the main component is abietic acid. These specifications do not cover substances derived from wood rosin, obtained by the solvent extraction of aged pine stumps, and substances derived from gum rosin, an exudate of living pine trees.

DESCRIPTION

Hard, yellow to pale amber-coloured solid

FUNCTIONAL USES

Emulsifier, density adjustment agent for 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 plate) corresponds with the typical infrared spectrum

Infrared spectra from commercially available products are requested.

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

PURITY

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

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

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

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

GLYCEROL ESTER OF WOOD ROSIN (TENTATIVE)

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 46th JECFA (1996) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI of 0 – 25 mg/kg bw for glycerol ester of gum rosin and glycerol ester of wood rosin was established at the 71st JECFA (2009).

Information required on batches of commercially available products:

- *representative infrared spectra, with identification of relevant peaks and conditions of analysis. Clean spectra are also required*
- *identification of the main resin acids with their relative proportions, obtained with updated chromatographic techniques*
- *other data or information useful for distinguishing among the glycerol esters of wood, gum and tall oil rosins*

SYNONYMS

INS No. 445

DEFINITION

Glycerol ester of wood rosin is a complex mixture of tri- and diglycerol esters of resin acids from wood rosin obtained by the solvent extraction of aged pine stumps followed by a liquid-liquid solvent refining process. The refined wood rosin is composed of approximately 90% resin acids and 10% neutrals (non-acidic compounds). 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 for 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 plate) corresponds with the typical infrared spectrum.

Infrared spectra from commercially available products are requested.

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

Specific gravity (Vol. 4)

d_{25}^{20} : 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 principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

LYCOPENE EXTRACT FROM TOMATO

New specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A group ADI “not specified” for lycopene from all sources was established at the 71st JECFA (2009).

SYNONYMS

Lycopene (tomato); INS 160d(ii)

DEFINITION

Lycopene extract from tomatoes is obtained by ethyl acetate extraction of the pulp of ripe red tomatoes (*Lycopersicon esculentum* L.) with subsequent removal of the solvent. The major colouring principle in tomato extract is lycopene; however, minor amounts of other carotenoid pigments may also be present. The product also contains oils, fats, waxes, and flavour components naturally occurring in tomatoes.

Chemical names

Ψ, Ψ -carotene
all-*trans*-lycopene
(all-*E*)-lycopene
(all-*E*)-2,6,10,14,19,23,27,31-octamethyl-
2,6,8,10,12,14,16,18,20,22,24,26,30-dotriacontatridecaene

C.A.S. number

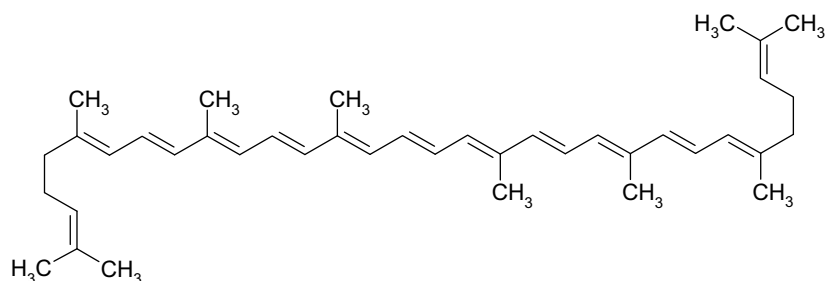
502-65-8 (lycopene)

Chemical formula

$C_{40}H_{56}$ (lycopene)

Structural formula

All-*trans*-lycopene, the major colouring principle



Formula weight

536.85 (lycopene)

Assay

Not less than 5% and not more than 15% total lycopenes.
Not less than 6.5% and not more than 16.5% total carotenoids
(calculated as lycopene)

DESCRIPTION

Dark-red viscous liquid

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

<u>Solubility</u> (Vol. 4)	Freely soluble in ethyl acetate and n-hexane; partially soluble in ethanol and acetone; and insoluble in water.
<u>Test for carotenoids</u>	The colour of the solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrate and 1 M sulfuric acid.
<u>Spectrophotometry</u> (Vol. 4)	A solution in n-hexane shows an absorption maximum at approximately 472 nm.

PURITY

<u>Sulfated Ash</u> (Vol. 4)	Not more than 1.0%, using a sample of 1-2 g
<u>Residual Solvents</u>	Ethyl acetate: Not more than 50 mg/kg See description under TESTS
<u>Lead</u> (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").
<u>Arsenic</u> (Vol. 4)	Not more than 3 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

<u>Residual solvents</u>	Ethyl acetate is determined by headspace gas chromatography. <u>Chromatographic system</u> - Detector: flame ionization - Column: Megabore fused silica (30 m x 0.53 mm I.D), coated with a 3 µm-film of 5% diphenyl-95% dimethyl polysiloxane - Carrier gas: nitrogen - Flow rate: 4 ml/min - Injector temperature: 180° - Detector temperature: 230° - Oven temperature: 5 min at 73°; to 160° at 25°/min; then 1 min at 160° - Injection mode: splitless 1:6
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- Run time: 9.5 min

Ethyl acetate stock solutions

- *Solution A (10,000 mg/kg)*: Accurately weigh 500 mg of ethyl acetate to a flask, and bring accurately to 50.00 g with diethylphthalate (use an ultrasonic bath to dissolve). The solution is stable at least for two months at room temperature.

- *Solution B (100 mg/kg)*: Accurately weigh 500 mg of Solution A to a flask, and bring accurately to 50.00 g with diethylphthalate (use an ultrasonic bath to dissolve). The solution is stable at least for two months at room temperature.

Ethyl acetate standard solutions

- *Solution C (5 mg/kg)*: Accurately weigh 500 mg of Solution B into a 20-mm headspace vial and bring accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial.

- *Solution D (10 mg/kg)*: Accurately weigh 1000 mg of Solution B, into a 20-mm headspace vial and bring accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial.

- *Solution E (17.5 mg/kg)*: Accurately weigh 1750 mg of Solution B into a 20-mm headspace vial and bring accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial.

- *Solution F (25 mg/kg)*: Accurately weigh 2500 mg of Solution B into a 20 mm headspace vial and bring accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial.

NOTE: The vials are pre-weighted.

Sample solution

Select a representative sample of 30 g sample from the lot. The sampling should be done after heating the sample lot to 40-50° and extensive mechanical stirring. Warm the sample to 50° in a water bath, mix well with a glass rod or a spatula and weigh accurately 5000 mg of the sample into a 20-mm headspace vial. Bring the weight of the sample accurately to 10.00 g (total weight), to within 0.1 mg, with diethylphthalate. Insert a 12-15 mm magnetic stirrer and seal the vial. Mix well using a magnetic stirrer.

Procedure

Place the four standard solutions (C, D, E and F) and the sample solution in a thermostatic water bath (70°) for exactly 2 h, stirring each one for 1 min every 30 min. Inject 1000 µl of each standard solution into the head-space gas chromatograph-FID system. Record the peak area and calculate the mean ratio of the standard concentration to peak area based on concentrations and peak areas of standard solutions C, D, E, and F. Inject 1000 µl of each sample solution, record the peak area and calculate the

concentration of the ethyl acetate (mg/kg), using the equation:

$$\text{Ethyl acetate (mg/kg)} = A_s \times \left(\frac{C_{ST}}{A_{ST}} \right) \times \frac{W_{tw}}{W_s}$$

where

A_s is the measured peak area of the sample solution;
 (C_{ST}/A_{ST}) is the mean ratio of the standard concentration to peak area based on concentrations and peak areas of standard solutions C, D, E, and F (mg/kg);

W_{tw} is the total weight of the sample solution (g); and

W_s is the sample weight (g).

METHOD OF ASSAY Total lycopenes are determined by HPLC. Total carotenoids are determined spectrophotometrically.

Total lycopene and Total carotenoids

TOTAL LYCOPENES

Reagents

- Dichloromethane (HPLC-grade)
- Acetonitrile (HPLC-grade)
- Methanol (HPLC-grade)
- n-Hexane (HPLC-grade)
- BHT (2,6 di-tert-butyl -4-methylphenol) (A.R.)
- Petroleum ether (spirit) b.p. 60-80°(A.R.)
- Ethanol (A.R.)
- N-ethyl-diisopropylamine
- All-*trans*-lycopene standard (purity 96% or higher, available from Lycored, P.O.B. 320, Industrial Zone, Beer-Sheva, 84102, Israel)

Chromatographic system

- HPLC system with a UV/VIS detector or a diode array detector, auto sampler or injector
- Detector: 472 nm
- Column: Select B (RP-C8) (250 x 4.6 mm, 5 µm) Merck no. 50984 or equivalent
- Mobile phase: acetonitrile:methanol:dichloromethane:n-hexane:N-ethyl-diisopropylamine 850:100:25:25:0.5 (v/v/v/v/v). Mix well and sonicate for 3-4 min in an ultrasonic bath
- Flow rate: 0.7 ml/min
- Injection volume: 10 µl
- Run time: 12 min

Diluent solution

Transfer 0.5 g BHT, 600 ml acetonitrile, 100 ml methanol, 150 ml dichloromethane and 150 ml n-hexane into a 1000-ml bottle. Mix well and sonicate for 3-4 min in an ultrasonic bath.

Lycopene standard stock solution (500 mg/l)

Weigh accurately (to ± 0.1 mg) about 50 mg all-*trans*-lycopene standard into a 100-ml volumetric flask and add 100 mg of α -tocopherol and 100 mg of BHT. Add toluene to volume and sonicate 1-2 min, mix well. Dispense to 8-ml amber vials. The solution is stable for six months when stored at -18° .

Lycopene standard solutions

Take one vial of the Lycopene standard stock solution and warm to 50° in a water bath for several minutes, shaking the solution occasionally to ensure that the lycopene particles are completely dissolved. Transfer 3 ml of this solution to a 25-ml amber volumetric flask and add the Diluent solution to volume and mix (Solution A). Take another vial of the Lycopene standard stock solution and treat as above. Transfer 4 ml of this solution to a second 25-ml amber flask and add the Diluent solution to volume and mix (Solution B). Solutions A and B are stable for at least 3 weeks if held at -18° . Prior to each use, determine spectrophotometrically the lycopene concentration in each solution (See Standardization of the Lycopene standard solutions).

BHT solution (5000 mg/l)

Weigh 2.5 g BHT into a 500-ml storage bottle and add 500 ml dichloromethane. Keep the solution protected from light. This solution is stable for 3 months.

Sample solutions

Introduce a representative sample of the tomato extract into a vial and close it. Place the vial in a water bath at 50° for 30 minutes. (NOTE: The temperature should not exceed 60°). Stir the solution using a glass rod. Weigh accurately (to ± 0.1 mg) 1.0 to 1.2 g of the sample into each of three 100-ml (V_A) volumetric flasks (samples 1, 2 and 3) and add 10 ml of BHT solution and 40 ml of dichloromethane to each flask. Homogenize the solutions using an ultrasonic bath, cool the solutions to room temperature and bring each to volume with dichloromethane and mix (Solutions C). Transfer 5 ml (V_B) of each Solution C to separate amber 50-ml (V_C) volumetric flasks. Bring each to volume with the Diluent solution and mix well (Solutions D).

Procedure

- Standardizing of the Lycopene standard solutions

Transfer 2.0 ml (V_D) of each of Solutions A and B into 100-ml (V_E) volumetric flasks and add 10 ml of ethanol and 10 ml of BHT solution. Bring the two solutions to volume with petroleum ether (Solutions E and F). Using a suitable UV/VIS spectrophotometer and 1-cm cell, determine the absorbances of these solutions at 472 nm using petroleum ether as a blank. Calculate the lycopene concentrations (C_{ST} mg/l) in Solutions A and B using the equation:

$$C_{ST}(\text{mg/l}) = \frac{A_{\text{max}} \times D \times 10000}{3450}$$

where

A_{\max} is the absorbance of either Solution E or Solution F (corresponding to Solutions A and B, respectively) at 472 nm corrected for the blank;
 D is the dilution factor V_E/V_D ;
 10000 is the scaling factor; and
 3450 is the specific absorbance ($A^{1\%_{1\text{ cm}}}$) of all-*trans*-lycopene in petroleum ether.

(NOTE: The lycopene concentrations of the standard Solutions A and B should be redetermined prior to each separate analysis.)

- Chromatographic analysis

Inject Solutions A and B into the chromatograph. Record the peak areas. Inject the three sample solutions (Solutions D) and record the peak areas of lycopene (the retention time of all isomers of lycopene is approximately 5 to 7 min and that for β -carotene is 8 to 9 min). The peak area of lycopene for the sample solutions should be between 80 and 120% of the standards, otherwise dilute the Solution C with the Diluent solution to bring the lycopene concentration to the desired range or increase the sample weight.

- Calculation

Calculate the percentage of total lycopenes in sample 1 (TL_{1A}) as follows:

$$TL_{1A}(\%) = \frac{A_S \times C_{ST} \times V_A \times D}{A_{ST} \times W_S} \times 100$$

where

A_S is the peak area of the sample;
 A_{ST} is the peak area of the standard Solutions A;
 C_{ST} is the lycopene concentration in the standard solution A (mg/l);
 V_A is the volume (l) used to dilute W_S to prepare solution C;
 D is the dilution factor V_C/V_B ; and
 W_S is the sample weight (mg).

Repeat the calculation for the remaining two samples to obtain TL_{2A} and TL_{3A} .

Use the same procedure to calculate the percent of total lycopenes in the samples using the peak area of the standard Solutions B. Record the results as TL_{1B} , TL_{2B} and TL_{3B} . Calculate the mean percentage of total lycopenes in tomato extract.

TOTAL CAROTENOIDS

Using a volumetric pipette, transfer 2 ml (V_F) of Solution D (see above) to an amber 100-ml (V_G) volumetric flask. Add 10 ml of ethanol, bring to volume with petroleum ether and mix well. This is sample Solution G.

Using a suitable UV/VIS spectrophotometer and 1-cm sample cells with covers, scan the spectrum of Solution G from 550 to 300 nm, using petroleum ether as the reference blank and measure the

absorbance at the absorbance maximum (approximately 472 nm).

The absorbance should be between 0.2 and 0.8. Calculate the percentage of total carotenoids (as lycopene) in the sample using the following equation:

$$\text{Total carotenoids (as lycopene) \%} = \frac{A \times D}{W_s \times 3450} \times 100$$

where

A is the absorbance of Solution G at 472 nm, corrected for the blank;

3450 is the specific absorbance ($A^{1\%}_{1\text{ cm}}$) of all-*trans*-lycopene in petroleum ether;

W_s is the weight of the sample (g); and

D is the dilution factor ($V_G \times V_C / V_F \times V_B$).

MODIFIED STARCHES

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 57th JECFA (2001) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI "not specified" was established at the 26th JECFA (1982) for all modified starches listed below except for acetylated oxidized starch for which an ADI "not specified" was established at the 57th JECFA (2001).

Modified starches comprise the following:

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

DEFINITION

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

C.A.S. numbers	Starch acetate:	9045-28-7
	Acetylated distarch adipate:	68130-14-3
	Hydroxypropyl starch:	9049-76-7

Hydroxypropyl distarch phosphate:	53124-00-8
Acetylated oxidized starch:	68187-08-6

DESCRIPTION

Most modified starches are white or off-white, odourless powders. According to the drying method these powders can consist of whole granules having the appearance of the original native starch, or aggregates consisting of a number of granules (pearl starch, starch-grits) or, if pre-gelatinized, of flakes, amorphous powder or coarse particles.

