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4

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

68th meeting 2007

World Health Organization

Food and Agriculture Organization of the United Nations

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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SPECIAL NOTE

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Geneva, 19 – 28 June, 2007

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INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 68th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Geneva on 19-28 June 2007. In addition, an analytical method of assay for four flavourings and their respective sodium salts 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?lang=en. The specifications for flavourings evaluated by JECFA, and previously published in FAO Food and Nutrition Paper 52 and subsequent Addenda, are included in a database for flavourings (flavouring agent) specifications which has been updated and modernized. All specifications for flavourings that have been evaluated by JECFA since its 44th meeting, including the 68th 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 www.fao.org/ag/agn/jecfa/index_en.stm. 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:
Asparaginase from *Aspergillus oryzae* expressed in *Aspergillus oryzae* (N)
Carrageenan (R)
Cyclotetraglucose (N)
Cyclotetraglucose syrup (N) Tentative
Ethyl maltol (R)
Isoamylase from *Pseudomonas amyloderamosa* (N)
Magnesium sulfate (R)
Maltol (R)
Nisin preparation (R)
Pectins (R)
Polyvinyl alcohol (R)
Processed *Euchema* seaweed (R)
Sodium chlorite (N)
Sodium hydrogen sulfate (N)
Steviol glycosides (R)
Sucrose esters of fatty acids (R)

When the specifications for heavy metals (as lead), other metals and arsenic in sweeteners, were reviewed by the Committee at its 63rd meeting in 2004, Sodium L(+)-tartrate was inadvertently omitted from the list of food additives with revised specifications. In the publication of FAO JECFA Monographs 1 (2005), the limit for heavy metals (as lead) was already brought in line with the limit for sodium potassium L(+)-tartarte, but the introductory paragraph did not reflect this an appropriate way. This has now been accomplished in the on-line version of the Combined Compendium of Food Additive Specifications and the specifications for sodium L(+)tartrate are not republished in this publication.

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.

New and revised INS numbers assigned to food additives by the Codex Alimentarius Commission at its 30th session in 2007, (ALINORM 07/30/12, Appendix XIII) have been introduced in the corresponding JECFA food additive specifications monographs in the on-line database, as appropriate, and these are not reproduced in this publication. In addition, correction of the INS number for trimagnesium phosphate to 343iii has been made.

The chemical abstract numbers (C.A.S.) for the following food additives have been revised in the specifications monographs in the on-line database and these are not reproduced in this publication:

Food additive	New C.A.S. number(s)
Aspartame	22839-47-0
Benzoic acid	65-85-0
Erythorbic acid	89-65-6
Sucrose acetate isobutyrate	137204-24-1; 27216-37-1; 126-13-6
Trimagnesium phosphate	7757-87-1

For sucrose acetate isobutyrate multiple C.A.S. numbers have been applied over the years and are still in use. The first number is a generic description, the second is more specific in terms of description and the third is precisely based on the molecular structure.

ASPARAGINASE FROM *ASPERGILLUS ORYZAE* EXPRESSED IN *A. ORYZAE*

New specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI "not specified" was established at the 68th JECFA (2007).

SYNONYMS Asparaginase

SOURCES Asparaginase is produced by submerged fed-batch fermentation of a genetically modified strain of *Aspergillus oryzae* which has a reduced capability for producing secondary metabolites and contains the asparaginase gene derived from *A. oryzae*. The enzyme is isolated from the fermentation broth by filtration to remove the biomass and concentrated by ultrafiltration and/or evaporation. The residual production microorganism is removed from the enzyme concentrate by germ filtration. The final product is formulated using food-grade stabilizing and preserving agents and standardized to the desired activity.

Active principles: Asparaginase

Systematic names and numbers: L-Asparagine amidohydrolase; EC 3.5.1.1; CAS No. 9015-68-3

Reactions catalysed: Hydrolysis of L-asparagine to L-aspartic acid and ammonia.

Secondary enzyme activities: No significant levels of secondary enzyme activities.

DESCRIPTION Light brown liquid

FUNCTIONAL USES Enzyme preparation.
Used in food processing to reduce the formation of acrylamide from asparagine and reducing sugars during baking or frying.

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

CHARACTERISTICS

IDENTIFICATION

Asparaginase activity: The sample shows asparaginase activity.
See description under TESTS.

TESTS

Asparaginase activity

Principle

Asparaginase catalyses the conversion of L-asparagine to L-aspartic acid and ammonia. Ammonia is subsequently combined with α-ketoglutarate to form L-glutamic acid. The reaction is catalysed by glutamate dehydrogenase in the presence of NADH, which is oxidized to NAD^+ with the concomitant loss of absorbance measured at 340 nm. The activity of asparaginase is determined by measuring the rate of consumption of NADH under standard conditions (pH=7.00±0.05; 37.0±0.5º) and is expressed in ASNU units.

One ASNU is defined as the amount of asparaginase that produces 1 micromole ammonia per minute under the conditions of the assay (pH=7.00±0.05; 37.0±0.5º).

Apparatus

Spectrophotometer (340 nm) with thermostatic control (37.0±0.5º) and a 1-cm light path.
Water bath with thermostatic control.
Vortex mixer.
Stopwatch

Reagents and solutions

(Note: use deionized water)

Sodium hydroxide 4 M: Weigh 16.0 g NaOH (Merck 106495 or equivalent). Dissolve in water in a 100-ml volumetric flask. Add water to volume and mix until fully dissolved. The solution is stable for 3 months at room temperature.

MOPS buffer, 0.1 M, pH 7, with Triton X-100, 0.1%: Weigh 20.9±0.5 g MOPS (Sigma M-1254 or equivalent) and dissolve in approximately 950 ml of water in a 1000-ml volumetric flask. Adjust the pH to 7.00±0.05 with 4 M NaOH. Add 1.0 ml of 100% Triton X-100 (Sigma T-9284 or equivalent). Add water to volume and mix. The solution must be used on the day of preparation.

L-Asparagine substrate solution: Weigh 0.25±0.02 g of L-asparagine (Sigma A-7094 or equivalent). Transfer to a 25-ml volumetric flask. Add 20 ml of the MOPS buffer with Triton X-100 and stir until dissolved. Add 0.011±0.001 g of NADH (Roche 107 735 or equivalent). Add 0.063±0.005 g of α-ketoglutarate (Sigma K-3752 or equivalent) and at least 2000 units of glutamate dehydrogenase (EC 1.4.1.3) (Fluka 49392 or equivalent). Remove the stirring magnet. Add MOPS buffer with Triton X-100 to volume and mix. The composition of the solution is: L-asparagine, 10 mg/ml; α-ketoglutarate, 2.5 mg/ml; NADH, 0.44 mg/ml; glutamate dehydrogenase, >80 U/ml. The solution is stable for about 2 hours at room temperature.

Control sample solution: Accurately weigh approximately 0.72 g of an asparaginase preparation with known activity (for example, 1301 ASNU/g; batch 115-11104; expiration date January 2026;

available from Novozymes A/S). Transfer to a 100-ml volumetric flask and add the MOPS buffer with Triton X-100 to volume. Mix until fully dissolved.

Blank: MOPS buffer, 0.1 M, pH 7, with Triton X-100, 0.1%.

Test sample solution: Accurately weigh at least 1 g of the asparaginase product into a 50 ml volumetric flask and add the MOPS buffer with Triton X-100 to volume. Repeat dilution if necessary to obtain the activity of approximately 0.4-1.0 ASNU/ml (corresponds to approximately 0.1-0.25 ABS/min).

Procedure

1. Set the temperature of the spectrophotometer and water bath at 37.0±0.5º.
2. Set the wavelength at 340 nm and use the MOPS buffer with Triton X-100 as a blank.
3. Equilibrate 2.4 ml of the L-asparagine substrate solution in the water bath for 10 min. Add 0.1 ml of the test or control sample solution, vortex briefly, and transfer 1 ml to a 1-cm quartz cuvette.
4. Place the cuvette in the spectrophotometer and immediately read the absorbance. If the absorbance exceeds 2.3, continue the assay. If the absorbance is below 2.3, prepare a new substrate solution. Read the absorbance every 10 sec between 3 and 5 min from the start of the reaction.
5. Plot the absorbance (A) versus time (min) and calculate the slope (ΔA/min).

(Note: Carry out steps 3-5 at least twice for each test or control sample solution. The results should agree within 15%.)

Calculations

Calculate the activity (pASNU/g) of the test or control sample as follows:

$$\text{pASNU/g} = \frac{\Delta\text{A/min} \times \text{Tv} \times \text{Dv} \times \text{F}}{\text{Sv} \times \varepsilon \times \text{d} \times \text{W}}$$

where:

ΔA/min is the absolute value of the decrease of absorbance per min for the test or control sample solution

Tv is the total assay volume (2.5 ml)

Dv is the dissolution volume of the test or control sample (before dilution) (ml)

F is the dilution factor

Sv is the volume of the enzyme solution used in the assay (0.1 ml)

ε is the extinction coefficient of NADH at 340 nm (6.3 ml μmol^{-1} cm^{-1})

d is the light path (1 cm)

W is the weight of the test or control sample (g)

Calculate the ratio (R) of the known-to-calculated activity for the control sample. If R≠1, multiply the activity of the test sample (pASNU/g) by R to obtain the corrected activity of the test sample (ASNU/g):

$$\text{ASNU/g} = \text{R} \times \text{pASNU/g}$$

Notes:
The method is specific for asparaginase activity when up to 6 g/l ammonia is generated in samples with asparaginase activity of 1200 ASNU/g.

Asparaginase activity may vary within ±15% between the replicates of the same sample.