FUNCTIONAL USES Thickener, stabilizer, binder, emulsifier

CHARACTERISTICS

IDENTIFICATION

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

Microscopy Passes test
See description under TESTS

Iodine stain Passes test
See description under TESTS

Copper reduction Passes test
See description under TESTS

Differentiation test Passes test for type of starch
See description under TESTS for:
1. Hypochlorite oxidized starch
2. Specific reaction for acetyl groups
3. Positive test for ester groups

PURITY

Sulfur dioxide Not more than 50 mg/kg for modified cereal starches
Not more than 10 mg/kg for other modified starches unless otherwise specified in Table I
See description under TESTS

Lead (Vol. 4) Not more than 2 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

Additional purity specifications for individual chemically modified starches See column 3 of Table I
See description under TESTS

TESTS

IDENTIFICATION TESTS

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

Principle
Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue.

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

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

Procedure
About 10 g of the sample is suspended in 25 ml water to which is added 20 ml of 0.4 N NaOH. After shaking for 1 h the starch is filtered off and the filtrate evaporated in an oven at 110°. The residue is dissolved in a few drops of water and transferred to a test tube. Add calcium hydroxide and heat the tube. If the sample is acetylated starch, acetone vapours are produced. These produce a blue colour

on a paper strip soaked in a fresh saturated solution of o-nitrobenzaldehyde in 2 N NaOH. The blue colour is more distinct when the original yellow colour of the reagents is removed with 1 drop of a 1 in 10 solution of hydrochloric acid.

3. Positive test for ester groups

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

PURITY TESTS

Sulfur dioxide

Scope

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

Principle

The sulfur dioxide is released from the sample in a boiling acid medium and is removed by a stream of carbon dioxide. The separated gas is collected in dilute hydrogen peroxide where it is oxidized to sulfuric acid and titrated with standard alkali.

Alternatively, the sulfuric acid may be determined gravimetrically as barium sulfate.

Apparatus

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

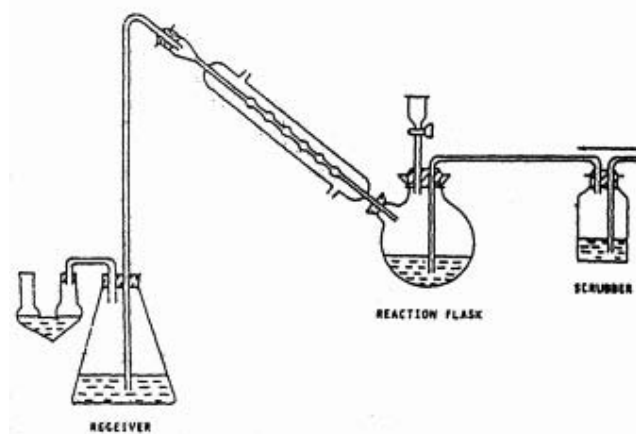


Figure 1

The assembly consists of a 1000-ml two-neck round-bottom boiling flask to which a gas-inlet tube, a 60-ml dropping funnel having a 2-mm bore stopcock, and a sloping Allihn reflux condenser are attached. A delivery tube connects the upper end of the condenser to the bottom of a 250-ml conical receiving flask, which is followed by a Peligot tube.

In operation, carbon dioxide is passed through the scrubber and

bubbled through the heated reaction mixture, sweeping sulfur dioxide through the condenser and into the receivers where it is absorbed quantitatively.

Preparation of solutions

Sodium carbonate solution: Dissolve approximately 15 g of Na_2CO_3 or 40 g of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ in distilled water, and dilute to 100 ml.
Hydrogen peroxide, 3%: Dilute 10 ml of C.P. (Chemical Purity) neutral 30% hydrogen peroxide (H_2O_2) with distilled water to 100 ml.

Procedure

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

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

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

where

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

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

<u>Modification</u>	<u>Process limitations</u>	<u>End-product specifications</u>
Dextrin roasted starch	Dry heat treatment with hydrochloric acid or ortho-phosphoric acid	Final pH 2.5-7.0
Acid treated starch	Treatment with hydrochloric acid or ortho-phosphoric acid or sulfuric acid	Final pH 4.8-7.0

<u>Modification</u>	<u>Process limitations</u>	<u>End-product specifications</u>
Alkaline treated starch	Treatment with sodium hydroxide or potassium hydroxide	Final pH 5.0-7.5
Bleached starch	Treatment with peracetic acid and/or hydrogen peroxide, or sodium hypochlorite or sodium chlorite, or sulfur dioxide or alternative permitted forms of sulfites, or potassium permanganate or ammonium persulfate	Added carbonyl group not more than 0.1% No residual reagent Residual sulfur dioxide not more than 50 mg/kg Residual manganese not more than 50 mg/kg
Enzyme-treated starch	Treatment in an aqueous solution at a temperature below the gelatinization point with one or more food-grade amylolytic enzymes	Residual sulfur dioxide not more than 50 mg/kg
Oxidized starch	Treatment with sodium hypochlorite	Carboxyl groups not more than 1.1% Residual sulfur dioxide not more than 50 mg/kg
Monostarch phosphate	Esterification with ortho-phosphoric acid, or sodium or potassium ortho-phosphate, or sodium tripolyphosphate	Phosphate calculated as phosphorus not more than 0.5% for potato or wheat, and not more than 0.4% for other starches
Distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride	Phosphate calculated as phosphorus not more than 0.5% for potato and wheat, and not more than 0.4% for other starches
Phosphated distarch phosphate	Combination of treatments for Monostarch phosphate and Distarch phosphate	Phosphate calculated as phosphorus not more than 0.5% for potato and wheat, and not more than 0.4% for other starches
Acetylated distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride combined with esterification with acetic anhydride or vinyl acetate	Acetyl groups not more than 2.5%; phosphate calculated as phosphorus not more than 0.14% for potato and wheat, and 0.04% for other starches; and vinyl acetate not more than 0.1 mg/kg

<u>Modification</u>	<u>Process limitations</u>	<u>End-product specifications</u>
Starch acetate	Esterification with acetic anhydride or vinyl acetate	Acetyl groups not more than 2.5%
Acetylated distarch adipate	Esterification with acetic anhydride and adipic anhydride	Acetyl groups not more than 2.5% and adipate groups not more than 0.135%
Hydroxypropyl starch	Esterification with propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg
Hydroxypropyl distarch phosphate	Esterification with sodium trimetaphosphate or phosphorus oxychloride combined with etherification by propylene oxide	Hydroxypropyl groups not more than 7.0%; propylene chlorohydrin not more than 1 mg/kg; and residual phosphate calculated as phosphorus not more than 0.14% for potato and wheat, and not more than 0.04% for other starches
Starch sodium octenylsuccinate	Esterification with octenylsuccinic anhydride	Octenylsuccinyl groups not more than 3%; and residual octenylsuccinic acid not more than 0.3%
Acetylated oxidized starch	Treatment with sodium hypochlorite followed by esterification with acetic anhydride	Acetyl groups not more than 2.5 % and carboxyl groups not more than 1.3 %

METHODS FOR ADDITIONAL PURITY SPECIFICATIONS

pH (Vol. 4)

As specified in Column 3 of Table 1
Suspend 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min (In the case of pre-gelatinized starches, 3 g should be suspended in 97 ml of water).

Carboxyl groups

As specified in Column 3 of Table 1.

Principle

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

in water and titrated with standard alkali.

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

Reagents

Hydrochloric Acid Solution, 0.10 N : Standardization unnecessary

Sodium Hydroxide Solution, 0.10 N : Standardized

Phenolphthalein Indicator, 1%

Procedure

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

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

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

Remove sample from bath and titrate while hot with standard 0.10 N sodium hydroxide solution to a phenolphthalein end-point. The end-point may be detected electrometrically at pH 8.3. A blank determination is run on the original sample to correct for native acid substances (Note 5). Weigh the same quantity of starch as taken for carboxyl titration, and slurry in 10 ml of distilled water. Stir at about 5-min intervals for 30 min.

Vacuum filter the slurry quantitatively through a medium porosity fritted-glass crucible or small funnel, and wash sample with 200 ml of distilled water. Transfer, gelatinize, and titrate the sample with standard 0.10 N sodium hydroxide in the same manner as the demineralized sample.

Calculation:

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

Notes and Precautions

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

2. Add 1 ml of 1% aqueous silver nitrate solution to 5 ml of filtrate. Turbidity or precipitation occurs within 1 min if chloride is present.
3. Heating on a hot plate or over a Bunsen burner is not recommended. Over-heating or scorching in amounts too small to be visible will cause sample decomposition and apparent high carboxyl results.
4. Thorough gelatinization facilitates rapid titration and accurate end-point detection.
5. A blank titration is run on a water-washed sample to correct for acidic components which are not introduced by oxidation or derivatization. Free fatty acids complexed with amylose in common corn starch are the principal contributors to the blank titre.
6. A correction for phosphate content in potato starch (deduction) should be made after determining the phosphorus content of the sample being examined.

The deduction is calculated:

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

where

P is the phosphorus content (%).

Manganese

As specified in Column 3 of Table 1.

Instrumentation

Atomic absorption spectrophotometer with manganese hollow cathode lamp.

Preparation of solutions

Standard solution: Prepare a solution containing 0.5 mg/l of manganese.

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

Procedure

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

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

Phosphorus

As specified in the Column 3 of Table 1.

Reagents

- Ammonium Molybdate Solution (5%): Dissolve 50 g of ammonium molybdate tetrahydrate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, in 900 ml of warm water, cool to room temperature, dilute to 1000 ml with water, and mix.
- Ammonium Vanadate Solution (0.25%): Dissolve 2.5 g of ammonium metavanadate, NH_4VO_3 , in 600 ml of boiling water, cool to 60 - 70°, and add 20 ml of nitric acid. Cool to room temperature, dilute to 1000 ml with water, and mix.
- Zinc Acetate Solution (10%): Dissolve 120 g of zinc acetate dihydrate, $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2\cdot 2\text{H}_2\text{O}$, in 880 ml of water, and filter through Whatman No. 2V or equivalent filter paper before use.
- Nitric Acid Solution (29%): Add 300 ml of nitric acid (sp. gr 1.42) to 600 ml of water, and mix.
- Standard Phosphorus Solution: (100 µg P in 1 ml): Dissolve 438.7 mg of monobasic potassium phosphate, KH_2PO_4 , in water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Standard Curve

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

Sample pre-treatment

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

For pregelatinized starch and other water-soluble starches, prepare a 1% to 2% aqueous paste, place it in a cellophane tube, and dialyze

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

Sample preparation

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

Procedure

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

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

where

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

Acetyl groups

As specified in Column 3 of Table 1.

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

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

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

where

N is the normality of hydrochloric acid solution; and

W is the weight of sample in grams.

Vinyl acetate

Headspace Gas Chromatographic method

Chromatographic system

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

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

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

Procedure

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

Adipate groups

As specified in Column 3 of Table 1.

Reagents and Solutions

N,N-Bis-trimethylsilyltrifluoroacetamide (BSTFA): Macherey-Nagel, D 5160 Dueren, Germany or equivalent.

Glutaric acid solution: Dissolve 1.00 g of glutaric acid (Merck or equivalent) in water and dilute to 1000 ml.

Adipic acid solution: Dissolve 1.00 g of adipic acid (UCB, Brussels, Belgium or equivalent) in 900 ml of warm water, cool to room temperature, dilute to 1000 ml and mix.

Apparatus

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

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

Calibration

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

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

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

Calculations

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

$$RF = \frac{H_i \times W_s}{H_s}$$

where

H_s and H_i is the peak heights of the standard adipic acid and glutaric acid, respectively; and
 W_s is the weight of the standard adipic acid.

RF should be verified weekly.

Total adipate

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

Free adipic acid

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

Calculation

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

$$A = \frac{H_x \times RF}{H_{ix} \times S \times 10}$$

where

A is the content of total adipate or free adipic acid respectively (%);
 H_x is the peak height of adipic acid in the actual sample preparation;
 H_{ix} is the peak height of glutaric acid in the actual sample preparation;
 RF is the response factor for adipic acid; and
 S is the weight of sample in the actual preparation (g).

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

Hydroxypropyl groups

As specified in Column 3 of Table 1

Ninhydrin reagent

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

Procedure

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

Calculation

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

where

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

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

and

W is the weight of sample (mg).

Propylene chlorhydrin

As specified in Column 3 of Table 1.

Gas Chromatographic system

Use a Hewlett-Packard Model 5750 or equivalent. A dual-column instrument equipped with a flame-ionization detector is recommended. An integrator should be part of the recording system.

Gas Chromatography column: Use a stainless steel column, 3 m x 3.2 mm (o.d.), packed with 10% Carbowax 20 M on 80/100-mesh Gas Chrom 2, or equivalent. After packing and prior to use, condition the column overnight at 200°, using a helium flow of 25 ml per min.

Concentrator: Use a Kuderna-Danish concentrator having a 500-ml flask, available from Kontes Glass Co., Vineland, N.J., USA, (Catalogue No. K-57000), or equivalent.

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

Reagents

Diethyl ether: Use anhydrous, analytical reagent-grade diethyl ether

Florisil: Use 60/100 mesh material, available from Floridin Co., 3 Penn Center, Pittsburgh, PA 15235, USA, or an equivalent product.

Propylene chlorohydrins: Use Eastman No. P1325 1-Chloro-2-propanol Practical, containing 25% 2-chloro-1-propanol, available from Eastman Kodak Co., Rochester, N.Y. 14650, USA or equivalent.

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

Sample Preparation

Transfer a blended representative 50.0 g sample into a Pressure Bottle, and add 125 ml of 2 N sulfuric acid. Clamp the top in place, and swirl the contents until the sample is completely dispersed. Place the bottle in a boiling water bath, heat for 10 min, then swirl the bottle to mix the contents, and heat in the bath for an additional 15 min. Cool in air to room temperature, then neutralize the hydrolyzed sample to pH 7 with 25% sodium hydroxide solution, and filter through Whatman No. 1 paper, or equivalent, in a Buchner funnel, using suction. Wash the bottle and filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, and stir with a magnetic stirring bar for 5 to 10 min, or until the sodium sulfate is completely dissolved. Transfer the solution into a 500-ml separator equipped with a teflon plug, rinse the flask with 25 ml of water, and combine the washings with the sample solution. Extract with five 50 ml portions of diethyl ether, allowing at least 5 min in each extraction for adequate phase separation. Transfer the combined ether extracts in a Concentrator, place the graduated receiver of the concentrator in a water bath maintained at 50 - 55°, and concentrate the extract to a volume of 4 ml.

(NOTE: Ether extracts of samples may contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. These residues are believed to be degradation products arising during the hydrolysis treatment. Analytical problems created by their presence can be avoided through application of a clean-up treatment performed as follows: Concentrate the ether extract to about 8 ml, instead of 4 ml specified above. Add 10 g of Florisil, previously heated to 130° for 16 h just before use, to a chromatographic tube of suitable size, then tap gently, and add 1 g of anhydrous sodium sulfate to the top of the column. Wet the column with 25 ml of diethyl ether, and quantitatively transfer the concentrated extract to the column with the aid of small portions of the ether. Elute with three 25-ml portions of the ether, collect all of the eluate, transfer it to a concentrator, and concentrate to a volume of 4 ml).

Cool the extract to room temperature, transfer it quantitatively to a 5.0-ml volumetric flask with the aid of small portions of diethyl ether,

dilute to volume with the ether, and mix.

Control Preparations

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

Procedure

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

Calculation

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

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

where

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

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

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

Degree of substitution of starch sodium octenyl succinate

Principle

The degree of substitution is determined by alkali consumed after acidification and thorough washing of the starch half ester.

Procedure

Weigh out 5.0 g of sample in a 150-ml beaker. Wet out with a few ml of reagent grade isopropyl alcohol. Add, by pipette 25 ml of 2.5 N hydrochloric acid in isopropanol, allowing the acid to wash down any sample on the sides of the beaker. Stir for 30 min on a magnetic stir plate. Add 100 ml of 90% isopropanol from a graduated cylinder. Stir for 10 min. Filter the sample through a Buchner funnel and wash the filter cake with 90% isopropanol until the filtrate is negative for chloride ions. Use 0.1 N AgNO₃ to check for chloride ions. Transfer the filter cake to a 600-ml beaker and rinse the Buchner funnel to wash any starch into the beaker. Bring to a 300-ml volume with distilled water. Place for 10 min in a boiling water bath with stirring. Titrate while hot with 0.1 N NaOH to the phenolphthalein end-point.

Calculation

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

where

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

Residual octenyl succinic acid in starch sodium octenyl succinate

Principle

HPLC method on 2-bromacetophenone-derivatised methanolic extract of the sample.

Extraction and Preparation of Sample Solution

Extract about 500 mg of the sample, accurately weighed, with 15 ml of methanol overnight under constant shaking. Filter the extraction mixture, wash the precipitate on the filter three times with 7 ml portions of methanol and combine all filtrates (about 80% of the residuals are extracted by this procedure). Add 1 ml of 0.16 N KOH in methanol to the extract. Dry the extract using a flash evaporator at 30° and dissolve the residue in 2 ml of methanol. Take 0.5 ml of residue solution to the reaction vial and add 0.5 ml of derivative 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 CH₃CN]. Add 2 ml CH₃CN to the reaction vial, cap it and heat for 30 min at 80°. Cool the reaction solution to room temperature and use it within 24 h.

Liquid Chromatography Analysis

- Column: Micro-Bondapack C18 (Waters) or equivalent, 20°
- Mobile Phase: Gradient elution of 70% to 80% methanol in water in 5 min
- Flow rate: 1.5 ml/min
- Detector: UV at 254 nm, attenuation 0.16 AUFS
- Injection volume: 5 µl

Preparation of Standard Curve

Prepare a 0.5 M solution of octenyl succinic acid anhydride (available from Milliken Chemical) (Solution A). With a syringe take 0.25 ml of Solution A and transfer into a 25-ml volumetric flask. Dilute to the mark with methanol (Solution B). Prepare three standards by transferring 0.5, 1 and 2 ml of Solution B into three 50-ml round bottom flasks and adding to each 1 ml of 0.16 N KOH in methanol. Dry each solution using a flash evaporator at 30° and dissolve the residue in 2.0 ml of methanol (Solution C1, C2 and C3). Place 0.5 ml of the residue solution in the reaction vial and add 0.5 ml derivative 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 CH₃CN]. Add 2 ml of CH₃CN to the reaction vials, cap them and heat for 30 min at 80°. Cool to room temperature and inject 5 µl into the Liquid Chromatograph (the derivative should be used immediately). The amount of residuals in each of the 5-µl injections are the following:

for Solution C1 0.2375 µg

for Solution C2 0.4750 µg

for Solution C3 0.9500 µg

Plot peak height from Liquid Chromatograph Chart versus µg of residuals per 5 ml of solution.

Calculation

Using the peak height of the unknown sample from the Liquid Chromatograph Chart, determine the level of residuals (calculated as octenyl succinic acid) in the injected volume from the standard curve.

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

where

V is the value from the graph; 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.

NISIN

Prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009), superseding specifications for Nisin Preparation prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI of 0-33,000 units/kg bw was established at the 12th JECFA (1968).

SYNONYMS

Nisin preparation; INS No. 234

DEFINITION

Nisin is a mixture of closely related antimicrobial polypeptides produced by strains of *Lactococcus lactis* subsp. *lactis*. The major polypeptide is Nisin A. Nisin is produced in a sterilized medium of non-fat milk solids or of a non-milk-based fermentation source, such as yeast extract and carbohydrate solids. Nisin can be recovered from the fermentation medium by various methods, such as injecting sterile, membrane filtration; acidification; salting out; and spray-drying. Non-fat milk solids or solids from other fermentation sources are present in the product. Nisin is available in the commerce as a preparation consisting of Nisin and sodium chloride and is stable at ambient temperatures and upon heating under acid conditions (maximum stability at pH 3).

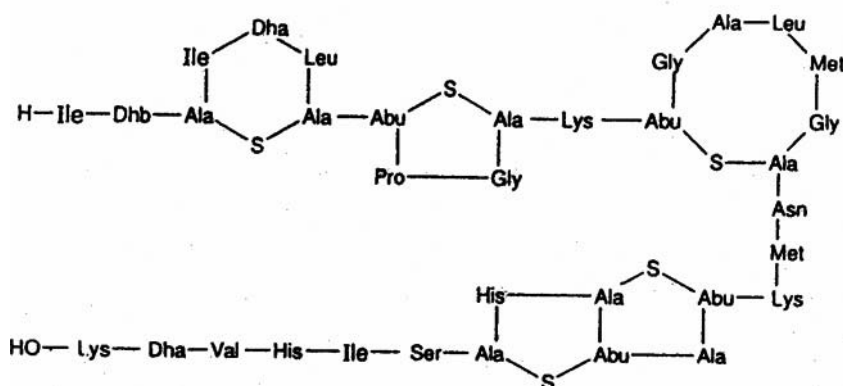
C.A.S. number

1414-45-5 (Nisin A)

Chemical formula

C₁₄₃H₂₃₀N₄₂O₃₇S₇ (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 and not less than 50% w/w sodium chloride

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

PURITY

Loss on drying (Vol. 4) Not more than 3.0% (105°, 2 h)
(See under "General methods, Inorganic Components.")

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").

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 sample

(See Volume 4 under "General Methods, Microbiological Analyses.")

TESTS

IDENTIFICATION TESTS

Differentiation from other antimicrobial substances Stability to acid
Suspend a 100-mg sample in 0.02 N hydrochloric acid as described in "Standard stock solution" under Method of Assay. Boil this solution for 5 min and determine the nisin activity as directed under Method of Assay. No significant loss of activity is noted following this heat treatment. The calculated nisin concentration of the boiled sample is 100% (+/- 5%) of the assay value. Adjust the pH of the nisin solution 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. Again determine the nisin concentration as directed under Method of Assay. Complete loss of the antimicrobial activity of nisin is observed following this treatment.

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. *L. lactis* will grow in 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.)

METHOD OF ASSAY 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 units/ml. Immediately before use, dilute the solution further with 0.02 N hydrochloric acid to give 50 units/ml. (NOTE: Nisin containing 2.5% nisin, minimum potency of 10⁶ IU/g, obtainable from Sigma, St Louis, USA or Fluka, Buchs, Switzerland, may be used for the Standard stock solution, as well as the preparation under the name of Nisaplin, available from Danisco, Copenhagen, Denmark).

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, the nisin content in the sample and standard is the same. Dilute the sample solution in 0.02 N hydrochloric acid to give an approximate concentration of 50 units of nisin per ml.

Resazurin solution

Prepare a 0.0125% w/v solution of resazurin in water immediately before 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 unit per ml sample and standard solutions into 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 is made in turn across each row of tubes containing the same nominal concentration, 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. Make the addition in the same order as for 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 tubes under fluorescent light in a black matt-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 row of tubes to find the nearest match in colour. Make further matches at the next two lower concentrations of the sample and standard. Interpolation of matches may be made at half dilution steps. As the standard tubes contain

known amounts of nisin, calculate the concentration of nisin in the sample solution. Obtain three readings of the solution and average them. Calculate the activity in terms of IU per gram of product.

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. Each ml of reacted 0.1 N silver nitrate is equivalent to 5.844 mg of NaCl. Calculate the percentage of sodium chloride in the sample taken by the equation:

$$\text{Sodium chloride \% (w/w)} = 100 \times 58.44(50 \times A - V \times B)/(W)$$

where

A is the concentration of the silver nitrate solution;
B is the concentration of the ammonium thiocyanate solution;
V is the volume (ml) of the ammonium thiocyanate consumed;
W is the weight of the sample (mg); and
58.44 is the formula weight of sodium chloride.

NITROUS OXIDE (TENTATIVE)

Tentative specifications prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 55th JECFA (2000) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI "Acceptable" was established at the 29th JECFA (1985).

Information is required on a capillary GC method for the assay of nitrous oxide.

SYNONYMS Dinitrogen oxide; Dinitrogen monoxide; INS No. 942

DEFINITION

Chemical names Nitrous oxide

C.A.S. number 10024-97-2

Chemical formula N₂O

Formula weight 44.01

Assay Not less than 99 % (v/v)

DESCRIPTION Colourless, odourless gas

FUNCTIONAL USES Propellant, antioxidant, packaging gas, foaming agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) 1 volume dissolves in 1.4 volumes of water (20° 760 mm Hg).
Freely soluble in alcohol; soluble in ether and in oils.

Chromatography The retention time of the major peak of the sample corresponds to that of nitrous oxide when analysed by gas chromatography using the conditions specified under the method of assay.

Carbon dioxide test Passes test
See description under TESTS

PURITY

Carbon monoxide Not more than 10 µl/l
See description under TESTS

Nitric oxide Not more than 1 µl/l

See description under TESTS

Nitrogen dioxide Not more than 1 µl/l
See description under TESTS

Halogens (as Cl) Not more than 5 µl/l
See description under TESTS

Ammonia Not more than 25 µl/l
See description under TESTS

TESTS

NOTE 1: The carbon dioxide identification test and purity tests are referenced from the Food Chemicals Codex, 6th Edition, 2008, p. 168.

Reprinted with permission from the US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD USA 20852.

NOTE 2: The identification and purity tests given below are designed to reflect the quality of nitrous oxide in both its vapour and its liquid phases, which are present in previously unopened cylinders. Reduce the sample cylinder pressure with a regulator. Withdraw the samples for the tests with the least possible release of sample gas consistent with proper purging of the sample apparatus. Measure the gases with a gas volume meter downstream from the detector tubes to minimize contamination of or change to the samples. Perform the tests in the sequence in which they are listed below.

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

IDENTIFICATION TESTS

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

PURITY TESTS

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

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

Nitrogen dioxide

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

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

Halogens (as Cl)

Pass 1000 ml of sample gas, released from the vapour phase of the contents of the sample gas cylinder, through a chlorine detector tube (Draeger CH 24301 or equivalent) at the rate specified for the tube. The indicator change corresponds to not more than 25 µl.

Ammonia

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

METHOD OF ASSAY Determine by Gas Chromatography using the following:

Apparatus

Gas chromatograph fitted with an appropriate gas sampling valve
Stainless steel; 2 m x 2 mm i.d. packed with silica gel for chromatography (250-350 µm)

Column and injector temperature: 60°

Thermal conductivity detector (130°)

Helium at 50 ml/min.

A data station with a suitable chromatography software to operate the instrument

Certified nitrous oxide gas (99.9%)

Procedure

Using a gas sampling valve inject standard gas taken from the liquid phase and record the area under the peak of nitrous oxide. Adjust the injection volume and operating conditions so that a good quantifiable peak for nitrous oxide is obtained (not less than the 35% of the full scale when using an integrator). Record area under the peak of nitrous oxide for the standard. Inject the sample gas taken from the liquid phase and record the area. Calculate the purity of sample gas from the peak areas of standard and sample, and purity of certified nitrous oxide standard.

OCTENYL SUCCINIC ACID MODIFIED GUM ARABIC

New specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A temporary ADI "not specified" was established at the 71st JECFA (2009).

SYNONYMS

Gum arabic hydrogen octenylbutandioate; Gum arabic hydrogen octenylsuccinate; OSA modified gum arabic; OSA modified gum acacia

DEFINITION

Octenyl succinic acid modified gum arabic is produced by esterifying gum arabic (*Acacia seyal*), or gum arabic (*Acacia senegal*) in aqueous solution with not more than 3% of octenyl succinic acid anhydride. It is subsequently spray dried.

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

DESCRIPTION

Off-white to light tan, free flowing powder

FUNCTIONAL USES

Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Freely soluble in water; insoluble in ethanol

Precipitate formation

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

pH (Vol. 4)

3.5 to 6.5 (5% solution)

Viscosity

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

PURITY

Degree of esterification

Not more than 0.6%
See description under TESTS

Loss on drying (Vol.4)

Not more than 15% (105°, 5h)

Total ash (Vol.4)

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.

<u>Tannin-bearing gums</u>	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.
<u>Residual octenyl succinic acid</u>	Not more than 0.3% See description under TESTS
<u>Microbiological criteria</u> (Vol. 4)	<i>Salmonella</i> species: absent in 25 g <i>Escherichia coli</i> : absent in 1 g
<u>Lead</u> (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

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

Procedure

Weigh 5.0 g of sample in a 150-ml beaker and wet it with a few ml of isopropanol. Add, by pipette 25 ml of 2.5 N hydrochloric acid in isopropanol and stir for 30 min on a magnetic stir plate. Add 100 ml of 90% isopropanol in water from a graduated cylinder and stir for 10 min. Filter the sample through a Buchner funnel and wash the filter cake with 90% isopropanol in water until the filtrate is negative for chloride ions checked by 0.1 N silver nitrate. Transfer the filter cake to a 600-ml beaker, rinse the Buchner funnel and bring to a 300-ml volume with distilled water. Place for 10 min in a boiling water bath while stirring and titrate while hot with 0.1 N sodium hydroxide using phenolphthalein TS as an indicator.

Calculation

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

where

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

Residual octenyl succinic acid

Principle: HPLC method on 2-bromacetophenone-derivatised methanolic extract of the sample.

Extraction and Preparation of Sample Solution

Extract about 500 mg of the sample, accurately weighed, with 15 ml of methanol overnight under constant shaking. Filter the extraction mixture, wash the precipitate on the filter three times with 7 ml portions of methanol and combine all filtrates (about 80% of the

residuals are extracted by this procedure). Add 1 ml of 0.16 N KOH in methanol to the extract. Dry the extract using a flash evaporator at 30° and dissolve the residue in 2 ml of methanol. Take 0.5 ml of residue solution to the reaction vial and add 0.5 ml of derivative 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 CH₃CN]. Add 2 ml CH₃CN to the reaction vial, cap it and heat for 30 min at 80°. Cool the reaction solution to room temperature and use it within 24 h.