CARRAGEENAN

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 57th JECFA (2001), published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI "not specified" for carrageenan and processed Eucheuma *seaweed was established at the 57th JECFA (2001).*

SYNONYMS

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

DEFINITION

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

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

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

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

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

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

processes.

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

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

FUNCTIONAL USES Thickener, gelling agent, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in ethanol; soluble in water at a temperature of about 80º, forming a viscous clear or slightly opalescent solution that flows readily; disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water.

Test for sulfate Dissolve a 100-mg sample in 20 ml of water (with heating if necessary), and add 3 ml of barium chloride TS and 5 ml of hydrochloric acid, dilute TS; filter if a precipitate forms. Boil the solution or the filtrate for 5 min. A white, crystalline precipitate appears.

Test for galactose and anhydrogalactose (Vol. 4) Proceed as directed in Vol.4 (under "General Methods, Organic Components, Gum Constituents Identification") using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present.

Identification of hydrocolloid and predominant type of copolymer Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80º, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel. To 50 ml of the solution or gel add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ("brittle") gel indicates a carrageenan of a predominantly kappa type, and a compliant ("elastic") gel indicates a predominantly iota type. If the solution does not gel, the carrageenan is of a predominantly lambda type.

Infrared absorption Passes test
See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 12% (105º to constant weight)

pH (Vol. 4)	Between 8 and 11 (1 in 100 suspension)
Viscosity	Not less than 5 cp at 75º (1.5% solution) See description under TESTS
Sulfate	Not less than 15% and not more than 40% (as SO_4^{2-}) on the dried basis See description under TESTS
Total ash	Not less than 15% and not more than 40% on the dried basis See description under TESTS.
Acid-insoluble ash (Vol. 4)	Not more than 1% Use the ash from the Total ash test
Acid-insoluble matter (Vol. 4)	Not more than 2% Use 2 g of sample obtained from part (a) of the procedure for sulfate determination.
Residual solvents (Vol. 4)	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination See description under TESTS
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50-g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenising the mixture in a high-speed blender. Total (aerobic) plate count: Not more than 5000 cfu/g *Salmonella* spp.: Negative per test *E. coli*: Negative in 1 g
Arsenic (Vol. 4)	Not more than 3 mg/kg Determine by the atomic absorption hydride technique. Use Method II for sample preparation.
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").
Cadmium (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").
Mercury (Vol.4)	Not more than 1 mg/kg Determine by the cold vapour atomic absorption technique

TESTS

IDENTIFICATION TESTS

Infrared absorption

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

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

Remove the clear supernatant, resuspend the residue in 200 ml of 2.5% potassium chloride solution, and centrifuge again. Coagulate the combined supernatants by adding 2 volumes of 85% ethanol or isopropanol (NOTE: Retain the sediment for use as directed below). Recover the coagulum, and wash it with 250 ml of the alcohol. Press the excess liquid from the coagulum, and dry it at 60º for 2 h. The product obtained is the non-gelling fraction (lambda-carrageenan).

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

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

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

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

PURITY TESTS

Sulfate

Principle
Hydrolysed sulfate groups are precipitated as barium sulfate.

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

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

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

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

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

Total ash

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

Calculate the percentage of total ash of the sample:

$(W_2/W_1) \times 100$

Retain the ash for the Acid-insoluble ash test.

Viscosity

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

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

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

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

Residual solvents (Vol.4)

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

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

Sample Preparation

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

CYCLOTETRAGLUCOSE

New specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). A temporary ADI "not specified" was established at the 68th JECFA (2007).

SYNONYMS Cyclotetraose, Cyclic nigerosyl-(1→6)-nigerose, cycloalternan, cycloalternanotetraose

DEFINITION Cyclotetraglucose has been found to occur naturally in sake lees (i.e., the sediment that forms during sake production), in sake itself, and in the cells of *Saccharomyces cerevisiae.* It is a non-reducing cyclic tetrasaccharide consisting of four D-glucopyranosyl units linked by alternating α(1→3) and α(1→6) glycosidic bonds. It is produced from hydrolyzed food-grade starch by the actions of a mixture of 6-α-glucosyltransferase α-isomaltosyltransferase derived from *Sporosarcina globispora*, and cyclodextrin glucosyltransferase derived from *Bacillus stearothermophilus.* After purification the product contains 0 to 5 molecules of water of crystallization per molecule of cyclotetraglucose.

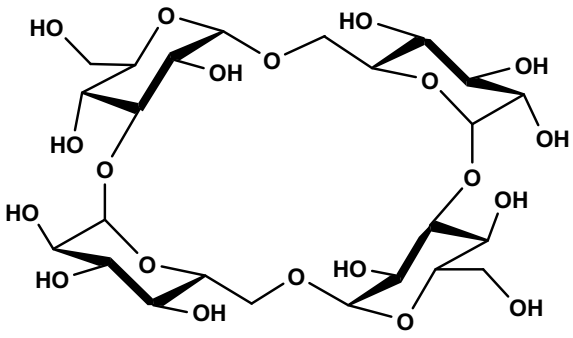
Chemical names *cyclo*[→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→]

Chemical formula $C_{24}H_{40}O_{20}$ (anhydrous)

C.A.S. number

Cyclotetraglucose, anhydrous:	159640-28-5
Cyclotetraglucose, monohydrate:	532945-75-8
Cyclotetraglucose, pentahydrate:	532945-76-9

Structural formula



Formula weight 648.56 (anhydrous)

Assay Not less than 98% on the anhydrous basis

DESCRIPTION Virtually odourless, white or almost white powder

FUNCTIONAL USES Carrier

CHARACTERISTICS

IDENTIFICATION

Melting range (Vol.4)	Decomposes above 300°
Solubility (Vol. 4)	Freely soluble in water
Anthrone reaction	Add 5 ml of Anthrone TS (Vol. 4) to 2 ml of a 0.1% aqueous solution of the test sample. React at 80º for 15 min. A deep blue colour develops.
Specific rotation (Vol.4)	$[\alpha]_D^{20}$ between +240º and +248º (10% solution)

PURITY

Water (Vol. 4)	Not more than 15.0% (Karl Fischer Method)
Total ash (Vol. 4)	Not more than 0.1% (500º, 5h)
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY Determine by HPLC (Vol. 4) using the following conditions:
NOTE: Use deionized water

Sample solution
Weigh accurately about 500 mg of test sample into a 50-ml volumetric flask and add about 40 ml of water. Dissolve the sample completely and dilute to the mark 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 of about 10 mg/ 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: 80º

Mobile phase: water
Flow rate: Adjust to obtain a retention time of 55 – 65 min
Injection volume: 20 µl
The retention time of cyclotetraglucose is approx. 62 min.

System suitability

Upon chromatography of a solution containing about 0.4% cyclotetraglucose and 0.4% glucose, the resolution (Vol. 4) is not less than 1.0 between glucose (first peak) and cyclotetraglucose (second peak).

Procedure

Inject the sample solution into the chromatograph, and measure the area of the cyclotetraglucose peak. Repeat for the standard solution. 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.

W_S and W_R are the weights (mg) of the test sample and cyclotetraglucose standard, respectively, corrected for water content.

CYCLOTETRAGLUCOSE SYRUP
(TENTATIVE)

New specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). A temporary ADI "not specified" was established at the 68th JECFA (2007).

Information is required on (1) the level of total saccharides and the test method and (2) the unidentified saccharides. The specifications will be withdrawn if the requested information is not made available by the end of 2008.

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 *Sporosarcina globispora*, and cyclodextrin glucosyltransferase derived from *Bacillus stearothermophilus*. The final product is a syrup or a spray-dried solid.

Assay Not less than --- % of total saccharides (information required) and 30 – 40% of cyclotetraglucose on the anhydrous basis

DESCRIPTION Slightly sweet tasting, colourless and odourless, clear viscous liquid or dry white crystalline mass.

FUNCTIONAL USES Carrier

CHARACTERISTICS

IDENTIFICATION

Chromatography The retention time for the major peak in a HPLC chromatogram of the sample corresponds to that for cyclotetraglucose in a chromatogram of cyclotetraglucose standard using the conditions described in the Method of Assay. The retention time of cyclotetraglucose is approx. 62 min.

PURITY

Water (Vol. 4) Not more than 30% for the syrup and not more than 10% for the syrup solids (Karl Fischer Method).

Total ash (Vol. 4) Not more than 0.05% on the anhydrous basis (500º, 5h)

Lead (Vol. 4) Not more than 1 mg/kg
Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of

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

Microbiological criteria (Vol.4)

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

Determine by HPLC (Vol. 4) using the following conditions:
NOTE: Use deionized water.

Sample solution
Weigh accurately about 1000 mg of test sample into a 50-ml volumetric flask and add about 40 ml of water. Dissolve the sample completely and dilute to the mark 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: 80º

Mobile phase: water
Flow rate: Adjust to obtain a retention time of 55–65 min
Injection volume: 20 µl
The retention time of cyclotetraglucose is approx. 62 min.

System suitability
Upon chromatography of a solution containing about 0.4% cyclotetraglucose and 0.4% glucose, the resolution (Vol. 4) is not less than 1.0 between glucose (first peak) and cyclotetraglucose (second peak).

Procedure
Inject the sample solution into the chromatograph, and measure the area of the cyclotetraglucose peak. Repeat for the standard solution. Calculate the percentage of cyclotetraglucose in the test sample as follows:

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

where:
A_S and A_R are the areas of the peaks due to cyclotetraglucose for the sample solution and standard solution, respectively.
W_S and W_R are the weights (mg) of the test sample and standard cyclotetraglucose, respectively, corrected for water content.