Liquid Chromatography Analysis

- Column: Micro-Bondapack C18 (Waters) or equivalent, 20°
- Mobile Phase: Gradient elution of 70% to 80% methanol in water in 5 min
- Flow rate: 1.5 ml/min
- Detector: UV at 254 nm, attenuation 0.16 AUFS
- Injection volume: 5 µl

Preparation of Standard Curve

Prepare a 0.5 M solution of octenyl succinic acid anhydride (available from Milliken Chemical) (Solution A). With a syringe take 0.25 ml of Solution A and transfer into a 25-ml volumetric flask. Dilute to the mark with methanol (Solution B). Prepare three standards by transferring 0.5, 1 and 2 ml of Solution B into three 50-ml round bottom flasks and adding to each 1 ml of 0.16 N KOH in methanol. Dry each solution using a flash evaporator at 30° and dissolve the residue in 2.0 ml of methanol (Solution C1, C2 and C3). Place 0.5 ml of the residue solution in the reaction vial and add 0.5 ml derivative 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 CH₃CN]. Add 2 ml of CH₃CN to the reaction vials, cap them and heat for 30 min at 80°. Cool to room temperature and inject 5 µl into the Liquid Chromatograph (the derivative should be used immediately). The amount of residuals in each of the 5-µl injections are the following:

for Solution C1 0.2375 µg

for Solution C2 0.4750 µg

for Solution C3 0.9500 µg

Plot peak height from Liquid Chromatograph Chart versus µg of residuals per 5 ml of solution.

Calculation

Using the peak height of the unknown sample from the Liquid Chromatograph Chart, determine the level of residuals (calculated as octenyl succinic acid) in the injected volume from the standard curve.

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

where

V is the value from the graph; 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.

PECTINS

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). A group ADI "not specified" was established for pectins and amidated pectins, singly or in combination at the 25th JECFA in 1981.

SYNONYMS

INS No. 440

DEFINITION

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

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

C.A.S. number

9000-69-5

DESCRIPTION

White, yellowish, light greyish or light brownish powder

FUNCTIONAL USES

Gelling agent, thickener, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Test for pectins

Passes test
See description under TESTS

Test for amide group

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

changes its colour from red to green, due to release of ammonia.

PURITY

Loss on drying (Vol. 4) Not more than 12% (105°, 2 h)

Sulfur dioxide Not more than 50mg/kg
See description under TESTS

Residual solvents (Vol. 4) Not more than 1% methanol, ethanol and 2-propanol, singly or in combination
See description under TESTS

Acid-insoluble ash (Vol. 4) Not more than 1%

Total insolubles Not more than 3%
See description under TESTS

Nitrogen content (Vol. 4) Not more than 2.5% after washing with acid and ethanol

Galacturonic acid Not less than 65% calculated on the ash-free and dried basis
See description under TESTS

Degree of amidation Not more than 25% of total carboxyl groups of pectin
See description under TESTS

Lead (Vol. 4) Not more than 5 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities.")

TESTS

IDENTIFICATION TESTS

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

	<u>Buffer pH</u> 7.0 *)	<u>Sample soln</u>	<u>Water</u>	<u>Enzyme soln</u> **)
Enzyme blank	0.5 ml	1.0 ml	1.0 ml	-
Sample blank	0.5 ml	-	1.5 ml	0.5 ml
Sample	0.5 ml	1.0 ml	0.5 ml	0.5 ml

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

***) Dilute pure pectate lyase 1:100 with buffer pH 7.0

Shake the solutions well, and measure the absorbance at 235 nm at 0 and 10 min.

Calculations

A_0 = absorbance at 0 min = Sample - (enzyme blank + sample blank)

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

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

PURITY TESTS

Sulfur dioxide

Suspend 100 g of the sample in 500 ml of methanol in a 1000-ml round-bottom flask, which is provided with a gas inlet tube reaching almost the bottom and connected to the neck with a reflux condenser. Prepare a glass joint connection from the condenser to an absorption flask or U-tube containing 10 ml of 3% hydrogen peroxide solution neutralized to methyl red TS. Connect the gas inlet tube with an oxygen-free source of carbon dioxide or nitrogen, and maintain a gas stream so as to cause steady bubbling. As soon as the apparatus is flushed free of air, pour 30 ml of hydrochloric acid solution (10 ml conc. HCl + 20 ml H₂O) into the reflux condenser, and immediately connect the absorption flask or U-tube. Heat slowly until methanol starts refluxing, and reflux gently for 2 h. Disconnect the apparatus and titrate the hydrogen peroxide solution against methyl red TS with 0.01 mol/l sodium hydroxide. Each ml of 0.01 mol/l sodium hydroxide corresponds to 0.32 mg of SO₂.

Total insolubles

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

Dry the filter paper with the residue at 105° for 1 h. Transfer to desiccator containing silica gel and leave to cool. Weigh the paper (M_2). Calculate the percentage of total insolubles from

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

Galacturonic acid and
Degree of amidation

Weigh 5 g of the sample to the nearest 0.1 mg, and transfer to a suitable beaker. Stir for 10 min with a mixture of 5 ml of hydrochloric acid TS, and 100 ml of 60% ethanol. Transfer to a fritted-glass filter tube (30 to 60 ml capacity) and wash with six 15-ml portions of the HCl-60% ethanol mixture, followed by 60% ethanol until the filtrate is free of chlorides. Finally wash with 20 ml of ethanol, dry for 2.5 h in an oven at 105°, cool and weigh. Transfer exactly one-tenth of the total net weight of the dried sample (representing 0.5 g of the original unwashed sample) to a 250-ml conical flask and moisten the sample with 2 ml of ethanol TS. Add 100 ml of recently boiled and cooled distilled water, stopper and swirl occasionally until a complete solution is formed. Add 5 drops of phenolphthalein TS, titrate with 0.1 mol/l sodium hydroxide and record the results as the initial titre (V_1).

Add exactly 20 ml of 0.5 mol/l sodium hydroxide TS, stopper, shake vigorously and let stand for 15 min. Add exactly 20 ml of 0.5 mol/l hydrochloric acid and shake until the pink colour disappears. Titrate with 0.1 mol/l sodium hydroxide to a faint pink colour which persists after vigorous shaking; record this value as the saponification titre (V_2).

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

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

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

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

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

Calculate mg of galacturonic acid by the formula:

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

The mg of galacturonic acid obtained in this way is the content of one-tenth of the weight of the washed and dried sample. To calculate % galacturonic acid on a moisture- and ash-free basis, multiply the number of mg obtained by $1000/x$, x being the weight in mg of the washed and dried sample.

NOTE 1: If the pectin is known to be of the nonamidated type, only V_1 and V_2 need to be determined and $(B - S)$ may be regarded as zero.

NOTE 2: For pectins from apple or citrus $(A - A_0)$ is usually insignificant in calculating galacturonic acid and degree of amidation.

NOTE 3: If desired, calculate degree of esterification (as % of total carboxyl groups) by the formula:

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

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

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

Residual solvents (Vol. 4) Apply Method I in Volume 4, General Methods, Organic Components.

Standard stock solution: To 500 ml of water in a 1000-ml volumetric flask, add about 5 g each of methanol, ethanol and 2-propanol, accurately weighed. Make up to the mark with water.

Internal standard solution: To 500 ml of water in a 1000-ml volumetric flask, add about 5 g of 2-butanol (W_{standard}), accurately weighed. Make up to the mark with water.

Blank Solution: Omit the blank determination

Samples: Store the sample in a cool, dry place. Mix the sample thoroughly before analysis.

Weigh accurately about 1 g of sample (W_{sample}) in a 100 ml beaker and mix with about 5 g of sucrose. Into a 100-ml Erlenmeyer flask

with magnetic stirrer bar, add 95 ml water and 1.0 ml internal

standard solution. While stirring fast, slowly add the pectin-sucrose mixture. Stopper the flask and stir for 2 h. The pectin must be completely dissolved. Accurately weigh about 1 g of this solution (M_{sample}) into a headspace vial for GC analysis.

Calibration solution: Pipette 2.0 ml of standard stock solution and 2.0 ml of internal standard solution into a 200-ml volumetric flask and make up to the mark with water. Accurately weigh about 1 g of this solution (M_{standard}) is filled into a head space vial and used for GC analysis.

Procedure

Continue the analysis as described in Vol.4 'Residual solvents', using the given conditions except for the sample heating temperature, which should be 70°, and syringe temperature, which should be 80°.

Calculation

Calculate the concentration of each residual solvent using the following equation:

$$\% \text{ of solvent} = \frac{R_{\text{sample}} \times W_{\text{s tan dard}} \times M_{\text{s tan dard}}}{R_{\text{s tan dard}} \times W_{\text{sample}} \times M_{\text{sample}}} \times 100$$

where

- R_{sample} is the relative peak area of the sample;
- R_{standard} is the relative peak area of the standard;
- W_{sample} is the weight of sample (g);
- W_{standard} is the weight of solvent used for the standard stock solution;
- M_{sample} is the weight of sample solution used for the GC analysis; and
- M_{standard} is the weight of Calibration solution used for the GC analysis.

SODIUM HYDROGEN SULFATE

Prepared at the 71st JECFA (2009), published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI “not specified” was established at the 71st JECFA (2009).

SYNONYMS	Sodium acid sulfate; nitre cake; sodium bisulfate; sulfuric acid, monosodium salt; INS no. 514(ii)
DEFINITION	Sodium chloride and sulfuric acid are combined at elevated temperatures to produce molten sodium hydrogen sulfate. The molten sodium hydrogen sulfate is sprayed and cooled to form a solid product with uniform particle size.
C.A.S. number	7681-38-1
Chemical formula	NaHSO ₄
Formula weight	120.06
Assay	Not less than 85%
DESCRIPTION	White crystals or granules
FUNCTIONAL USES	Acidifier, acidity regulator
CHARACTERISTICS	
IDENTIFICATION	
<u>Solubility</u> (Vol. 4)	Freely soluble in water
<u>Sodium</u> (Vol. 4)	Passes test
<u>Sulfate</u> (Vol. 4)	Passes test
PURITY	
<u>Loss on drying</u> (Vol.4)	Not more than 0.8% (105°, 3h, use 25 g of sample)
<u>Water-insoluble matter</u> (Vol. 4)	Not more than 0.05% (Use 50 g of sample and 300 ml hot water)
<u>Lead</u> (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”).
<u>Selenium</u> (Vol. 4)	Not more than 5 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample

preparation may be based on principles of methods described in Volume 4 (under "General Methods, Metallic Impurities").

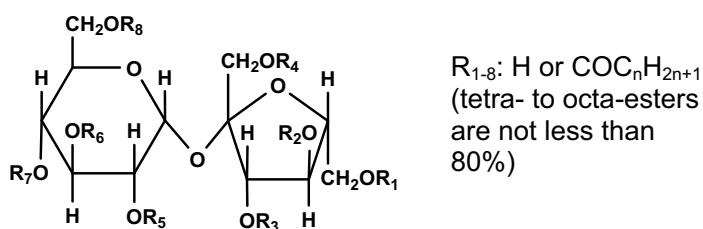
METHOD OF ASSAY Accurately weigh about 5 g of sample into a 250-ml conical flask, dissolve in about 125 ml of water, and add phenolphthalein TS. Titrate with 1 N sodium hydroxide. Each milliliter of sodium hydroxide is equivalent to 120.06 mg of NaHSO_4 .

SUCROSE OLIGOESTERS TYPE I

New specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A group ADI of 0 - 30 mg/kg bw for sucrose esters of fatty acids, sucroglycerides and sucrose oligoesters type I and type II was established at the 71st JECFA (2009).

SYNONYMS	Sucrose fatty acid esters (high-esterified); Sucrose oligoesters (high-esterified); INS No. 473a
DEFINITION	Sucrose oligoesters type I contains mainly tetra- to octa-fatty acid esters of sucrose, though the content of hepta- and octa-esters is not more than 50%. They are prepared from sucrose and methyl esters of food fatty acids such as stearic acid, palmitic acid, oleic acid, lauric acid and erucic acid by interesterification in the presence of an alkaline catalyst. Only the following solvents may be used for the production: dimethyl sulfoxide, isobutanol and methyl ethyl ketone.

Structural formula



Assay	Total content of tetra- to octa-esters: not less than 80% Content of hepta- and octa-esters: not more than 50% Content of octa-esters: not more than 20%
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DESCRIPTION White to red-brown powders, soft solid, stiff gels or colourless to red-brown viscous liquid

FUNCTIONAL USES Emulsifier, stabilizer, tableting aid

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4) Insoluble in water

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

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

PURITY

<u>Sulfated ash</u> (Vol.4)	Not more than 2% Test 1 g of the sample (Method I)
<u>Acid value</u> (Vol.4)	Not more than 6
<u>Free sucrose</u>	Not more than 1 % See description under TESTS
<u>Dimethyl sulfoxide</u>	Not more than 2 mg/kg See description under TESTS
<u>Isobutanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methyl ethyl ketone</u>	Not more than 10 mg/kg See description under TESTS
<u>Lead</u> (Vol.4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Free sucrose Determine by gas liquid chromatography described in Volume 4 using the following conditions.

Standard solutions

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

Internal standard solution

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

Chromatography conditions

Column: 100%-Dimethylpolysiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)

Carrier gas: Helium

Flow rate: 1.5 ml/min

Detector: Flame-ionization detector (FID)

Temperatures:

- injection: 280°

- column: Hold for 1 min at 100°, then 100-300° at 12°/min, hold for 45 min at 300°
- detector: 320°

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

Procedure

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

Standard curve

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

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

Calculate the percentage of free sucrose from:

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

Dimethyl sulfoxide

Determine by gas liquid chromatography described in Volume 4 using following conditions.

Standard solutions

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

Chromatography conditions

Column: 10% PEG 20M and 3% KOH on Chromosorb W AW DMCS 60/80 mesh (2 m x 3 mm i.d.) or equivalent. Raise the oven temperature to 180° at a rate of 10°/min and let stabilize for 24 to 48 h with 30 to 40 ml/min of nitrogen for conditioning

Carrier gas: Nitrogen

Flow rate: 30 ml/min

Detector: Flame photometric detector (using 394 nm sulfur filter)

Temperatures

- injection: 210°

- column: 160°

The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3 min.

Procedure

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

Standard curve

Prepare daily by injecting 3 µl of each of the standard solutions into the chromatograph.

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

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

where

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

Methanol, isobutanol, and methyl ethyl ketone

Determined by gas chromatography with a head space sampler using the following methods.

Standard solutions

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

Procedure:

Weigh accurately 1 g of the sample into each of four sample vials. To one vial add 5 µl of water, to the second, third and fourth, add, respectively, standard solutions A, B and C, and seal them quickly with a septum. (The concentrations of each solvent after adding 5 µl of standard solutions A, B and C to 1 g of the sample are equal to 20, 10 and 5 mg/kg of sample, respectively). Place the sample vials in a head space sampler and analyse using the following conditions: Column: 100% Polydimethylsiloxane (30 m x 0.53 mm i.d. with 1.5 µm film)

Carrier gas: Nitrogen

Flow rate: 3.5 ml/min

Detector: FID

Temperatures:

- injection: 110°

- column: 40°

- detector: 110°

Head space sampler:

- sample heat insulating temperature: 80°

- sample heat insulating period: 40 min

- syringe temperature: 85°

- sample gas injection: 1.0 ml

Calculation

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

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

where

w_i is the x-intercept of relationship line using the standard addition method (μg); and

W is the weight of sample (g).