ETHYL MALTOL

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 65th JECFA (2005) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-2 mg/kg bw was established at the 18th JECFA (1974).

SYNONYMS INS No. 637

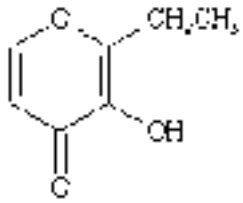
DEFINITION Ethyl maltol is obtained by chemical synthesis

Chemical names 2-Ethyl-3-hydroxy-4-pyrone

C.A.S. number 4940-11-8

Chemical formula $C_7H_8O_3$

Structural formula



Formula weight 140.14

Assay Not less than 99.0%, calculated on the anhydrous basis

DESCRIPTION White, crystalline powder with a cotton-candy odour

FUNCTIONAL USES Flavour enhancer, flavouring agent (see 'Flavouring agents' monograph No. 1481)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4) Sparingly soluble in water; soluble in ethanol and in propylene glycol

Melting range (Vol. 4) 89 - 93º

Ultraviolet absorption (Vol. 4) The ultraviolet spectrum of a 10 mg/l solution of the sample in 0.1 N hydrochloric acid shows an absorption maximum at about 276 nm

PURITY

Water (Vol. 4) Not more than 0.5% (Karl Fischer method)

Sulfated ash (Vol. 4) Not more than 0.2% (use 5 g sample)

Lead (Vol. 4) Not more than 1 mg/kg

Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 under “General Methods, Metallic Impurities”.

METHOD OF ASSAY

Standard solution

Transfer about 50 mg of Ethyl Maltol Reference Standard (available from the United States Pharmacopoeia, 12601 Twinbrook Parkway, Rockville, MD 20852, USA), or equivalent, accurately weighed, into a 250-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix. Pipet 5 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Sample solution

Transfer about 50 mg of the sample, accurately weighed, into a 250-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix. Pipet 5 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Procedure

Determine the absorbance of each solution in a 1-cm quartz cell at the absorption maximum (about 276 nm) using 0.1 N hydrochloric acid as the blank.

Calculate the percent of Ethyl maltol in the sample by the formula:

$$\% \text{ of Ethyl maltol} = 100 \times W_S \times A_A / (A_S \times W_A)$$

where

A_A is the absorbance of the sample solution
A_S is the absorbance of the standard solution
W_A is the weight in mg of the sample in the sample solution
W_S is the weight in mg of the reference standard in the standard solution

ISOAMYLASE FROM *PSEUDOMONAS AMYLODERAMOSA*

New specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI "not specified" was established at the 68th JECFA (2007).

SYNONYMS

Debranching enzyme; α-1,6-glucan hydrolase

SOURCES

Isoamylase is produced by submerged fed-batch pure culture fermentation of *Pseudomonas amyloderamosa*. The enzyme is isolated from the fermentation broth by filtration to remove the biomass and concentrated by ultrafiltration. The final product is formulated using food-grade stabilizing and preserving agents.

Active principles: Isoamylase

Systematic names and numbers: Glycogen α-1,6-glucanohydrolase; EC 3.2.1.68; CAS No. 9067-73-6

Reactions catalysed: Hydrolysis of α-1,6-D-glucosidic linkages in glycogen, amylopectin and their β-limit dextrins.

Secondary enzyme activities: Low levels of cellulase, lipase, and protease.

DESCRIPTION

Yellow to brownish liquid

FUNCTIONAL USES

Enzyme preparation.
Used in the production of food ingredients from starch.

GENERAL SPECIFICATIONS

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

CHARACTERISTICS

IDENTIFICATION

Isoamylase activity: The sample shows isoamylase activity.
See description under TESTS.

TESTS

Isoamylase activity

Principle

Isoamylase activity is determined by incubating the enzyme with soluble waxy corn starch as a substrate in the presence of iodine for 30 min under standard conditions (pH=3.5; 40.0±0.1º) and measuring absorbance of the reaction mixture at 610 nm. The change in absorbance represents the degree of hydrolysis of the substrate. Isoamylase activity is calculated in isoamylase activity units (IAU) per gram of the enzyme preparation. One IAU is defined as the amount of isoamylase that increases absorbance of the reaction mixture by 0.008 in 30 min under the conditions of the assay.

Apparatus
Spectrophotometer (UV/VIS).
Thermostated water bath with agitator
Test tubes (18x180 mm)
Vortex mixer
Digital timer or stopwatch.

Reagents and solutions
Substrate solution: Accurately weigh 4.17 g (dried basis) of soluble waxy corn starch (Hayashibara Biochemical Laboratories, Inc., Cat. No. CS 101, or equivalent) in a 50-ml beaker. Add approximately 30 ml of water and stir to produce a suspension. Heat to boiling approximately 300 ml of water in a 500-ml beaker with stirring. Slowly add the starch slurry to the boiling water. Rinse the empty beaker with a small amount of water and add the rinse to the boiling water. Boil the starch suspension for 5 min. Quantitatively transfer the starch solution to a 500-ml volumetric flask and cool to room temperature under running water. Stir the solution continuously during cooling. Add 50 ml of 1 M acetate buffer solution (pH 3.5) and dilute with water to volume. The solution should be freshly prepared.

Acetate buffer solution (1 M; pH 3.5): Mix 1 M acetic acid and 1 M sodium acetate to obtain pH 3.5. The solution is stable for up to 3 months at room temperature.

Acetate buffer stock solution (1M; pH 4.5): Mix 1 M acetic acid and 1 M sodium acetate to obtain pH 4.5. The solution is stable for up to 3 months at room temperature.

Acetate buffer working solution (0.01M; pH 4.5): Transfer 1 ml of the acetate buffer stock solution (1M, pH 4.5) to a 100-ml volumetric flask and add water to volume. The solution should be freshly prepared.

Iodine solution (0.01 N): Transfer 10 ml of 0.1 N iodine solution to a 100-ml volumetric flask and add water to volume. The solution should be freshly prepared.

Sample solution: Accurately weigh (to four decimal places) approximately 3g of the sample into a 1000-ml volumetric flask and add the acetate buffer working solution (0.01 M, pH 4.5) to volume. Repeat the dilution with the acetate buffer working solution as necessary to obtain the activity of approximately 25-50 IAU/ml. The solution should be freshly prepared.

Procedure
Blank solution: Place 3.0 ml of freshly prepared substrate solution in a test tube and incubate in a water bath at 40.0±0.1º for 10 min. Add 0.5 ml of the sample solution and mix rapidly. After 30 sec, transfer 0.5 ml of the reaction mixture to a test tube containing 15 ml of 0.02 N sulfuric acid and mix rapidly.

Test solution: Incubate the remaining reaction mixture in a water bath at 40.0±0.1º for 30 min and 30 sec. Transfer 0.5 ml of the reaction mixture to a test tube containing 15 ml of 0.02 N sulfuric

acid and mix rapidly.

Let the blank and test solutions stand at 25±1° for at least 15 min. Then add 0.5 ml of 0.01 N iodine solution to both solutions and let the solutions stand at 25±1° for another 15 min. Read the absorbance of each solution at 610 nm against water in a 10-mm cell.

Calculations

Calculate the activity of the sample in IAU/g according to the following equation:

$$\text{Activity(IAU/g)} = \frac{(E_{30} - E_0) \times V \times D \times F}{0.004 \times W}$$

where:

E_0 is the absorbance of the blank solution,
E_{30} is the absorbance of the test solution,
V is the volume of the volumetric flask in which the sample was initially dissolved (ml),
D is the dilution of the sample solution,
W is the sample weight (g),
0.004 is obtained by multiplying the absorbance corresponding to one IAU (0.008) by the volume of the sample solution used in the experiment (0.5 ml), and
F is the correction factor that accounts for discrepancies between various batches of the substrate. This factor is determined for each new batch of the substrate by measuring the isoamylase activity of the same sample of the enzyme preparation using the old and new batch of the substrate. F is calculated by dividing the result obtained with the old batch by that obtained with the new batch of the substrate.

MAGNESIUM SULFATE

Prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 4 (2007), superseding the specifications prepared at the 63rd JECFA (2004) and published the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI "not specified" was established at the 68th JECFA (2007).

SYNONYMS Epsom salt (heptahydrate); INS No.518

DEFINITION Magnesium sulfate occurs naturally in sea water, mineral springs and in minerals such as kieserite and epsomite. It is recovered from them or by reacting sulfuric acid and magnesium oxide. It is produced with one or seven molecules of water of hydration or in a dried form containing the equivalent of between 2 and 3 waters of hydration.

Chemical names: Magnesium sulfate

C.A.S. number: Monohydrate: 14168-73-1
Heptahydrate: 10034-99-8
Dried: 15244-36-7

Chemical formula: Monohydrate: $MgSO_4.H_2O$
Heptahydrate: $MgSO_4.7H_2O$
Dried: $MgSO_4.xH_2O$, where x is the average hydration value (between 2 and 3)

Formula weight: Monohydrate: 138.38
Heptahydrate: 246.47

Assay: Not less than 99.0 % and not more than 100.5% on the ignited basis

DESCRIPTION Colourless crystals, granular crystalline powder or white powder. Crystals effloresce in warm, dry air.

FUNCTIONAL USES Nutrient; flavour enhancer; firming agent; and processing aid (fermentation aid in the production of beer and malt beverages)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Freely soluble in water, very soluble in boiling water, and sparingly soluble in ethanol.