METHOD OF ASSAY 1. Tetra- to octa-esters

Determine by HPLC using the following conditions:

Procedure

Accurately weigh 250 mg of the sample into a 100-ml volumetric flask. Dissolve and dilute to volume with tetrahydrofuran and mix. Filter through a 0.5 μm membrane filter. Inject 80 μl of the sample into the chromatograph.

Chromatography conditions

Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G1000HXL, G2000HXL, G3000HXL, G4000HXL (each 30 cm x 7.8 mm i.d., 5 μm particles for solvent manufactured by Tosoh in series or equivalent)

Mobile phase: HPLC-grade tetrahydrofuran

Flow rate: 0.8 ml/min

Detector: RI

Temperatures:

- Column: 40°

- Detector: 40°

Record the chromatogram for about 50 min

Identification of the peaks

More highly esterified components elute earlier and tetra- to octa-esters elute as one peak. Their retention times are dependent on the variety of esterified fatty acids and chromatography conditions. Their retention times at these conditions are described in Table 1. The reference products are available from Mitsubishi Chemical Corporation (Tokyo, Japan) or Dai-ichi Kogyo Seiyaku Co. Ltd (Kyoto, Japan) to confirm the retention time.

Table 1. The retention time (min) of mono-, di-, tri- and tetra- to octa-esters esterified with main fatty acids

Esterified fatty acid	Mono-esters	Di-esters	Tri-esters	Tetra- to octa-esters
Lauric acid	40.0	38.2	37.0	36.2
Palmitic acid	39.3	37.2	36.0	35.1
Stearic acid	39.0	37.0	35.7	34.9
Oleic acid	39.1	37.1	35.9	35.0
Erucic acid	38.5	36.3	35.1	34.3

Calculate the percentage of tetra- to octa-esters ($E_{\text{tet-oct}}$) from:

$$E_{\text{tet-oct}}(\%) = 100 A_{\text{tet-oct}}/T$$

where

$A_{\text{tet-oct}}$ is the sum of peak areas for tetra- to octa-esters; and
 T is the sum of all peak areas eluting within 43 min.

2. Hepta- and octa-esters

The percentage of the sum of hepta- and octa-esters ($E_{\text{hep+oct}}$), and the percentage of octa-esters (E_{oct}) is calculated by two steps. The ratio of hepta- and octa-esters, and the ratio of octa-esters in sum of tetra- to octa-esters are determined by HPLC. Then $E_{\text{hep+oct}}$ and E_{oct} are calculated using $E_{\text{tet-oct}}$ obtained above in Method 1.

Procedure

Accurately weigh 1g of the sample into a 50-ml volumetric flask and add a solution for the mobile phase (tetrahydrofuran/methanol=50/50 (vol/vol)) to the mark. Filter through a 0.5 μm membrane filter. Inject 20 μl of the sample into the chromatograph.

Chromatography conditions

Column: reversed phase C18 columns (150mm x 4.6 mm i.d.; ODS-2 manufactured by GL Science or equivalent)

Mobile phase: tetrahydrofuran/methanol=50/50 (vol/vol)

Flow rate: 0.8 ml/min

Detector: RI

Temperatures:

- Column: 40°

- Detector: 40°

Record the chromatogram for about 16 min

Identification of the peaks

The retention times of tetra-, penta-, hexa-, hepta- and octa-esters are dependent on the variety of esterified fatty acids and chromatography conditions. Their retention times at these conditions are described in Table 2. The reference products are available from Mitsubishi Chemical Corporation (Tokyo, Japan) or Dai-ichi Kogyo Seiyaku Co. Ltd (Kyoto, Japan) to confirm the retention time.

Table 2. The retention time (min) of tetra- to octa-esters esterified with main fatty acids

Esterified fatty acid	Tetra-esters	Penta-esters	Hexa-esters	Hepta-esters	Octa-esters
Lauric acid	2.5	2.7	3.0	3.4	4.0
Palmitic acid	2.9	3.5	4.3	5.5-5.9*	7.5-9.3*
Stearic acid	3.1	3.7	4.8	6.1-7.0*	7.9-10.7*
Oleic acid	2.8	3.2	3.8	4.7	5.4-6.7*
Erucic acid	3.3	4.1	5.4	7.5	11.0

*: the retention time range, because highly esterified components have been shown as several unresolved peaks

Calculate the percentage of the sum of hepta- and octa-esters ($E_{\text{hep+oc}}$) and the percentage of octa-esters (E_{oct}) as follows:

$$E_{\text{hep+oct}}(\%) = (B_{\text{hep+oct}}/T_{\text{tet-oct}}) \times E_{\text{tet-oct}}$$
$$E_{\text{oct}}(\%) = (B_{\text{oct}}/T_{\text{tet-oct}}) \times E_{\text{tet-oct}}$$

where

$B_{\text{hep+oct}}$ is the sum of peak areas of hepta-esters and octa-esters;

B_{oct} is the peak areas for octa-esters;

$T_{\text{tet-oct}}$ is the sum of peak areas from tetra- to octa-esters; and

$E_{\text{tet-oct}}$ (%) is the percentage of tetra- to octa-esters measured by Method 1 described above.

SUCROSE OLIGOESTERS TYPE II

New specifications prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009). A group ADI of 0 - 30 mg/kg bw for sucrose esters of fatty acids, sucroglycerides and sucrose oligoesters type I and type II was established at the 71st JECFA (2009).

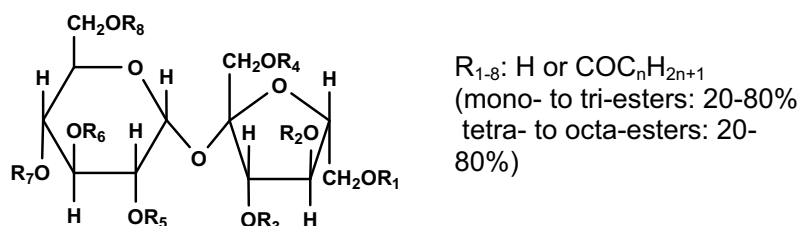
SYNONYMS

Sucrose fatty acid esters; Sucrose oligoesters; INS No. 473a

DEFINITION

Sucrose oligoesters type II contains both mono- to tri-esters and tetra- to octa-fatty acid esters of sucrose. Their composition and properties are between Sucrose esters of fatty acids and Sucrose oligoesters type I. They are prepared from sucrose and methyl esters of food fatty acids such as stearic acid, palmitic acid, erucic acid and behenic acid by interesterification in the presence of an alkaline catalyst. Only the following solvents may be used for the production: dimethyl sulfoxide, isobutanol, and methyl ethyl ketone.

Structural formula



Assay

Total content of mono- to tri-esters: between 20 - 80%
Total content of tetra- to octa-esters: between 20 - 80%
Content of hepta- and octa-esters: not more than 20%
Content of octa-esters: not more than 10%

DESCRIPTION

White to red-brown powders, soft solid, stiff gels or colourless to red-brown viscous liquid

FUNCTIONAL USES Emulsifier, stabilizer, tableting aid

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4)

Insoluble in water

Fatty acids

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

Sugars

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

green.

PURITY

<u>Sulfated ash</u> (Vol.4)	Not more than 2% Test 1 g of the sample (Method I)
<u>Acid value</u> (Vol.4)	Not more than 6
<u>Free sucrose</u>	Not more than 1 % See description under TESTS
<u>Dimethyl sulfoxide</u>	Not more than 2 mg/kg See description under TESTS
<u>Isobutanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methanol</u>	Not more than 10 mg/kg See description under TESTS
<u>Methyl ethyl ketone</u>	Not more than 10 mg/kg See description under TESTS
<u>Lead</u> (Vol.4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Free sucrose Determine by gas liquid chromatography described in Volume 4 using the following conditions.

Standard solutions

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

Internal standard solution

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

Chromatography conditions

Column: 100%-Dimethylpolysiloxane (30 m x 0.32 mm i.d. with 0.25 µm film)

Carrier gas: Helium

Flow rate: 1.5 ml/min

Detector: Flame-ionization detector (FID)

Temperatures:

- injection: 280°

- column: Hold for 1 min at 100°, then 100-300° at 12°/min, hold for 45 min at 300°
- detector: 320°

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

Procedure

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

Standard curve

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

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

Calculate the percentage of free sucrose from:

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

Dimethyl sulfoxide

Determine by gas liquid chromatography described in Volume 4 using following conditions.

Standard solutions

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

Chromatography conditions

Column: 10% PEG 20M and 3% KOH on Chromosorb W AW DMCS 60/80 mesh (2 m x 3 mm i.d.) or equivalent. Raise the oven temperature to 180° at a rate of 10°/min and let stabilize for 24 to 48 h with 30 to 40 ml/min of nitrogen for conditioning

Carrier gas: Nitrogen

Flow rate: 30 ml/min

Detector: Flame photometric detector (using 394 nm sulfur filter)

Temperatures

- injection: 210°

- column: 160°

The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3 min.

Procedure

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

Standard curve

Prepare daily by injecting 3 µl of each of the standard solutions into the chromatograph.

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

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

where

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

Methanol, isobutanol, and methyl ethyl ketone

Determined by gas chromatography with a head space sampler using the following methods.

Standard solutions

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

Procedure

Weigh accurately 1 g of the sample into each of four sample vials. To one vial add 5 µl of water, to the second, third and fourth, add, respectively, standard solutions A, B and C, and seal them quickly with a septum. (The concentrations of each solvent after adding 5 µl of standard solutions A, B and C to 1 g of the sample are equal to 20, 10 and 5 mg/kg of sample, respectively). Place the sample vials in a head space sampler and analyse using the following conditions: Column: 100% Polydimethylsiloxane (30 m x 0.53 mm i.d. with 1.5 µm film)

Carrier gas: Nitrogen

Flow rate: 3.5 ml/min

Detector: FID

Temperatures

- injection: 110°

- column: 40°

- detector: 110°

Head space sampler:

- sample heat insulating temperature: 80°

- sample heat insulating period: 40 min

- syringe temperature: 85°

- sample gas injection: 1.0 ml

Calculation

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

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

where

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

METHOD OF ASSAY 1. Mono- to tri-esters and tetra- to octa-esters

Determine by HPLC using the following conditions:

Procedure

Accurately weigh 250 mg of the sample into a 100-ml volumetric flask. Dilute to volume with tetrahydrofuran and mix. Filter through a 0.5 μm membrane filter. Inject 80 μl of the sample into the chromatograph.

Chromatography conditions

Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G1000HXL, G2000HXL, G3000HXL, G4000HXL (each 30 cm x 7.8 mm i.d., 5 μm particle for solvent manufactured by Tosoh in series or equivalent)

Mobile phase: HPLC-grade degassed tetrahydrofuran

Flow rate: 0.8 ml/min

Detector: RI

Temperatures:

- Column: 40°

- Detector: 40°

Record the chromatogram for about 50 min

Identification of the peaks

More highly esterified components elute earlier and tetra- to octa-esters elute as one peak. Their retention times are dependent on the variety of esterified fatty acids and chromatography conditions. Their retention times at these conditions are described in Table 1. The reference products are available from Mitsubishi Chemical Corporation (Tokyo, Japan) or Dai-ichi Kogyo Seiyaku Co. Ltd (Kyoto, Japan) to confirm the retention time.

Table 1. The retention time (min) of mono-, di-, tri- and tetra- to octa-esters esterified with main fatty acids

Esterified fatty acid	Mono-esters	Di-esters	Tri-esters	Tetra- to octa-esters
Stearic acid	39.0	37.0	35.7	34.9
Erucic acid	38.5	36.3	35.1	34.3
Behenic acid	38.2	36.2	35.0	34.2

Calculate the percentage of mono- to tri esters ($E_{\text{mono-tri}}$) and tetra- to octa-esters ($E_{\text{tet-oct}}$) in the sample from:

$$E_{\text{mono-tri}} (\%) = 100 A_{\text{mono-tri}}/T$$

$$E_{\text{tet-oct}} (\%) = 100 A_{\text{tet-oct}}/T$$

where

$A_{\text{mono-tri}}$ is the sum of peak areas for mono- to tri-esters;
 $A_{\text{tet-oct}}$ is the sum of peak areas for tetra- to octa-esters; and
 T is the sum of all peak areas eluting within 43 min.

2. Hepta- and octa-esters

The percentage of the sum of hepta- and octa-esters ($E_{\text{hep+oct}}$), and the percentage of octa-esters (E_{oct}) is calculated by two steps. The ratio of hepta- and octa-esters, and the ratio of octa-esters in sum of tetra- to octa-esters are determined by HPLC. Then $E_{\text{hep+oct}}$ and E_{oct} are calculated using $E_{\text{tet-oct}}$ obtained above in Method 1.

Procedure

Accurately weigh 1g of the sample into a 50-ml volumetric flask and add a solution for the mobile phase (tetrahydrofuran/methanol=50/50 (vol/vol)) to the mark. Filter through a 0.5 μm membrane filter. Inject 20 μl of the sample into the chromatograph.

Chromatography conditions

Column: reversed phase C18 columns (150mm x 4.6 mm i.d.; ODS-2 manufactured by GL Science or equivalent)

Mobile phase: tetrahydrofuran/methanol=50/50 (vol/vol)

Flow rate: 0.8 ml/min

Detector: RI

Temperatures:

- Column: 40°

- Detector: 40°

Record the chromatogram for about 16 min

Identification of the peaks

The retention times of tetra-, penta-, hexa-, hepta- and octa-esters are dependent on the variety of esterified fatty acids and chromatography conditions. Their retention times at these conditions are described in Table 2. The reference products are available from Mitsubishi Chemical Corporation (Tokyo, Japan) or Dai-ichi Kogyo Seiyaku Co. Ltd (Kyoto, Japan) to confirm the retention time.

Table 2. The retention time (min) of tetra- to octa-esters for some type of SOE Type I

Esterified fatty acid	Tetra-esters	Penta-esters	Hexa-esters	Hepta-esters	Octa-esters
Stearic acid	3.1	3.7	4.8	6.1-7.0*	7.9-10.7*
Erucic Acid	3.3	4.1	5.4	7.5	11.0
Behenic acid	4.5	5.5	7.1	10.2	14.8

*: the retention time range, because highly esterified components have been shown as several unresolved peaks

Calculate the percentage of the sum of hepta- and octa-esters

($E_{\text{hep+oct}}$) and the percentage of octa-esters (E_{oct}) as follows:

$$E_{\text{hep+oct}}(\%) = (B_{\text{hep+oct}}/T_{\text{tet-oct}}) \times E_{\text{tet-oct}}$$
$$E_{\text{oct}}(\%) = (B_{\text{oct}}/T_{\text{tet-oct}}) \times E_{\text{tet-oct}}$$

where

$B_{\text{hep+oct}}$ is the sum of peak areas of hepta-esters and octa-esters;

B_{oct} is the peak areas for octa-esters;

$T_{\text{tet-oct}}$ is the sum of peak areas from tetra- to octa-esters; and

$E_{\text{tet-oct}}$ (%) is the percentage of tetra- to octa-esters measured by Method 1 described above.

TANNIC ACID

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 39th JECFA (1992), published in the combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI 'not specified' was established at the 35th JECFA (1989).

SYNONYMS

Tannins (food grade), gallotannic acid, INS No. 181

DEFINITION

Consists of gallotannins obtained by solvent extraction from certain natural sources; the substance is not an acid in the chemical sense. The common name "Tannic acid" has been adopted to distinguish the commercial substance from other tannins, such as condensed tannins. These specifications relate only to hydrolysable gallotannins, i.e., those which yield gallic acid on hydrolysis. Hydrolysable gallotannins may be obtained from nutgalls, the excrescences which form on young twigs of various *Quercus* species, e.g., *Q. infectoria*; these include Chinese and Aleppo tannins. They may also be obtained from various Sumac species, e.g. *Rhus coriaria*, *R. galabra*, *R. thypia*; these include Sicilian and American sumacs. All of these consist essentially of polydigalloyl esters of glucose. A further source of hydrolysable gallotannins is the seed pods of Tara (*Caesalpinia spinosa*); these tannins consist essentially of the polydigalloyl esters of quinic acid. These specifications do not apply to many other kinds of tannins which occur in nature, including condensed (non-hydrolysable) tannins and hydrolysable ellagitannins.

Assay

Not less than 96% on dried basis

DESCRIPTION

Amorphous powder, glistening scales or spongy mass, varying in colour from yellowish white to light brown; odourless or with a faint, characteristic odour

FUNCTIONAL USES

Clarifying agent, flavouring agent, flavour adjunct

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Soluble in water, acetone and ethyl alcohol; insoluble in ether; 1 g dissolves in about 1 ml of warm glycerine.

Colour reaction

To a 1 in 10 solution add a small quantity of ferric chloride TS. A bluish black colour or precipitate forms

Precipitate formation

A solution of the sample when added to a solution of either albumin or gelatin produces a precipitate

<u>Test for gallic acid</u>	Passes test after hydrolysis See description under TESTS
PURITY	
<u>Loss on drying</u> (Vol. 4)	Not more than 7% (105°, 2 h)
<u>Sulfated ash</u> (Vol. 4)	Not more than 1% Test 2.0 g of the sample (Method I)
<u>Gums or dextrin</u>	Dissolve 1 g in 5 ml of water, filter, and to the filtrate add 10 ml of ethanol; no turbidity is produced within 15 min
<u>Resinous substances</u>	Dissolve 1 g in 5 ml of water, filter and dilute the filtrate to 15 ml; no turbidity is produced
<u>Condensed tannins</u>	Not more than 0.5% See description under TESTS
<u>Residual solvent</u> (Vol. 4)	Not more than 25 mg/kg acetone or ethyl acetate, singly or in combination See description under TESTS
<u>Lead</u> (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under "General Methods, Metallic Impurities").

TESTS

IDENTIFICATION TESTS

<u>Test for gallic acid</u>	Proceed as directed under <i>Chromatography (thin-layer chromatography)</i> (see Volume 4). <u>Sample preparation</u> Add 20 mg of tannase to 100 ml of 1 in 1000 solution of the sample. Incubate at 37° for 2 h. <u>Standard preparation</u> 1 in 1000 solution of gallic acid. <u>Developing solvent</u> A mixture of 5 volumes of chloroform, 4 volumes of ethyl formate and 1 volume of formic acid. <u>Spraying reagent</u> <i>Solution A</i> : Prepare a 1% solution of ferric chloride (FeCl ₃) in 10% ethanol <i>Solution B</i> : Prepare a 1% solution of potassium ferricyanate (K ₃ Fe(CN) ₆) in 50% ethanol Mix 1 volume of solution A and 1 volume of solution B.
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Adsorbent

Silica gel

Procedure

Apply 10 µl of the sample preparation and 10 µl of the standard preparation. Stop the development when the solvent front has advanced about 15 cm, dry in air, and spray the reagent. The R_f value of the sample and that of the reference standard are identical.

PURITY TESTS

Condensed tannins

The following HPLC method can be used for the determination of condensed tannins in the sample.

Sample preparation

Dissolve 200 mg of the sample in 100 ml of a mixture of 100 volumes of 10% methanol and 0.1 volume of phosphoric acid.

Standard preparation

Dissolve 10 mg of rutin in 100 ml of methanol.

Conditions

Column: Merck Hibar, LiChrosorb RP 18, 250 mm x 4.6 mm id, 5 µ (Art. 50333), or equivalent.

Eluent

A: 0.2% H₃PO₄ in water; B: 0.2% H₃PO₄ in methanol

Gradient elution:

Time (min)	% A	%B
0	90	10
65	25	75
66	0	100
75	0	100
76	90	10
95	90	10

Flow rate: 1 ml/min.

Detection wavelength: 350 nm

Injection volume: 10 µl

Calculate the content of the condensed tannins as follows:

$$\% \text{ condensed tannins (as rutin)} = \frac{A \times W_o}{A_o \times W} \times 100$$

where

A is peak area of the sample (condensed tannins);

A_o is peak area of the reference standard (rutin);

W is weight in mg of the sample taken; and

W_o is weight in mg of the reference standard taken.

Residual solventsReagents

- Acetone

- Ethyl acetate

Standard solutions

Place 1 g of acetone and 1 g of ethyl acetate in a volumetric flask and add water to total volume of 100 ml, and prepare 0.02 - 0.4 g/100 ml solutions by dilution of this solution.

Procedure

Place 1 g (1.0±0.1 g) of powdered sample in a sample vial. Add 5 µl of water to the sample vial and seal it quickly with a septum. Set the sample vial in a pre-conditioned gas chromatograph and start the analysis under the below-mentioned conditions.

Standard

Take 1 g of tannic acid free of solvent or with known residual solvent content in a sample vial, add 5 µl of the standard solution and seal it quickly with a septum. Set the sample vial in a pre-conditioned gas chromatograph and start the analysis under the following conditions and obtain standard curves for each solvent.

Determine by *Gas chromatography*, using a head space sampler under the following conditions:

- Column: 100% methyl polysiloxane 30 m x 0.53 mm id, 1 µm film thickness
- Column conditioning: Heat to 60° for 2-3 h with approximately 10 ml/min of nitrogen
- Carrier gas: Nitrogen
- Flow rate: 5 ml/min
- Detector: Flame ionization
- Temperatures
- injection port: 110°
- column: 40°
- detector: 110°

Head space sampler

- Sample heating temp.: 80°
- Sample heating time: 40 min
- Syringe temperature: 85°
- Sample gas injection: 0.4 ml

Calculation

$$C_i \text{ (mg/kg)} = \left(\frac{A_i \times f_i}{W} \right)$$

where

C_i is the concentration of solvent (mg/kg);

A_i is peak area of solvent (Area units);

f_i is the slope of the standard curve (µg/Area units); and

W is weight of sample in g.

METHOD OF ASSAY Sample test

Accurately weigh about 2.0 g of the sample (W), transfer to a 500-

ml volumetric flask, add water to dissolve and make up to the volume with water. Transfer 100 ml of this solution into a 300 ml Erlenmeyer flask and add 7.2 g of Hide Powder (a suitable grade is available from L.H. Lincoln & Son, Inc., Tanning Materials, Coudersport, Pennsylvania, 16915 USA). Shake the flask for 20 min. Let stand for 10 min. and filter through a G₄-filter. The filtrate shall be clear. Pipette 50 ml of the filtrate into a tared crystallizing dish. Evaporate to dryness on a steam bath and heat in an oven at 105° for 1 h. Cool in a desiccator and weigh (a).

Blank test:

On each lot of Hide Powder (a suitable grade of Hide Powder may be obtained from L.H. Lincoln & Son, Inc., Tanning Materials, Coudersport, Pennsylvania, 16915 USA) a blank test has to be carried out. Weigh 7.2 g of Hide Powder EFT into a 300 ml Erlenmeyer containing 100 ml water. Proceed as directed for the Sample test, beginning with "Shake the flask for 20 min...". Cool in a desiccator and weigh (b).

Calculation

$$\% \text{ Tannic Acid} = \left(\frac{(W - (a - b)) \times 1000}{W} \right)$$

where

- a is the weight of dry matter found in the sample test (g);
- b is the weight of dry matter found in the blank test (g); and
- W is weight of sample, on dry basis (g).

NOTE: the multiplication factor 1000 = 10 (500 ml/50 ml) x 100 (percent).

TITANIUM DIOXIDE

Prepared at the 71st JECFA (2009) and published in FAO JECFA Monographs 7 (2009), superseding specifications prepared at the 67th JECFA (2006) and published in FAO JECFA Monographs 3 (2006). An ADI "not limited" was established at the 13th JECFA (1969).

SYNONYMS

Titania; CI Pigment white 6; CI (1975) No. 77891; INS No. 171

DEFINITION

Titanium dioxide is produced by either the sulfate or the chloride process. Processing conditions determine the form (anatase or rutile structure) of the final product.

In the sulfate process, sulfuric acid is used to digest ilmenite (FeTiO_3) or ilmenite and titanium slag. After a series of purification steps, the isolated titanium dioxide is finally washed with water, calcined, and micronized.

In the chloride process, chlorine gas is reacted with a titanium-containing mineral under reducing conditions to form anhydrous titanium tetrachloride, which is subsequently purified and converted to titanium dioxide either by direct thermal oxidation or by reaction with steam in the vapour phase. Alternatively, concentrated hydrochloric acid can be reacted with the titanium-containing mineral to form a solution of titanium tetrachloride, which is then further purified and converted to titanium dioxide by hydrolysis. The titanium dioxide is filtered, washed, and calcined.

Commercial titanium dioxide may be coated with small amounts of alumina and/or silica to improve the technological properties of the product.

C.A.S. number 13463-67-7

Chemical formula TiO_2

Formula weight 79.88

Assay Not less than 99.0% on the dried basis (on an aluminium oxide and silicon dioxide-free basis)

DESCRIPTION

White to slightly coloured powder

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water, hydrochloric acid, dilute sulfuric acid, and organic solvents. Dissolves slowly in hydrofluoric acid and hot concentrated sulfuric acid.

Colour reaction Add 5 ml sulfuric acid to 0.5 g of the sample, heat gently until

fumes of sulfuric acid appear, then cool. Cautiously dilute to about 100 ml with water and filter. To 5 ml of this clear filtrate, add a few drops of hydrogen peroxide; an orange-red colour appears immediately.

PURITY

<u>Loss on drying</u> (Vol. 4)	Not more than 0.5% (105°, 3 h)
<u>Loss on ignition</u> (Vol. 4)	Not more than 1.0% (800°) on the dried basis
<u>Aluminium oxide and/or silicon dioxide</u>	Not more than 2%, either singly or combined See descriptions under TESTS
<u>Acid-soluble substances</u>	Not more than 0.5%; Not more than 1.5% for products containing alumina or silica. Suspend 5 g of the sample in 100 ml 0.5 N hydrochloric acid and place on a steam bath for 30 min with occasional stirring. Filter through a Gooch crucible fitted with a glass fibre filter paper. Wash with three 10-ml portions of 0.5 N hydrochloric acid, evaporate the combined filtrate and washings to dryness, and ignite at a dull red heat to constant weight.
<u>Water-soluble matter</u> (Vol. 4)	Not more than 0.5% Proceed as directed under acid-soluble substances (above), using water in place of 0.5 N hydrochloric acid.
<u>Impurities soluble in 0.5 N hydrochloric acid</u>	
<u>Antimony</u>	Not more than 2 mg/kg See description under TESTS
<u>Arsenic</u>	Not more than 1 mg/kg See description under TESTS
<u>Cadmium</u>	Not more than 1 mg/kg See description under TESTS
<u>Lead</u>	Not more than 10 mg/kg See description under TESTS
<u>Mercury</u> (Vol. 4)	Not more than 1 mg/kg Determine using the cold vapour atomic absorption technique. Select a sample size appropriate to the specified level

TESTS

PURITY TESTS

Impurities soluble in 0.5 N hydrochloric acid

Antimony, arsenic, cadmium and lead (Vol.4) Transfer 10.0 g of sample into a 250-ml beaker, add 50 ml of 0.5 N hydrochloric acid, cover with a watch glass, and heat to boiling on a hot plate. Boil gently for 15 min, pour the slurry into a 100- to

150-ml centrifuge bottle, and centrifuge for 10 to 15 min, or until undissolved material settles. Decant the supernatant through Whatman No. 4 filter paper, or equivalent, collecting the filtrate in a 100-ml volumetric flask and retaining as much as possible of the undissolved material in the centrifuge bottle. Add 10 ml of hot water to the original beaker, washing off the watch glass with the water, and pour the contents into the centrifuge bottle. Form a slurry, using a glass stirring rod, and centrifuge. Decant through the same filter paper, and collect the washings in the volumetric flask containing the initial extract. Repeat the entire washing process two more times. Finally, wash the filter paper with 10 to 15 ml of hot water. Cool the contents of the flask to room temperature, dilute to volume with water, and mix.

Determine antimony, cadmium, and lead using an AAS/ICP-AES technique appropriate to the specified level. Determine arsenic using atomic absorption hydride technique.

Aluminium oxide

Reagents and sample solutions

Ammonium acetate buffer solution

In a 1000-ml volumetric flask, dissolve 77 g of ammonium acetate in about 500 ml of water, add 10 ml of glacial acetic acid and dilute to volume with water.

Diammonium hydrogen phosphate solution

In a 1000-ml volumetric flask, dissolve 150 g of diammonium hydrogen phosphate in about 700 ml of water, adjust pH to 5.5 using a 1 in 2 solution of hydrochloric acid, then dilute to volume with water.

Zinc Sulfate solution (0.01 N)

Dissolve 2.9 g of zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) in sufficient water and make up to 1000 ml in a volumetric flask. Standardize the solution as follows: Dissolve 500 mg of high-purity (99.9%) aluminium wire, accurately weighed, in 20 ml of concentrated hydrochloric acid, heating gently to effect solution, then transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and mix. Transfer a 10 ml aliquot of this solution into a 500 ml Erlenmeyer flask containing 90 ml of water and 3 ml of concentrated hydrochloric acid, add 1 drop of methyl orange TS and 25 ml of 0.02 M disodium ethylenediaminetetraacetate (EDTA). Add, dropwise, ammonia solution (1 in 5) until the colour is just completely changed from red to orange-yellow. Then, add 10 ml of ammonium acetate buffer solution and 10 ml of diammonium hydrogen phosphate solution. Boil the solution for 5 min, cool it quickly to room temperature in a stream of running water, add 3 drops of xylenol orange TS, and mix.

Using zinc sulfate solution as titrant, titrate the solution to the first yellow-brown or pink end-point colour that persists for 5-10 sec. (NOTE: This titration should be performed quickly near the end-point by adding rapidly 0.2 ml increments of the titrant until the first colour change occurs; although the colour will fade in 5-10 sec, it is the true end-point. Failure to observe the first colour change will result in an incorrect titration. The fading end-point

does not occur at the second end-point)

Add 2 g of sodium fluoride, boil the mixture for 2-5 min, and cool in a stream of running water. Titrate this solution, using the zinc sulfate solution as titrant, to the same fugitive yellow-brown or pink end-point as described above.