Test for magnesium (Vol. 4): Passes test

Test for sulfate (Vol. 4): Passes test

PURITY

Loss on ignition (Vol. 4)	Monohydrate: between 13.0 and 16.0 %, Heptahydrate: between 40.0 and 52.0 %, Dried: between 22.0 and 32.0 % (105º, 2h, then 400º to constant weight)
pH (Vol. 4)	Between 5.5 and 7.5 (1 in 20 solution)
Chloride (Vol. 4)	Not more than 0.03% Test 1g of the sample as described under “Chloride Limit Test” using 0.9 ml of 0.01 N hydrochloric acid in the control
Arsenic (Vol. 4)	Not more than 3 mg/kg Determine by the atomic absorption hydride technique. Use Method I for sample preparation.
Iron (Vol. 4)	Not more than 20 mg/kg Use 1 ml of Iron Standard TS
Selenium (Vol. 4)	Not more than 30 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”).
Lead (Vol. 4)	Not more than 2mg/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 0.5 g of the ignited sample, dissolve in 5 ml of hydrochloric acid TS, Dilute, dilute with water to 100 ml, and mix. Transfer 50 ml of this solution into a 250-ml conical flask; add 10 ml of Ammonia/Ammonium Chloride Buffer TS and 0.1 ml of Eriochrome Black TS. Titrate with 0.05 M disodium EDTA until the colour of red-purple solution changes to blue. Each ml of 0.05 M disodium EDTA is equivalent to 12.04 mg of $MgSO_4$.

MALTOL

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 65th JECFA (2005) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-1 mg/kg bw was established at the 25th JECFA (1981).

SYNONYMS INS No. 636

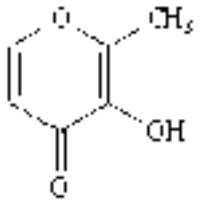
DEFINITION Maltol is obtained by chemical synthesis

Chemical names 3-Hydroxy-2-methyl-4-pyrone

C.A.S. number 118-71-8

Chemical formula $C_6H_6O_3$

Structural formula



Formula weight 126.11

Assay Not less than 99.0%, calculated on the anhydrous basis

DESCRIPTION White crystalline powder having a characteristic caramel-butterscotch odour

FUNCTIONAL USES Flavour enhancer, flavouring agent (see 'Flavouring agents' monograph No. 1480)

CHARACTERISTICS

IDENTIFICATION

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

Melting range (Vol. 4) 160 - 164º

Ultraviolet absorption (Vol. 4) The ultraviolet spectrum of a 10 mg/l solution of the sample in 0.1 N hydrochloric acid shows an absorption maximum at about 274 nm

PURITY

Water (Vol. 4) Not more than 0.5% (Karl Fischer)

Sulfated ash (Vol. 4)

Not more than 0.2% (use 5 g sample)

Lead (Vol. 4)

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

METHOD OF ASSAY

Standard solution
Transfer about 50 mg of Maltol Reference Standard (available from the United States Pharmacopoeia, 12601 Twinbrook Parkway, Rockville, Md. 20852, USA), or equivalent, accurately weighed, into a 250-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix. Pipet 5 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Assay solution
Transfer about 50 mg of the sample, accurately weighed, into a 250-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix. Pipet 5 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 N hydrochloric acid, and mix.

Procedure
Determine the absorbance of each solution in a 1-cm quartz cell at the absorption maximum (about 274 nm) using 0.1 N hydrochloric acid as the blank.

Calculate the percent of Maltol in the sample by the formula:

$$\% \text{ of Maltol} = 100 \times W_S \times A_A / (A_S \times W_A)$$

where

A_A is the absorbance of the sample solution
A_S is the absorbance of the standard solution
W_A is the weight in mg of sample in the sample solution
W_S is the weight in mg of the reference standard in the standard solution

NISIN PREPARATION

Prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 4 (2007), superseding specifications for nisin prepared at the 12th JECFA (1968) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). Metals and arsenic specifications revised at the 63rd JECFA (2004). An ADI of 0-33,000 units/kg bw was established at the 12th JECFA (1968).

SYNONYMS INS No. 234

DEFINITION Nisin is a mixture of closely related antimicrobial polypeptides produced by strains of *Lactococcus lactis* subsp. *lactis*. The structure of a major component of nisin is shown below. Nisin may be 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, compressed air (froth concentration); membrane filtration; acidification; salting out; and spray-drying.

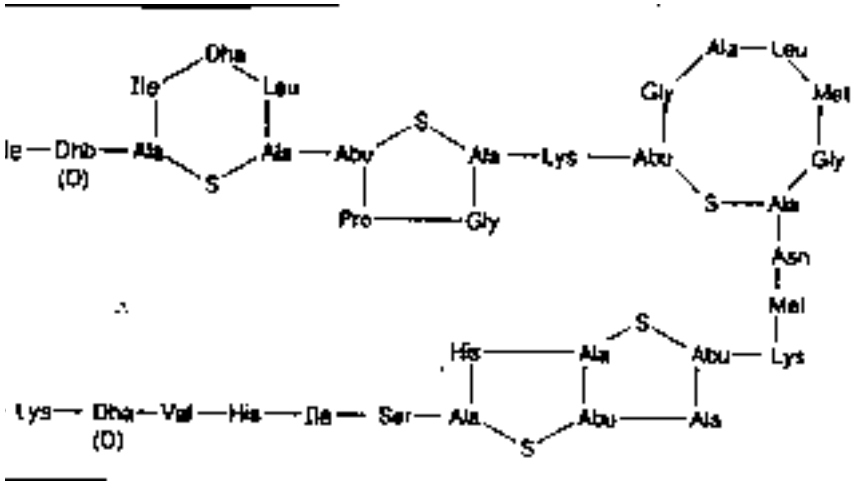
Nisin preparation consists of nisin and sodium chloride with an activity of not less than 900 units per mg. The activity is adjusted by addition of sodium chloride. Non-fat milk solids or solids from other fermentation sources are present in the preparation. Nisin preparation is stable at ambient temperatures and upon heating under acid conditions (maximum stability at pH 3).

(NOTE: The International Unit for nisin activity is the amount of nisin required to inhibit one cell of *Streptococcus agalactiae* in 1 ml of broth. A standard preparation has been defined by Tramer and Fowler, *J.Sci.Fd.Agric.*, 15, 522 (1964) as 10^6 IU of nisin per gram of preparation.)

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

Chemical formula $C_{143}H_{230}N_{42}O_{37}S_7$

Structural formula Abu=alpha-aminobutyric acid, Dha=dehydroalanine, Dhb=dehydrobutyrine



Formula weight	Ca. 3354
Assay	Not less than 900 IU of nisin per milligram and not less than 50% sodium chloride

DESCRIPTION White to light brown micronized powder

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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)
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

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.

Using the method of assay described below, determine the nisin activity. 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 5N sodium hydroxide. Heat the solution at 65º for 30 min, and then cool. Adjust the pH to 2.0 by adding hydrochloric acid dropwise. Again determine the nisin concentration using the assay method described below. 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 *Lactococcus lactis* (ATCC 11454, NCIMB 8586) in sterile skim (<1% fat) milk by incubating for 18 h at 30º. Prepare one or more flasks containing 100 ml of litmus milk, and sterilize at 121º 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 30º 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. This test will not differentiate nisin from subtilin.

METHOD OF ASSAY

*Determination of nisin activity (*Based on the method of Friedman and Epstein, *J. Gen. Microbiol*. 5: 830, 1951)

Preparation of the test organism

*Lactococcus lactis sbsp .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 30º. 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 30º for 90 min. Use immediately.

Standard stock solution

Dissolve an accurately weighed quantity of standard nisin in 0.02N hydrochloric acid to give a solution containing 5 000 units/ml. Immediately before use, dilute the solution further with 0.02N hydrochloric acid to give 50 units/ml. (NOTE: Nisin preparation containing 2.5 % nisin, minimum potency of 10^6 IU/g, obtainable from Sigma, St Louis, USA or Fluka, Buchs, Switzerland, may be used for the Standard Stock Preparation, as well as, the preparation under the name of Nisaplin, available from Danisco, Copenhagen, Dennmark).

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 estimated concentration of 50 units of nisin per ml.

Resazurin solution
Prepare a 0.0125% 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. 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 30º for 15 min, then cool in an ice water bath while adding 1 ml resazurin solution to each. The addition is made with an automatic pipetting device, in the same order used for the addition of inoculated milk. Thoroughly mix the contents of the tubes by shaking. Continue incubation at 30º in a water-bath for a further 3-5 min.

Examine the tubes under fluorescent light in a black matt-finish cabinet. The sample tube of the highest concentration which shows the first clear difference in colour (i.e. has changed from blue to mauve) is compared with tubes of the standard row to find the nearest 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 preparation.

Determination of sodium chloride

Accurately weigh about 100 mg of sample, and transfer to a porcelain casserole. Add 100 ml water, 2 ml 2% dextrin soln, and 1 ml 0.1% dichlorofluoroscein soln. Mix, and titrate with 0.1 N silver nitrate soln until the silver chloride flocculates and the mixture acquires a faint pink colour.

$$\text{Sodium chloride, \% (w/w)} = \frac{V \times N \times 100 \times 58.5}{W}$$

where:

V is the volume of silver nitrate solution consumed (ml),
N is the normality of the silver nitrate solution,
58.5 is the formula weight of sodium chloride, and
W is the weight of the sample (mg).

PECTINS

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 57th JECFA (2001) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). 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 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 30º. 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% (105º, 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:

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

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

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

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

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

PURITY TESTS

Sulfur dioxide

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

Total insolubles

Dry a 70 mm glass fiber filter paper (GF/B (Whatman code 1821 070) in an oven with fan set at 105º 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 50º)

water that has been filtered through GF/B paper.
Dry the filter paper with the residue at 105º 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

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 105º, 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 25 ml 0.125 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 20.00 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 20 ml distilled water. Record the required volume, in ml, as A_0. The acetate ester titre is (A - A_0). Calculate degree of amidation (as % of total carboxyl groups) by the formula:

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

Calculate mg of galacturonic acid by the formula:

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

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

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

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

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

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

Residual solvents (Vol. 4) 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:

$$\%\ \text{of solvent} = \frac{R_{sample} \times W_{standard} \times M_{standard}}{R_{standard} \times W_{sample} \times M_{sample} \times 1000} \times 100$$

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
$M_{standard}$	is the weight of Calibration solution used for the GC analysis

POLYVINYL ALCOHOL

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 63rd JECFA (2004) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 50 mg/kg bw was established at 61st JECFA (2003).

SYNONYMS Vinyl alcohol polymer, PVOH, INS No. 1203

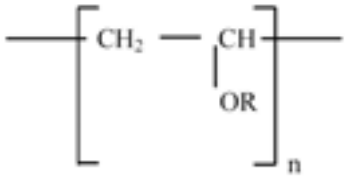
DEFINITION Polyvinyl alcohol is a synthetic resin prepared by the polymerization of vinyl acetate, followed by partial hydrolysis of the ester in the presence of an alkaline catalyst. The physical characteristics of the product depend on the degree of polymerization and the degree of hydrolysis.

Chemical names: Ethenol homopolymer

C.A.S. number: 9002-89-5

Chemical formula: $(C_2H_3OR)_n$ where R=H or $COCH_3$ (randomly distributed)

Structural formula



Where R=H or $COCH_3$ (randomly distributed)

DESCRIPTION Odourless, translucent, white or cream-coloured granular powder.

FUNCTIONAL USES Coating, binder, sealing agent and surface-finishing agent.

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Soluble in water, sparingly soluble in ethanol.

pH (Vol. 4): 5.0 – 6.5 (1 in 25)

Infrared spectrum (Vol. 4): The infrared absorption spectrum of a potassium bromide dispersion of the sample corresponds to that of a polyvinyl alcohol standard (see Appendix).

Colour reaction A: Dissolve 0.01 g of the sample in 100 ml of water with warming and let the solution cool to room temperature. To 5 ml of the solution, add one drop of iodine TS and a few drops of boric acid solution (1 in 25). A blue colour is produced.

Colour reaction B	Dissolve 0.5 g of the sample in 10 ml of water with warming and let the solution cool to room temperature. Add 1 drop of iodine TS to 5 ml of solution and allow to stand. A dark red to blue colour is produced.
Precipitation reaction	Add 10 ml of ethanol to the remaining 5 ml of solution prepared for Colour reaction B. A white, turbid or flocculent precipitate is formed.

PURITY

Loss on drying (Vol. 4)	Not more than 5.0% (105º, 3 h)
Residue on ignition (Vol. 4)	Not more than 1.0%
Water insoluble substances (Vol. 4)	Not more than 0.1% Substitute a 100-mesh screen for the sintered-glass filter specified in Volume 4
Particle size	Not less than 99.0% material to pass through a 100 mesh sieve Determine by sieving for 30 min 100g of sample through a 100 mesh sieve and weigh the material passing through the sieve.
Methanol and methyl acetate	Not more than 1.0 % of each See description under TESTS
Acid value	Not more than 3.0 See description under TESTS
Ester value	Between 125 and 153 mg KOH/g See description under TESTS
Degree of hydrolysis	Between 86.5 and 89.0% See description under TESTS
Viscosity	4.8 - 5.8 mPa•s (4% solution at 20°) See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Vol. 4 (under “General Methods, Metallic Impurities).

TESTS

PURITY TESTS

Methanol and methyl acetate	Place 2.0 g of the sample into a 100 ml screw-cap bottle, and add a magnetic stirrer. Add 98 ml of water and 30 µl of acetone. Close the bottle tightly with the screw cap and heat in a water-bath, stirring continuously. Once the solution becomes clear, remove the bottle from the water bath and allow it to cool to room temperature.

Prepare a standard by taking 2 ml of a mixed solution of methanol and methyl acetate (1.2 % v/v solution), 98 ml of water and 30µl acetone; proceed as above starting from “close the bottle…Temperature”.

GC Conditions:

Column:	Sunpak A (3.2 mm i.d. x 3 m) or equivalent
Column temperature:	160º
Injector temperature:	160º
Detector temperature:	160º

Inject 0.4 ± 0.1µl of the standard solution into the gas chromatograph and record the peak areas (PAs) for methanol, methyl acetate and acetone. Inject 0.4 ± 0.1 µl of the sample solution and record the peak areas (PAs) for methanol, methyl acetate, and acetone.
Calculate the methanol and methyl acetate content using the formulae:

$$\text{Methanol (wt\%)} = PA(\text{methanol})/PA(\text{acetone}) \times PR_1 \times 0.024 \times 100/2$$

$$\text{Methyl acetate (wt \%)} = PA(\text{methyl acetate})/PA(\text{acetone}) \times PR_2 \times 0.024 \times 100/2$$

where

0.024 is the conversion factor to obtain the masses of methanol and methyl acetate added to 30 µl acetone (density = 0.8) for the methanol/methyl acetate standard; and

PR_1 and PR_2 are the peak area ratios PA(acetone)/PA(methanol) and PA(acetone)/PA(methyl acetate), respectively, of the standard 1.2% methanol and methyl acetate aqueous solutions.

Acid value

Add 200 ml of water and a stir bar into a 500-ml round-bottom flask, attach a reflux condenser and begin heating in a boiling water bath. Add 10.0 g of the sample and continue heating for 30 min while stirring continuously. Remove the flask from the water bath and continue stirring until the solution reaches room temperature. Quantitatively transfer this solution to a 250-ml volumetric flask and dilute to volume with water. Take 50 ml of the solution, add 1 ml of phenolphthalein TS and titrate with 0.05 M potassium hydroxide until the pink colour persists for 15 sec; record the titre in ml (V). Calculate the acid value, A:

$$A = 5.0(56.1 \times V \times M)/W$$

where

56.1 is the formula weight of KOH,
M is the molarity of the KOH solution, and
W is the weight of sample (g).

Ester value

Accurately weigh about 1.0 g of sample into a 250-ml round-bottom flask, add 25 ml 0.5 M alcoholic potassium hydroxide, 25.0 ml of water and a few glass beads. Attach a condenser and allow the contents to reflux for 30 minutes in a boiling water-bath. Let cool to room temperature, remove the condenser, add 1 ml of phenolphthalein TS and titrate immediately with 0.5 M hydrochloric acid; record the titre in ml (V_1).

Carry out a blank test under the same conditions. Titrate with 0.5 M hydrochloric acid and record the titre in ml (V_2). Calculate the saponification value, S:

$$S = 56.1(V_2 - V_1) \times M/W$$

where

56.1 is the formula weight of KOH,
M is the molarity of the hydrochloric acid solution, and
W is the weight of the sample in (g).

Calculate the ester value, E:

$$E = S - A$$

where

S is the saponification value and
A is acid value.

Degree of hydrolysis

Convert the saponification value obtained during the determination of the ester value to the "dried basis" (S_{db}):

$$S_{db} = (S \times 100)/(100 - LOD)$$

where

LOD is Loss on Drying

The degree of hydrolysis is:

$$100 - [7.84\ S_{db}/(100 - 0.075\ S_{db})]$$

Viscosity

Calibration of *capillary-type* viscometers

An oil of known viscosity is used to determine the viscometer constant (k*)*.

Ostwald-Type Viscometer: Fill the tube with the exact amount of oil (adjusted to 20.0±0.1°), as specified by the manufacturer. Use either pressure or suction to adjust the meniscus of the column of liquid in the capillary tube to the level of the top graduation line. Allow the liquid to flow into the reservoir against atmospheric pressure by opening both the filling and capillary tubes. If either tube is not open, false values might be obtained. Record the time (seconds), for the liquid to flow from the upper mark to the lower mark of the capillary tube (efflux time).

Ubbelohde-Type Viscometer: Place a quantity of the oil (adjusted to 20.0 ± 0.1°) in the filling tube, and transfer to the capillary tube by gentle suction. Keep the air vent tube closed in order to prevent bubble formation in the liquid. Adjust the meniscus of the

column of liquid in the capillary tube to the level of the top graduation line. Allow the liquid to flow into the reservoir against atmospheric pressure by opening both the filling and capillary tubes. If either tube is not open, false values might be obtained. Record the efflux time (seconds).

The viscosity constant for *capillary-type* viscometers is given by:

$$k = v/dt,$$

where v is the known viscosity (mPa•s) of the oil used for viscometer calibration; d is the density (g/ml) of the liquid tested at 20º/20º; and t (seconds) is the efflux time.