Calculate mass (mg) of Al_2O_3 per ml of zinc sulfate solution (T) from the formula

$$T = 18.896 W/V$$

where

W is the mass (g) of aluminium wire;

V is the ml of the zinc sulfate solution consumed in the second titration;

$18.896 = (R \times 1000 \text{ mg/g} \times 10 \text{ ml/2})/1000 \text{ ml}$; and

R is the ratio of the formula weight of aluminium oxide to that of elemental aluminium.

Sample Solution A

Accurately weigh 1.0 g of the sample and transfer to a 250-ml high-silica glass Erlenmeyer flask. Add 10 g of sodium bisulfate ($\text{NaHSO}_4 \cdot \text{H}_2\text{O}$). (*Note:* Do not use more sodium bisulfate than specified, as an excess concentration of salt will interfere with the EDTA titration later on in the procedure.) Begin heating the flask at low heat on a hot plate, and then gradually raise the temperature until full heat is reached. (*Caution:* perform this procedure in a well ventilated area) When spattering has stopped and light fumes of SO_3 appear, heat in the full flame of a Meeker burner, with the flask tilted so that the fusion of the sample and sodium bisulfate is concentrated at one end of the flask. Swirl constantly until the melt is clear (except for silica content), but guard against prolonged heating to avoid precipitation of titanium dioxide. Cool, add 25 ml sulfuric acid solution (1 in 2), and heat until the mass has dissolved and a clear solution results. Cool, and dilute to 120 ml with water. Introduce a magnetic stir bar into the flask.

Sample Solution B

Prepare 200 ml of an approximately 6.25 M solution of sodium hydroxide. Add 65 ml of this solution to Sample Solution A, while stirring with the magnetic stirrer; pour the remaining 135 ml of the alkali solution into a 500-ml volumetric flask.

Slowly, with constant stirring, add the sample mixture to the alkali solution in the 500-ml volumetric flask; dilute to volume with water, and mix. (*Note:* If the procedure is delayed at this point for more than 2 hours, store the contents of the volumetric flask in a polyethylene bottle.) Allow most of the precipitate to settle (or centrifuge for 5 min), then filter the supernatant liquid through a very fine filter paper. Label the filtrate Sample Solution B.

Sample Solution C

Transfer 100 ml of the Sample Solution B into a 500-ml Erlenmeyer flask, add 1 drop of methyl orange TS, acidify with hydrochloric acid solution (1 in 2), and then add about 3 ml in excess. Add 25 ml of 0.02 M disodium EDTA, and mix. [*Note:* If

the approximate Al_2O_3 content is known, calculate the optimum volume of EDTA solution to be added by the formula: $(4 \times \% \text{Al}_2\text{O}_3) + 5 \text{ ml}$

Add, dropwise, ammonia solution (1 in 5) until the colour is just completely changed from red to orange-yellow. Then add 10 ml each of ammonium acetate and diammonium hydrogen phosphate solution and boil for 5 min. Cool quickly to room temperature in a stream of running water, add 3 drops of xylenol orange TS, and mix. If the solution is purple, yellow-brown, or pink, bring the pH to 5.3 - 5.7 by the addition of acetic acid. At the desired pH, a pink colour indicates that not enough of the EDTA solution has been added, in which case, discard the solution and repeat this procedure with another 100 ml of Sample Solution B, using 50 ml, rather than 25 ml, of 0.02 M disodium EDTA.

Procedure

Using the standardized zinc sulfate solution as titrant, titrate Sample Solution C to the first yellow-brown or pink end-point that persists for 5-10 sec. (*Important: See Note under "0.01 Zinc sulfate"*). This first titration should require more than 8 ml of titrant, but for more accurate work a titration of 10-15 ml is desirable.

Add 2 g of sodium fluoride to the titration flask, boil the mixture for 2-5 min, and cool in a stream of running water. Titrate this solution, using the standardized zinc sulfate solution as titrant, to the same fugitive yellow-brown or pink end-point as described above.

Calculation

Calculate the percentage of aluminium oxide (Al_2O_3) in the sample taken by the formula:

$$\% \text{Al}_2\text{O}_3 = 100 \times (0.005VT)/S$$

where

V is the number of ml of 0.01 N zinc sulfate consumed in the second titration;

T is the mass of Al_2O_3 per ml of zinc sulfate solution;

S is the mass (g) of the sample taken; and

$0.005 = 500 \text{ ml} / (1000 \text{ mg/g} \times 100 \text{ ml})$.

Silicon dioxide

Accurately weigh 1 g of the sample and transfer to a 250-ml high-silica glass Erlenmeyer flask. Add 10 g of sodium bisulfate ($\text{NaHSO}_4 \cdot \text{H}_2\text{O}$). Heat gently over a Meeker burner, while swirling the flask, until decomposition and fusion are complete and the melt is clear, except for the silica content, and then cool. (*Caution: Do not overheat the contents of the flask at the beginning, and heat cautiously during fusion to avoid spattering.*)

To the cooled melt add 25 ml of sulfuric acid solution (1 in 2) and heat carefully and slowly until the melt is dissolved. Cool, and carefully add 150 ml of water by pouring very small portions down the sides of the flask, with frequent swirling to avoid over-heating and spattering. Allow the contents of the flask to cool, and filter through fine ashless filter paper, using a 60 degree gravity funnel.

Rinse out all the silica from the flask onto the filter paper with sulfuric acid solution (1 in 10). Transfer the filter paper and its contents into a platinum crucible, dry in an oven at 120°, and heat the partly covered crucible over a Bunsen burner. To prevent flaming of the filter paper, first heat the cover from above, and then the crucible from below.

When the filter paper is consumed, transfer the crucible to a muffle furnace and ignite at 1000° for 30 min. Cool in a desiccator, and weigh. Add 2 drops of sulfuric acid (1 in 2) and 5 ml of concentrated hydrofluoric acid (sp.gr. 1.15), and carefully evaporate to dryness, first on a low-heat hot plate (to remove the HF) and then over a Bunsen burner (to remove the H₂SO₄). Take precautions to avoid spattering, especially after removal of the HF. Ignite at 1000° for 10 min, cool in a desiccator, and weigh again. Record the difference between the two weights as the content of SiO₂ in the sample.

METHOD OF ASSAY Accurately weigh about 150 mg of the sample, previously dried at 105° for 3 hours, and transfer into a 500-ml conical flask. Add 5 ml of water and shake until a homogeneous, milky suspension is obtained. Add 30 ml of sulfuric acid and 12 g of ammonium sulfate, and mix. Initially heat gently, then heat strongly until a clear solution is obtained. Cool, then cautiously dilute with 120 ml of water and 40 ml of hydrochloric acid, and stir. Add 3 g of aluminium metal, and immediately insert a rubber stopper fitted with a U-shaped glass tube while immersing the other end of the U-tube into a saturated solution of sodium bicarbonate contained in a 500-ml wide-mouth bottle, and generate hydrogen. Allow to stand for a few minutes after the aluminium metal has dissolved completely to produce a transparent purple solution. Cool to below 50° in running water, and remove the rubber stopper carrying the U-tube. Add 3 ml of a saturated potassium thiocyanate solution as an indicator, and immediately titrate with 0.2 N ferric ammonium sulfate until a faint brown colour that persists for 30 seconds is obtained. Perform a blank determination and make any necessary correction. Each ml of 0.2 N ferric ammonium sulfate is equivalent to 7.990 mg of TiO₂.

ANALYTICAL METHODS

The following analytical methods were prepared by the Committee at the 69th meeting. This method will be made available in the on-line edition of Volume 4 of the Combined Compendium of Food Additive Specifications.

GLYCEROL ESTERS OF ROSINS

Ring and ball softening point method

The ring-and-ball softening point is defined as the temperature at which a disk of the sample held within a horizontal ring is forced downward a distance of 1 in. (25.4 mm) under the weight of a steel ball as the sample is heated at a prescribed rate in a water or glycerol bath.

Apparatus

The apparatus illustrated in Figures 1 and 2 consists of the components described in the following paragraphs.

Ring

A brass-shouldered ring conforming to the dimensions shown in Figure 1a should be used. If desired, the ring may be attached by brazing or other convenient manner to a brass wire of about 13 B & S gauge (0.06 to 0.08 in., or 1.52 to 2.03 mm, in diameter) as shown in Figure 2a.

Ball

A steel ball, 3/8 in. (9.53 mm) in diameter, weighing between 3.45 and 3.55 g, should be used.

Ball-Centering Guide

A guide for centering the ball, constructed of brass and having the general shape and dimensions, as illustrated in Figure 1c may be used if desired.

Container

Use a heat-resistant glass vessel, such as an 800-ml low-form Griffin beaker, not less than 3.34 in. (8.5 cm) in diameter and not less than 5 in. (12.7 cm) in depth from the bottom of the flare.

Support for Ring and thermometer

Any convenient device for supporting the ring and thermometer may be used, provided that it meets the following requirements: (1) the ring shall be supported in a substantially horizontal position; (2) when using the apparatus shown in Figure 1d, the bottom of the ring shall be 1.0 in. (25.4 mm) above the horizontal plate below it, the bottom surface of the horizontal plate shall be at least 0.5 in. (13 mm) and not more than 0.75 in. (18 mm) above the bottom of the container, and the depth of the liquid in the container shall be not less than 4.0 in. (10.2 cm); (3) when using the apparatus shown in Figure 1e, the bottom of the ring shall be 1.0 in. (25.4 mm) above the bottom of the container, with the bottom end of the rod resting on the bottom of the container, and the depth of the liquid in the container shall be not less than 4.0 in. (10.2 cm), as shown in Figure 1 a, b and c; and (4) in both assemblies, the thermometer shall be suspended so that the bottom of the bulb is level with the bottom of the ring and within 0.5 in. (13 mm) but not touching the ring.

Thermometers (mercury-in-glass)

Depending upon the expected softening point of the sample, use either an ASTM 15C low-softening-point thermometer (-2° to 80°) or an ASTM 16C high-softening-point thermometer (30° to 200°).

Stirrer

Use a suitable mechanical stirrer rotating between 500 and 700 rpm. To ensure uniform heat distribution in the heating medium, the direction of the shaft rotation should move the liquid upward. (See Figure 2d for recommended dimensions.)

Sample Preparation

Select a representative sample of the material under test consisting of freshly broken lumps free of oxidized surfaces. Scrape off the surface layer of samples received as lumps immediately before use, avoiding inclusion of finely divided material or dust. The amount of sample taken should be at least twice that necessary to fill the desired number of rings, but in no case less than 40 g. Immediately melt the sample in a clean container, using an oven, hot plate, or sand or oil bath to prevent local overheating. Avoid incorporating air bubbles in the melting sample, which must not be heated above the temperature necessary to pour the material readily without inclusion of air bubbles. The time from the beginning of heating to the pouring of the sample shall not exceed 15 min. Immediately before filling the rings; preheat them to approximately the same temperature at which the sample is to be poured. While being filled, the rings should rest on an amalgamated brass plate. Pour the sample into the rings so as to leave an excess on cooling. Cool for at least 30 min, and then cut off the excess material cleanly with a slightly heated knife or spatula. Use a clean container and a fresh sample if the test is repeated.

Procedure

Materials having softening points above 80° : Fill the glass vessel with glycerol to a depth of not less than 4.0 in. (10.2 cm) and not more than 4.25 in. (10.8 cm). The starting temperature of the bath shall be 32° . For resins (including rosin), the glycerol should be cooled to not less than 45° below the anticipated softening point, but in no case lower than 35° . Position the axis of the stirrer shaft near the back wall of the container, with the blades clearing the wall and with the bottom of the blades 0.75 in. (18 mm) above the top of the ring. Unless the ball-centering guide is used, make a slight indentation in the center of the sample by pressing the ball or a rounded rod, slightly heated for hard materials, into the sample at this point. Suspend the ring containing the sample in the glycerol bath so that the lower surface of the filled ring is 1.0 in. (25.4 mm) above the surface of the lower horizontal plate (see Figure 1d), which is at least 0.5 in. (13 mm) and not more than 0.75 in. (18 mm) above the bottom of the glass vessel, or 1.0 in. (25.4 mm) above the bottom of the container (see Figure 2e). Place the ball in the glycerol but not on the test specimen. Suspend an ASTM high-softening-point thermometer (16C) in the glycerol so that the bottom of its bulb is level with the bottom of the ring and within 0.5 in. (13 mm) but not touching the ring. Maintain the initial temperature of the glycerol for 15 min, and then, using suitable forceps, place the ball in the center of the upper surface of the sample in the ring. Begin stirring, and continue stirring at 500 to 700 rpm until completion of the determination. Apply heat at such a rate that the temperature of the glycerol is raised 5° per min, avoiding the effects of drafts by using shields if necessary.

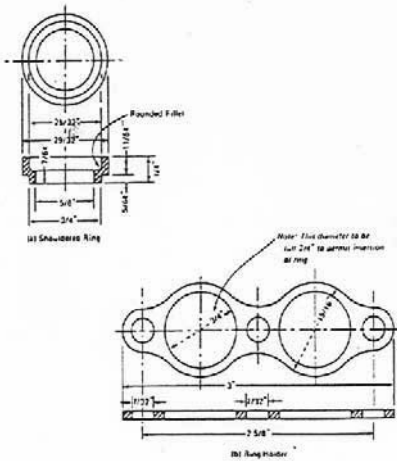
[Note: The rate of rise of the temperature shall be uniform and shall not be averaged over the test period. Reject all tests in which the rate of rise exceeds $\pm 0.5^{\circ}$ for any minute period after the first three.]

Record as the softening point the temperature of the thermometer at the instant the sample touches the lower horizontal plate (see Figure 1d) or the bottom of the container (see Figure 2e). Make no correction for the emergent stem of the thermometer.

Materials having softening points of 80° or below: Follow the above procedure, except use an ASTM low-softening-point thermometer (15C) and use freshly boiled water cooled to 5° as the heating medium. For resins (including rosins), use water cooled to not less than 45° below the anticipated softening point, but in no case lower than 5°.