Procedure

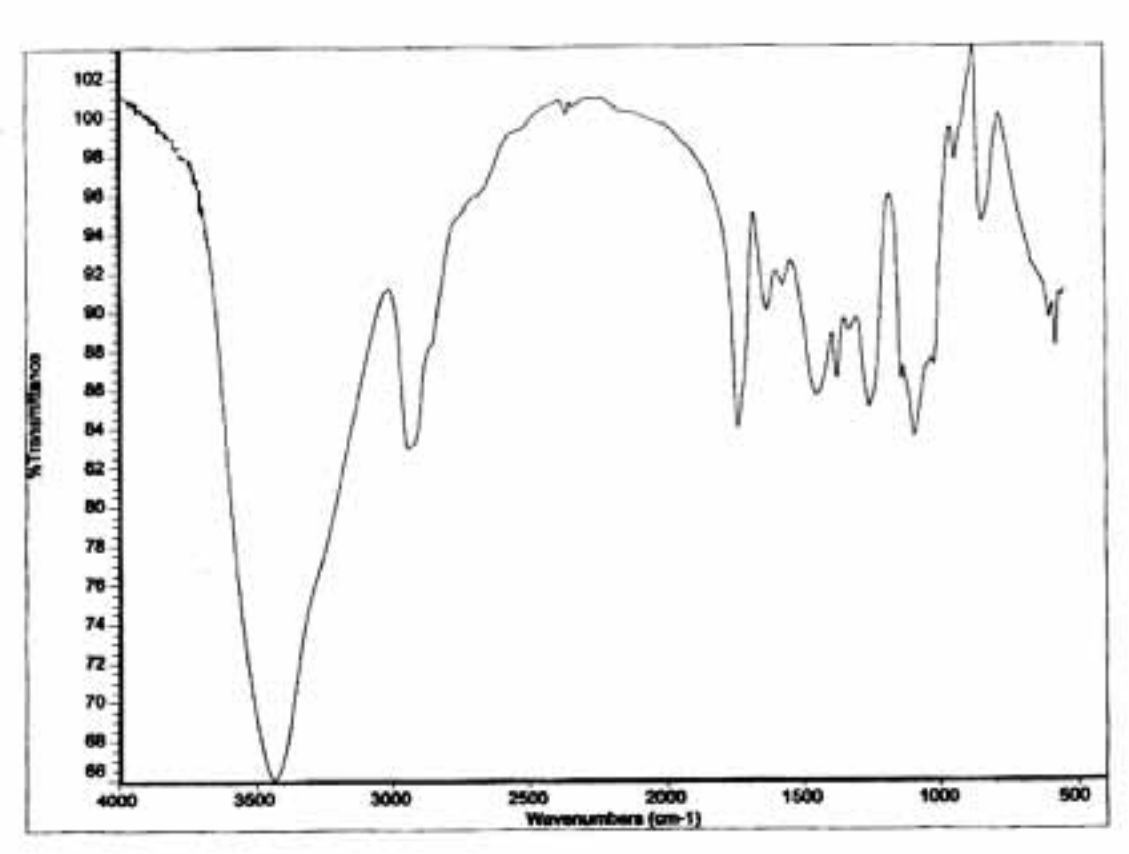
Weigh a quantity of undried sample equivalent to 6.00 g on the dried basis. Into a tared 250-ml flask containing a magnetic stir bar and approximately 140 ml of water, quickly (seconds) transfer the sample, while simultaneously stirring slowly and continuously. Once the sample appears thoroughly saturated, slowly increase the stirring rate to minimize the entrainment of air in the mixture. Heat the mixture to 90º, and maintain it at this temperature for approximately 5 minutes; discontinue heating and continue stirring for 1 hour. Add water in small amounts to attain a total mixture weight of 150 g, and resume stirring until the mixture appears homogenous. Filter the mixture through a tared 100-mesh screen into a 250 ml conical flask, cool the filtrate to about 15º, mix, and determine its viscosity at 20º using an appropriate viscometer (follow the manufacturer's instructions). NOTE: The temperature at which the viscosity measurement is made must be strictly controlled.

For measurements using *capillary-type* viscometers, the viscosity is given by:

$$v = kdt$$

where t is the efflux time for the sample solution and d is its density at 20º.

Appendix



POLYVINYL ALCOHOL

PROCESSED *EUCHEUMA* SEAWEED

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding specifications prepared at the 57th JECFA (2001) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A group ADI "not specified" for carrageenan and processed Eucheuma *seaweed was established at the 57th JECFA (2001).*

SYNONYMS PES, PNG-carrageenan, semi-refined carrageenan; INS No. 407a

DEFINITION A substance with hydrocolloid properties obtained from either *Eucheuma cottonii* or *E. spinosum* (from the *Rhodophyceae* class of red seaweeds). In addition to carrageenan polysaccharides, processed eucheuma seaweed may contain up to 15% of insoluble algal cellulose and minor amounts of other insoluble matter. Articles of commerce may include sugars for standardization purposes or salts to obtain specific gelling or thickening characteristics. It is distinguished from carrageenan (INS No. 407) by its higher content of cellulosic matter and by the fact that it is not solubilized and precipitated during processing.

The functional component of the product obtained from *E. cottonii* is kappa-carrageenan (a copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose). From *E. spinosum* it is iota-carrageenan (a copolymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose-2-sulfate).

Processing consists of soaking the cleaned seaweed in alkali for a short time at elevated temperatures. The material is then thoroughly washed with water to remove residual salts followed by purification, drying, and milling to a powder. Alcohols that may be used during purification are restricted to methanol, ethanol, and isopropanol.

DESCRIPTION Light tan to white coarse to fine powder

FUNCTIONAL USES Thickener, gelling agent, stabilizer, emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Forms cloudy viscous suspensions in water; insoluble in ethanol
A 1 g sample disperses and partially dissolves in 100 ml of water at 80^{o} giving a cloudy opalescent solution. (The sample disperses in water more readily if first moistened with alcohol, glycerol, or a saturated solution of glucose or sucrose in water).

Test for sulfate Dissolve a 100-mg sample in 20 ml of water. Heat to boiling, cool to room temperature, and add 3 ml of barium chloride TS and 5 ml of hydrochloric acid, dilute TS. Filter the mixture. Boil the filtrate for 5 min. A white, crystalline precipitate appears.

Test for galactose and anhydrogalactose (Vol.4)	Proceed as directed in Volume 4 (under "General Methods, Organic Components, Gum Constituents Identification") using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present.
Identification of hydrocolloid and predominant type of copolymer	Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80°, with constant stirring until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. The solution becomes viscous and may form a gel. To 50 ml of the solution or gel, add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ("brittle") gel indicates a carrageenan of a predominantly kappa-type. A compliant ("elastic") gel indicates a predominantly iota-type.
Infrared absorption	Passes test See description under TESTS
PURITY	
Loss on drying (Vol. 4)	Not more than 12% (105° to constant weight)
pH (Vol. 4)	Between 8 and 11 (1 in 100 suspension)
Viscosity	Not less than 5 cp at 75° (1.5% solution) See description under TESTS
Sulfate	Not less than 15% and not more than 40% (as SO_4^{2-}) on the dried basis See description under TESTS
Total ash	Not less than 15% and not more than 30% on the dried basis See description under TESTS
Acid-insoluble ash (Vol. 4)	Not more than 1% Use the ash from the Total ash test
Acid-insoluble matter (Vol. 4)	Not less than 8% and not more than 15% on the dried basis Use 2 g of sample obtained from part (a) of the procedure for sulfate determination
Residual solvents (Vol. 4)	Not more than 0.1% of ethanol, isopropanol, or methanol, singly or in combination See description under TESTS
Microbiological criteria (Vol. 4)	Initially prepare a 10^{-1} dilution by adding a 50 g sample to 450 ml of Butterfield's phosphate-buffered dilution water and homogenizing the mixture in a high speed blender. Total (aerobic) plate count: Not more than 5000 cfu/g *Salmonella* spp.: Negative per test *E. coli*: Negative in 1 g

Arsenic (Vol. 4) — Not more than 3 mg/kg
Determine by the atomic absorption hydride technique. Use Method II for sample preparation.

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").

Cadmium (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").

Mercury (Vol.4) — Not more than 1 mg/kg
Determine by the cold vapour atomic absorption technique

TESTS

IDENTIFICATION TESTS

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

Iota- and kappa-carrageenan have strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. Other characteristic absorption bands and their intensities relative to the absorbance at 1050 cm^{-1} are as follows:

Wave number (cm^{-1})	*Molecular Assignment*	*Absorbance Relative to 1050 cm^{-1}*	
		Kappa	Iota
1220-1260	ester sulfate	0.6-1.0	0.9-1.2
928-933	3,6-anhydrogalactose	0.3-0.6	0.2-0.6
840-850	galactose-4-sulfate	0.3-0.5	0.2-0.4
800-805	3,6-anhydrogalactose-2-sulfate	0.0-0.2	0.2-0.4

PURITY TESTS

Sulfate

Principle:
Hydrolysed sulfate groups are precipitated as barium sulfate.

Procedure:
(a) Disperse an accurately weighed 15 g sample of commercial

product into 500 ml of 60% w/w isopropanol/water at room temperature. Stir gently for4 h. Filter through ash-free filter paper. Discard the filtrate. Wash the material remaining on the filter paper with two 15-ml portions of 60% isopropanol/water. Dry the material at 105° to constant weight.
Approximately 1 g of the dried matter is to be used for part (b). The remainder should be retained for determination of Total ash, Acid-insoluble matter, and viscosity.

(b) Accurately weigh a 1 g sample (W_1) obtained from part (a), Transfer the sample to a 100-ml long-neck round-bottom flask and add 50 ml of 0.2 N hydrochloric acid. Fit a condenser, preferably one with at least 5 condensing bulbs, to the flask and reflux for 1 h. Add 25 ml of a 10% (by volume) hydrogen peroxide solution and resume refluxing for about 5 h or until the solution becomes completely clear. Transfer the solution to a 600-ml beaker, bring to a boil, and add dropwise 10 ml of a 10% barium chloride solution. Heat the reaction mixture for 2 h on a boiling water bath. Filter the mixture through ash-free slow-filtration filter paper. Wash with boiling distilled water until the filtrate is free from chloride. Dry the filter paper and contents in a drying oven. Gently burn and ash the paper at 800° in a tared porcelain or silica crucible until the ash is white. Cool in a desiccator.

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

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

Total ash

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

$(W_2/W_1) \times 100$.

Retain the ash for the Acid-insoluble ash test.

Viscosity

Transfer 7.5 g of the dried sample obtained from part (a) under the procedure for sulfate determination into a tared, 600-ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g and heat in a water bath, with continuous agitation, until a temperature of 80º is reached (20-30 min). Add 7.5 g of diatomaceous earth or perlite filter aid.

Stir for two minutes. Add water to adjust for loss by evaporation. Filter the solution through a Büchner funnel (pre-heated with hot water to 80º) equipped with a coarse filter paper. Place the filter assembly in a vacuum receiver bottle.

Filter 200 ml of solution. Cool to 76-77º, and heat in a constant temperature bath at 75º. Pre-heat the bob and guard of a Brookfield LVF viscometer to approximately 75º in water. Dry the bob and guard and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0-100 scale.

If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter or equivalent.

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

Residual solvents
(Vol. 4)

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

Prepare standard, blank, and calibration solutions as directed under Method1.

Sample Preparation

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

SODIUM CHLORITE

New specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI of 0.03 mg/kg bw for chlorite was established at the 68th JECFA (2007).

SYNONYMS Chlorous acid sodium salt

DEFINITION Sodium chlorite is manufactured by first reducing, chemically or electrochemically, sodium chlorate, in the presence of hydrochloric acid, to produce chlorine dioxide. The chlorine dioxide is then reduced with hydrogen peroxide in a sodium hydroxide solution to yield a solution containing 30 to 50 percent sodium chlorite, which can be dried to give a solid with ca. 80% sodium chlorite.

Alternatively, chlorine dioxide may be obtained by reacting together sodium chlorate, hydrogen peroxide, and sulfuric acid. The chlorine dioxide is then reduced with hydrogen peroxide in sodium hydroxide solution to yield a solution of sodium chlorite, which can be neutralized with sulfuric acid. The resulting solution may be dried to a solid and the sodium chlorite content may be adjusted to ca. 80% by the addition of sodium chloride, sodium sulfate, or sodium carbonate.

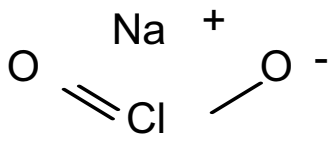
Sodium chlorite is marketed as a solid or an aqueous solution.

C.A.S. number 7758-19-2

Chemical formula $NaClO_2$

Formula weight 90.44

Structural Formula



Assay 79-86%

DESCRIPTION White crystalline powder, solutions can be colourless to greenish yellow

FUNCTIONAL USES Antimicrobial agent (for use in antimicrobial washing solutions)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water, sparingly soluble in polar solvents and insoluble in non-polar solvents.

Chlorite Add 2 g of sample into a 250-ml Erlenmeyer flask and add 50 g of deionized water to dissolve. Add 10 ml of 1.0*N* hydrochloric acid and swirl flask until a yellow colour appears. Add 2 g potassium iodide and swirl to dissolve. The solution turns brown and a grey precipitate

forms.

Sodium (Vol. 4)	Passes test
PURITY	
Loss on drying (Vol.4)	Not more than 6% (105º, 24 h, use 5 g of sample)
Sodium carbonate	Not more than 8% on the dried basis See description under TESTS
Sodium hydroxide	Not more than 3% on the dried basis See description under TESTS
Sodium sulfate	Not more than 5% on the dried basis See description under TESTS
Sodium chlorate	Not more than 4% on the dried basis See description under TESTS
Sodium chloride	Not more than 19% on the dried basis See description under TESTS
Lead (Vol. 4)	Not more than 5 mg/kg on the dried basis 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 Vol. 4 (under "General Methods, Metallic Impurities").

TESTS

PURITY TESTS

Sodium chlorate and Sodium sulfate

Determination by Ion Chromatography

Apparatus

Ion Chromatograph with anion suppressor, autosampler, conductivity detector and a data station (Dionex or equiv.)
Analytical Column: [Dionex AS9-HC, 2 mm or 4 mm or equiv.]
Guard column: [Dionex AG9-HC, 2 mm or 4mm or equiv.]
Mobile phase: 9 mM Na_2CO_3 at flow rate of 0.4 ml/min

Reagents

Deionized water: Ultra high quality (UHQ) passing through 0.20 µm filter, Millli Q or equiv.

Mobile phase [sodium carbonate solution (9mM)]: Dissolve 1.91 g Na_2CO_3 in deionized water and dilute to 2 liters.

Chlorate (ClO_3^-) stock standard solution (1000 mg/l): Transfer 0.1275 g of sodium chlorate, accurately weighed, into a 100-ml volumetric flask. Dissolve the sample in deionized water, make up to volume and mix.

Sulfate (SO_4^{2-}) stock standard solution (1000 mg/l): Transfer 0.1814 g of potassium sulfate, accurately weighed, into a 100-ml volumetric

flask. Dissolve the sample in deionized water, make up to volume, and mix.

Ethylenediamine (EDA) preservation solution (100 mg/ml): Dissolve 2.5 g of EDA in 25 ml of deionized water. Prepare fresh monthly. *Surrogate solution:* Transfer 0.065 g of potassium dichloroacetate into a 100-ml volumetric flask, dissolve in deionized water, make up to volume, and mix. (prepare fresh every 3 months)

Working standard solutions for standard curve: Prepare a series of 5 standards covering the entire calibration range by diluting the stock chlorate and sulfate standards with deionized water in suitable volumetric flasks. Add enough EDA preservation solution to the chlorate working standards to obtain a final concentration of 50 mg/l, in order to normalize any bias from the presence of EDA in analysis samples.

Sample solutions: Accurately weigh about 2.0 g of solid sample, dissolve in deionized water, quantitatively transfer into a 100-ml volumetric flask, make up to volume, and mix. Prepare sample solution fresh prior to analysis. If analysis is delayed, refrigerate solution. Draw solution into a 10-ml plastic syringe, attach a 0.45 µm filter and filter into an auto sampler vial.

Procedure

Construction of standard curve: Set up the ion chromatograph, purge the column with the mobile phase and check the base line stability. Inject 10 µl each of working standards for analysis of sulfate and 50 µl each for analysis of chlorate using the 2 mm column (inject 50 and 200 µl samples, respectively, when using 4 mm column). Construct standard curves for sulfate and chlorate and check curves for linearity ($R^2 > 0.99$). Verify calibration curve acceptability each day prior to analysis by injecting a middle standard as a sample and confirm acceptance (error shall not be more than 5%). If the error is more than 5%, repeat construction of standard curve.

System calibration: Transfer a 10.0 ml aliquot of sample solution and 20 µl of the surrogate solution to a 20 ml disposable plastic beaker and mix. Draw the solution into a 10-ml plastic syringe, attach a 0.45 µm filter and filter the solution into an auto-sampler vial. Inject the solution [10 µl for determination of sulfate and 50 µl for determination of chlorate using a 2 mm column (inject 50 and 200 µL samples, respectively, when using 4 mm column)]. Record retention times and peak areas. Calculate the surrogate recovery from the surrogate concentration obtained and surrogate concentration fortified. The percent surrogate recovery shall fall between 90-115%. If the surrogate recovery falls outside the 90-115% window, an analysis error is evident and the analysis should be repeated.

Inject sample solutions. If the analyte response exceeds the calibration range then dilute and inject again. Obtain the concentration of sulfate and chlorate from the respective standard curves and calculate the sulfate and chlorate content in the samples from the weight of sample and concentration of analyte in the injected solution.

Sodium Chloride

Determination by potentiometric titration with silver nitrate solution

Apparatus

pH meter capable of reading millivolts (mV) and equipped with a chloride-specific-ion electrode or silver/silver chloride electrodes

Procedure

Electrode calibration: Connect the electrodes to the pH meter and set the meter to read mV. Transfer about 2 g of sample into a beaker and dissolve in water. Place the electrodes in the solution. Titrate with 0.1N silver nitrate (0.5 ml increments), recording the volume of titrant added along with the mV reading after each incremental addition. Plot the titer value (ml) on the X-axis and mV on the Y-axis. Determine the mV reading at the inflection point of the titration curve and use this point as the endpoint in subsequent titrations. Repeat the endpoint determination on a regular basis.

Accurately weigh about 1 g of solid sample and dissolve in about 100 ml of deionized water in a beaker. Add 2 drops of phenolphthalein indicator and nitric acid (1:3 by volume) drop-wise until the phenolphthalein endpoint is reached (alternatively, test with pH test paper). Place the electrodes in sample solution. Titrate with 0.1N silver nitrate solution until the endpoint determined in the electrode calibration is reached. Record the titer value in ml (T).

Calculation:

$$\mathrm{NaCl},\%(\mathrm{w/w}) = \frac{\mathrm{TxNx5.845}}{\mathrm{W}}$$

where:

58.45 is the formula weight of sodium chloride,
N is the normality of silver nitrate solution,
W is the weight of sample (g), and

the factor 5.845 is $\frac{58.45\mathrm{x}100}{1000}$

METHOD OF ASSAY Determination of sodium chlorite content by an iodometric method

Accurately weigh about 4.0 g of solid sample (W), dissolve in deionized water, quantitatively transfer into a 100-ml volumetric flask, make up to volume and mix. Pipet 50 ml of into a 500-ml volumetric flask, dilute to volume and mix to obtain the test sample solution.