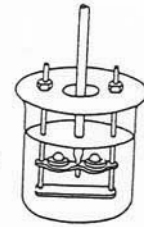
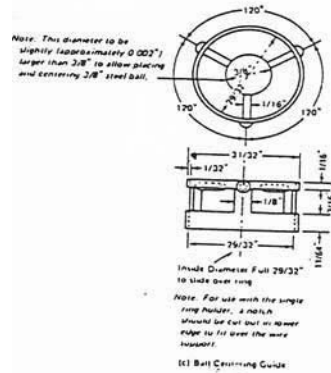
Apparatus - Ring and Ball Softening Point

(a) Shouldered Ring



(b) Ring Holder

(c) Bell Centering Guide



(d) Assembly Apparatus with Two Rings

Figure 1. Shouldered Ring, Ring Holder Ball-Centering Guide, and Assembly of Apparatus Showing Two Rings

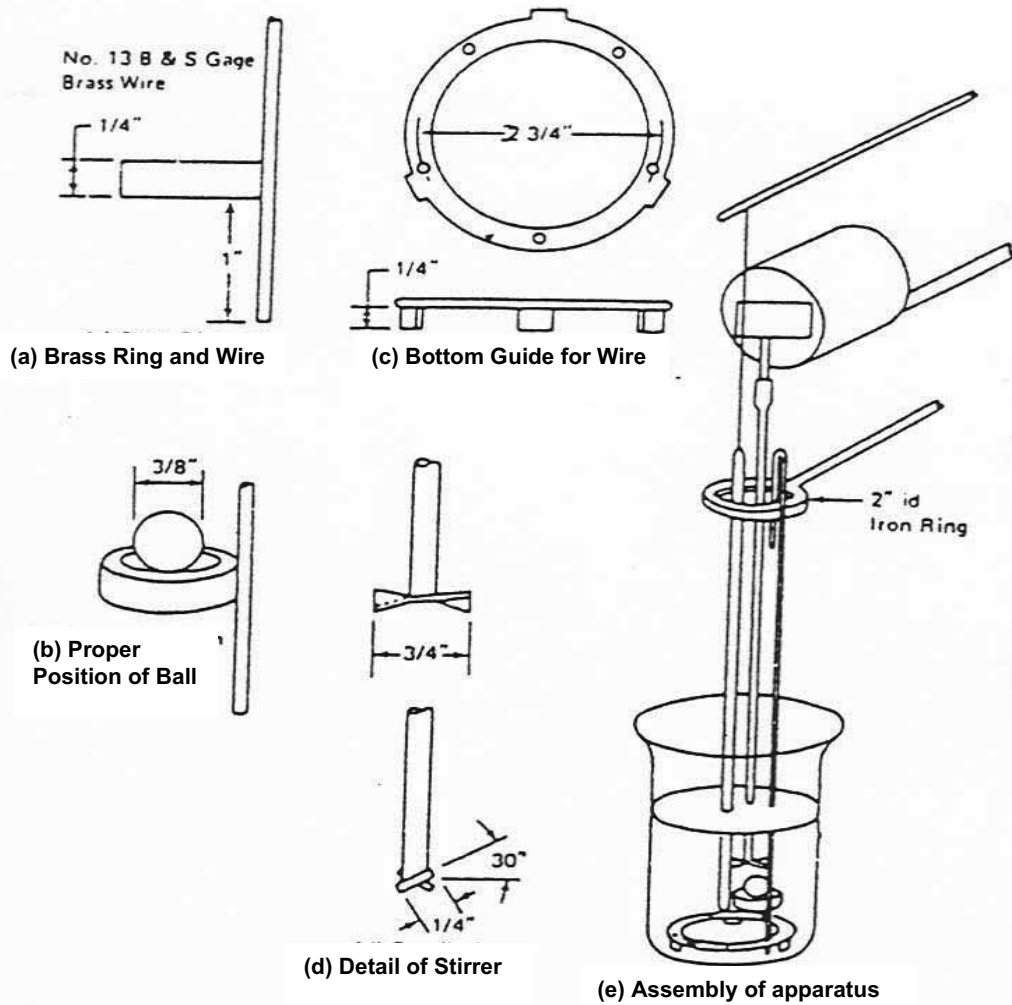


Figure 2. Assembly of Apparatus Showing Stirrer and Single Shouldered Ring.

Figures 1 and 2 are referenced from the Food Chemicals Codex, 6th Edition, 2008, p. 1161 (figures 40 and 41).

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ANNEX 1: SUMMARY OF RECOMMENDATIONS FROM THE 71ST JECFA MEETING

Toxicological recommendations and information on specifications

1. Food additives and ingredients evaluated toxicologically or assessed for dietary exposure

Food additive	Specifications ^a	Acceptable daily intake (ADI) and other toxicological recommendations ^b
Branching glycosyltransferase from <i>Rhodothermus obamensis</i> expressed in <i>Bacillus subtilis</i>	N	The Committee allocated an ADI “ not specified ” for branching glycosyltransferase from <i>Rhodothermus obamensis</i> expressed in <i>Bacillus subtilis</i> used in the specified applications and in accordance with Good Manufacturing Practice.
Cassia gum	N, T	The Committee allocated an ADI “ not specified ” for cassia gum that complies with the tentative specifications established at the current meeting, when used in the applications specified and in accordance with Good Manufacturing Practice. The Committee decided to make the specifications tentative pending submission of data on a suitable and validated method for determination of anthraquinones at a level of 0.5 mg/kg and below, by the end of 2010.
Cyclamic acid and its salts (dietary exposure assessment)		Of the four maximum use levels (250, 500, 750 and 1000 mg/kg) that the Committee considered at the request of the Codex Committee on Food Additives (CCFA) for cyclamates in beverages covered by General Standard for Food Additives (GSFA) Food Category 14.1.4, only the lowest level of 250 mg/kg was not likely to lead to dietary exposures exceeding the ADI for high consumers, including children. Moreover, it was noted that a maximum use level of 350 mg/kg also resulted in dietary exposures for high consumers, including children that were less than the ADI.
Cyclotetraglucose and cyclotetraglucose syrup	R (cyclotetraglucose syrup)	The Committee removed the temporary designation and established an ADI “ not specified ” for cyclotetraglucose and cyclotetraglucose syrup. The specifications for cyclotetraglucose syrup were revised, and the tentative designation was removed.
Ferrous ammonium phosphate	N	The newly available information on the toxicity of iron did not indicate a need to revise the provisional maximum tolerable daily intake (PMTDI) of 0.8 mg/kg body weight. Consideration of the toxicity of ammonium and phosphate did not indicate a need to revise the Committee’s previous evaluations of these ions. The Committee concluded that ferrous ammonium phosphate is acceptable for use as a source of iron for dietary fortification, provided that the total intake of iron does not exceed the PMTDI. Products, including ferrous ammonium phosphate, that are intended to provide a source of additional iron should not be consumed by individuals with any type of iron storage disease, except under medical supervision.
Glycerol ester of gum rosin (GEGR)	N, T	The Committee decided to include GEGR in the ADI for glycerol esters of wood rosin (GEWR) of 0–25 mg/kg body weight, thereby establishing a group ADI of 0–25 mg/kg body weight for GEWR and GEGR . The specifications for GEGR were made tentative pending the submission of infrared spectra that correspond to the

		commercially available products, data on the resin acid composition obtained with updated chromatographic techniques, and additional information on methods that enables the identification of the individual glycerol esters of rosins and their differentiation. This information should be submitted by the end of 2010.
Glycerol ester of tall oil rosin (GETOR)	N, T	<p>The Committee concluded in principle that the data from GEWR could be used in the evaluation of GETOR; however, the Committee did not have adequate information on the composition of GETOR, considering that the source material and production processes are different, which may result in different by-products.</p> <p>The Committee decided that it could not evaluate GETOR without additional information on its composition in order to clarify the extent and significance of any differences relative to other glycerol esters of rosins.</p> <p>The specifications for GETOR were made tentative pending the submission of infrared spectra that correspond to the commercially available products, data on the resin acid composition obtained with updated chromatographic techniques, and additional information on methods that enables the identification of the individual glycerol esters of rosins and their differentiation. The Committee also requested information on the identity of the sulfur compounds in the commercial product. This information should be submitted by the end of 2010.</p>
Lycopene from all sources		<p>The Committee decided to revise the group ADI established at the sixty-seventh meeting and replace it with a group ADI “not specified” for lycopene from all sources when used as food colour. Hence, the previous group ADI of 0–0.5 mg/kg for lycopene has been withdrawn.</p> <p>The group ADI “not specified” applies to synthetic lycopene, lycopene derived from the fungus <i>Blakeslea trispora</i> and lycopene extract from tomato that comply with the specifications, when used in accordance with Good Manufacturing Practice.</p>
Lycopene extract from tomato	N	The Committee established a group ADI “not specified” for synthetic lycopene, lycopene derived from the fungus <i>Blakeslea trispora</i> and lycopene extract from tomato , when used as food colour, that comply with the specifications, and when used in accordance with Good Manufacturing Practice.
Mineral oil (low and medium viscosity) class II and class III		The Committee was informed that finalization of the requested studies has been delayed. The Committee decided to further extend the temporary group ADI , but noted that the temporary group ADI will be withdrawn at the end of 2011 if the data are not submitted by that time.
Octenyl succinic acid (OSA) modified gum arabic	N	The Committee decided to allocate a temporary ADI “not specified” for OSA modified gum arabic used in the applications specified and in accordance with Good Manufacturing Practice. The ADI is temporary pending submission of data by the end of 2011 showing hydrolysis of OSA modified gum arabic to confirm the validity of using gum arabic data in the evaluation of OSA modified gum arabic.
Sodium hydrogen sulfate	R	The Committee allocated an ADI “not specified” for sodium hydrogen sulfate, in line with the principles established for ionizable salts at its twenty-ninth meeting, when used in the applications specified and in accordance

		with Good Manufacturing Practice. Specifications were revised to include a new technological use.
Sucrose oligoesters (SOE) type I and type II	N	The Committee considered it appropriate to include SOE type I and type II in a group ADI of 0–30 mg/kg body weight for sucrose esters of fatty acids, sucroglycerides and SOE type I and type II . The Committee emphasized that this evaluation is valid only for the material as specified.

^a N: new specifications prepared; R: existing specifications revised; S: existing specifications maintained; T: tentative specifications.

^b ADI 'not specified' is used to refer to a food substance of very low toxicity which, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary intake of 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.

2. Food additives considered for specifications only

Food Additive	Specifications^a
Diacetyltartaric and fatty acid esters of glycerol	R
Ethyl lauroyl arginate	R
Glycerol ester of wood rosin	R, T
Nisin preparation	R
Nitrous oxide	R, T
Pectins	R
Starch sodium octenyl succinate	R
Tannic acid	R
Titanium dioxide	R
Triethyl citrate	R

^a N: New specifications prepared; R: Existing specifications revised; T: tentative specifications; W: Existing specifications withdrawn.

ANNEX 2: RECOMMENDATIONS AND FURTHER INFORMATION REQUIRED

Recommendation

To better assess chronic dietary exposure, the Committee recommends the use of food consumption data collected over a period of more than 1 day with an averaging of the amounts of food consumed per day. Moreover, the Committee recommends that food consumption data collected over a few days be adjusted by using food frequency questionnaires on a comparable population where these data are available.

Further information required

Cassia gum

Information is required on a suitable and validated method for determination of anthraquinones in cassia gum at a level of 0.5 mg/kg and below. This information should be submitted by the end of 2010.

Glycerol ester of gum rosin (GEGR)

The Committee requested that it be provided with full reports of the two 90-day toxicity studies with GEGR in rats fed dietary concentrations of up to 1.0% to confirm the validity of the comparison of glycerol ester of wood rosin (GEWR) with GEGR.

The specifications were made tentative pending the submission of infrared spectra that correspond to the commercially available products, data on the resin acid composition obtained with updated chromatographic techniques, and additional information on methods that enables the identification of the individual glycerol esters of rosins and their differentiation. This information should be submitted by the end of 2010.

Glycerol ester of tall oil rosin (GETOR)

The Committee did not have adequate information on the composition of GETOR, as the source material and production processes are different, which may result in different by-products. Therefore, the Committee decided that it could not evaluate GETOR without additional information on its composition in order to clarify the extent and significance of any differences relative to other glycerol esters of rosins.

The specifications were made tentative pending the submission of infrared spectra that correspond to the commercially available products, data on the resin acid composition obtained with updated chromatographic techniques, and additional information on methods that enables the identification of the individual glycerol esters of rosins and their differentiation. The Committee also requested information on the identity of the sulfur compounds in the commercial products. This information should be submitted by the end of 2010.

Glycerol ester of wood rosin (GEWR)

The specifications were made tentative pending the submission of infrared spectra that correspond to the commercially available products, data on the resin acid composition obtained with updated chromatographic techniques, and additional information on methods that enables the identification of the individual glycerol esters of rosins and their differentiation. This information should be submitted by the end of 2010.

Mineral oil (low and medium viscosity) class II and class III

The Committee at its current meeting was informed that studies are under way but that technical problems had been encountered that will delay the finalization of the requested studies. The Committee received confidential information on the studies and nature of the problems and, based on this, decided to

further extend the temporary group ADI. The Committee noted that the temporary group ADI will be withdrawn at the end of 2011 if the data are not submitted by that time.

Nitrous oxide

The revised specifications were made tentative, as information on a capillary gas chromatographic assay method was required. This information should be submitted by the end of 2010.

Octenyl succinic acid (OSA) modified gum Arabic

The ADI is temporary pending submission of data by the end of 2011 showing hydrolysis of OSA modified gum arabic to confirm the validity of using gum arabic data in the evaluation of OSA modified gum arabic.

CORRIGENDA

**COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS
FAO JECFA Monographs 4 (2007).**

Page 61, Steviol glycosides. The chemical name for Rebaudioside A is corrected to read: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester.

**COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS
FAO JECFA Monographs 5 (2008).**

Page 75, Steviol glycosides. The chemical name for Rebaudioside A is corrected to read: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-16-en-18-oic acid, β-D-glucopyranosyl ester.

**COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS
FAO JECFA Monographs 6 (2009).**

Page 4, last line: The word honey is corrected to residues.

Page 122, 3rd paragraph first line: The word boiler is corrected to broiler.

Page 288: The data for the veterinary drug Doramectin are missing and the information is inserted between the entries for Diminazene and Enrofloxacin as follows:

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Doramectin	0-1	Full	62 (2004)	10	Muscle	Cattle Pigs	Doramectin
				5	Muscle		
				100	Liver	Cattle, Pigs	
				30	Kidney		
				150	Fat		
15	Milk	Cattle					

Page 290: The data for Lincomycin is corrected with respect to the number and year of the last meeting JECFA meeting at which the veterinary drug was on the agenda. The correct text is: 62 (2004).

Page 293: The data for Thiabendazole is corrected with respect to the tissues in the last line of the entry. The correct text for MRL 100 µg/kg for Cattle, goat is Milk as follows:

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Tiabendazole (Thiabendazole)	0-100	Full	58 (2002)	100	Muscle, Liver, Kidney, Fat	Cattle, Pigs, Goat, Sheep	Sum of tiabendazole + 5-hydroxy tiabendazole
				100	Milk	Cattle, Goat	

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- 2 Residue evaluation of certain veterinary drugs – Joint FAO/WHO Expert Committee on Food Additives 66th meeting 2006 (E)
- 3 Compendium of food additive specifications – Joint FAO/WHO Expert Committee on Food Additives 67th meeting 2006 (E)
- 4 Compendium of food additive specifications – Joint FAO/WHO Expert Committee on Food Additives 68th meeting 2006 (E)
- 5 Compendium of food additive specifications – Joint FAO/WHO Expert Committee on Food Additives 69th meeting 2006 (E)
- 6 Residue evaluation of certain veterinary drugs – Joint FAO/WHO Expert Committee on Food Additives 70th meeting 2006 (E)

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COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

71st meeting 2009

This document contains food additive specifications monographs, analytical methods and other information, prepared at the seventy-first meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, from 16 to 24 June 2009. The specifications monographs provide information on the identity and purity of food additives used directly in foods or in food production. The main three objectives of these specifications are to identify the food additive that has been subjected to testing for safety, to ensure that the additive is of the quality required for use in food or in processing, and to reflect and encourage good manufacturing practice. This publication and other documents produced by JECFA contain information that is useful to all those who work with or are interested in food additives and their safe use in food.