Pipette 25 ml of sample solution into a 500 ml narrow-mouth Erlenmeyer flask, add 100 ml of deionized water and 10 ml 0.5N Hydrochloric acid. Add about 2 g of potassium iodide, stopper the flask, mix and keep in a dark place for 2 min. Titrate the liberated iodine with 0.1N Sodium thiosulfate until most of the iodine colour has disappeared. Add 2 ml Starch TS and titrate until the blue colour is discharged. Record the titer value in ml (T_1). Conduct a blank determination and record the titer value in ml (T_2).

Calculation

$$NaClO_2\%(w/w) = \frac{(T_1 - T_2)xNx90.44}{W}$$

where:

22.61 is the equivalent weight of sodium chlorite,
N is the normality of sodium thiosulfate,
W is the weight of sample (g), and

the factor 90.44 is $\frac{22.61x100x500x100}{1000x50x25}$

SODIUM HYDROGEN SULFATE

New specifications prepared at the 68th JECFA (2007), published in FAO JECFA Monographs 4 (2007). No ADI has been allocated to sodium hydrogen sulfate for use in production of acidified sodium chlorite. An ADI "not specified' was established for sodium sulfate at the 57th JECFA (2001) and no ADI was allocated for sulfuric acid at the 20th JECFA (1976).

SYNONYMS Sodium acid sulfate; nitre cake; sodium bisulfate; sulfuric acid, monosodium salt.

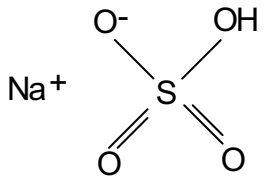
DEFINITION Sodium chloride and sulfuric acid are combined at elevated temperatures to produce molten sodium hydrogen sulfate. The molten sodium hydrogen sulfate is sprayed and cooled to form a solid product with uniform particle size.

C.A.S. number 7681-38-1

Chemical formula $NaHSO_4$

Formula weight 120.06

Structural Formula



Assay Not less than 85%

DESCRIPTION White crystals or granules

FUNCTIONAL USES For use in antimicrobial washing solutions

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water

Sodium (Vol. 4) Passes test

Sulfate (Vol. 4) Passes test

PURITY

Loss on drying (Vol.4) Not more than 0.8% (105º, 3h, use 25 g of sample),

Water-insoluble matter (Vol. 4) Not more than 0.05% (Use 50 g of sample and 300 ml hot water)

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”).
Selenium (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, dissolve in 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 $NaHSO_4$.

STEVIOL GLYCOSIDES

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 63rd JECFA (2004), in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). A temporary ADI of 0-2 mg/kg bw (expressed as steviol) was established at the 63rd JECFA (2004).

SYNONYMS INS no. 960

DEFINITION The product is obtained from the leaves of *Stevia rebaudiana* Bertoni. The leaves are extracted with hot water and the aqueous extract is passed through an adsorption resin to trap and concentrate the component steviol glycosides. The resin is washed with methanol to release the glycosides and product is recrystallized with methanol. Ion-exchange resins may be used in the purification process. The final product may be spray-dried.

Stevioside and rebaudioside A are the component glycosides of principal interest for their sweetening property Associated glycosides include rebaudioside C, dulcoside A, rubusoside, steviolbioside, and rebaudioside B generally present in preparations of steviol glycosides at levels lower than stevioside or rebaudioside A.

Chemical name

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

Rebaudioside A: 13-[(2-O-β-D-glucopyranosyl-3-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]kaur-6-en-8-oic acid, β-D-glucopyranosyl ester

C.A.S. number

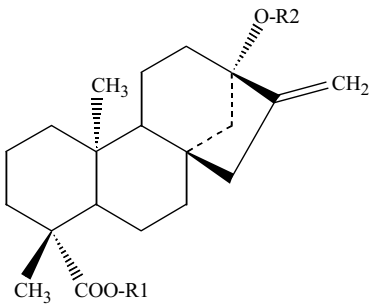
Stevioside:	57817-89-7
Rebaudioside A:	58543-16-1

Chemical formula

Stevioside:	$C_{38}H_{60}O_{18}$
Rebaudioside A:	$C_{44}H_{70}O_{23}$

Structural formula

The seven named steviol glycosides:



Compound name	*R1*	*R2*
Stevioside	*β*-Glc	*β*-Glc-*β*-Glc(2→1)
Rebaudioside A	*β*-Glc	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)
Rebaudioside C	*β*-Glc	*β*-Glc-*α*-Rha(2→1) | *β*-Glc(3→1)
Dulcoside A	*β*-Glc	*β*-Glc-*α*-Rha(2→1)
Rubusoside	*β*-Glc	*β*-Glc
Steviolbioside	H	*β*-Glc-*β*-Glc(2→1)
Rebaudioside B	H	*β*-Glc-*β*-Glc(2→1) | *β*-Glc(3→1)

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

Formula weight

Stevioside: 804.88
Rebaudioside A: 967.03

Assay

Not less than 95% of the total of the seven named steviol glycosides, on the dried basis.

DESCRIPTION

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

FUNCTIONAL USES

Sweetener

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Freely soluble in water and in ethanol

Stevioside and rebaudioside A

The main peak in the chromatogram obtained by following the procedure in Method of Assay corresponds to either stevioside or rebaudioside A.

pH (Vol. 4)

Between 4.5 and 7.0 (1 in 100 solution)

PURITY

Total ash (Vol. 4)

Not more than 1%

Loss on drying (Vol. 4)

Not more than 6% (105º, 2h)

Residual solvents (Vol. 4) Not more than 200 mg/kg methanol
(Method I in Vol. 4, General Methods, Organic Components, Residual Solvents)

Arsenic (Vol. 4) Not more than 1 mg/kg
Determine by the atomic absorption hydride technique (Use Method II to prepare the test (sample) solution)

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

METHOD OF ASSAY Determine the percentages of the individual steviol glycosides by high pressure liquid chromatography (Volume 4).

Standards
Stevioside, >99.0% purity and rebaudioside A, >97% purity (available from Wako pure Chemical Industries, Ltd. Japan).

Mobile phase
Mix HPLC-grade acetonitrile and water (80:20). Adjust the pH to 3.0 with phosphoric acid (85% reagent grade). Filter through 0.22 µm Millipore filter or equivalent.

Standard solutions
(a) Accurately weigh 50 mg of dried (105º, 2 h) stevioside standard into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with mobile phase.
(b) Repeat with previously dried rebaudioside A standard.

Sample solution
Accurately weigh 60-120 mg of dried (105º, 2 h) sample into a 100-ml volumetric flask. Dissolve with mobile phase and dilute to volume with the mobile phase.

Chromatography Conditions

Column: Supelcosil LC-NH2 or equivalent (length: 15-30 cm; inner diameter: 3.9-4.6 mm)

Mobile phase: A 80:20 mixture of acetonitrile and water (see above)

Flow rate: Adjust so that the retention time of rebaudioside A is about 21 min.

Injection volume: 5-10 µl

Detector: UV at 210 nm

Column temperature: 40º

Procedure

Equilibrate the instrument by pumping mobile phase through it until a drift-free baseline is obtained. Record the chromatograms of the

sample solution and of the standard solutions.

The retention times relative to rebaudioside A (1.00) are:

0.45-0.48 for stevioside	0.12-0.16 for rubusoside
0.25-0.30 for dulcoside A	0.35-0.41 for steviolbioside
0.63-0.69 for rebaudioside C	0.73-0.79 for rebaudioside B

Measure the peak areas for the seven steviol glycosides from the sample solution (the minor components might not be detected). Measure the peak area for stevioside for the standard solution.

Calculate the percentage of each of the seven steviol glycosides, *X,* in the sample from the formula:

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

where

W_S is the amount (mg) of stevioside in the standard solution

W is the amount (mg) of sample in the sample solution

A_S is the peak area for stevioside from the standard solution

A_X is the peak area of *X* for the sample solution

f_X is the ratio of the formula weight of *X* to the formula weight of stevioside: 1.00 (stevioside), 0.98 (dulcoside A), 1.20 (rebaudioside A), 1.18 (rebaudioside C), 0.80 (rubusoside), 0.80 (steviolbioside), and 1.00 (rebaudioside B).

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

SUCROSE ESTERS OF FATTY ACIDS

Prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007), superseding tentative specifications prepared at the 65th JECFA (2005) and published in the Combined Compendium of Food Additive Specifications, FAO JECFA Monographs 1 (2005). An ADI of 0-30 mg/kg bw for this substance together with sucroglycerides was established at the 49th JECFA (1997).

SYNONYMS Sucrose fatty acid esters, INS No. 473

DEFINITION Mono-, di- and tri-esters of sucrose with food fatty acids, prepared from sucrose and methyl and ethyl esters of food fatty acids by esterification in the presence of a catalyst or by extraction from sucroglycerides. Only the following solvents may be used for the production: dimethylformamide, dimethyl sulfoxide, ethyl acetate, isopropanol, propylene glycol, isobutanol and methyl ethyl ketone.

Assay Not less than 80% of sucrose esters

DESCRIPTION Stiff gels, soft solids or white to slightly greyish white powders

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol.4) Sparingly soluble in water, soluble in ethanol

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

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

PURITY

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

Acid value (Vol.4) Not more than 6

Free sucrose Not more than 5%
See description under TESTS

Dimethylformamide	Not more than 1 mg/kg See description under TESTS
Dimethyl sulfoxide	Not more than 2 mg/kg See description under TESTS
Ethyl acetate, isopropanol and propylene glycol	Not more than 350 mg/kg, singly or in combination See description under TESTS
Isobutanol	Not more than 10 mg/kg See description under TESTS
Methanol	Not more than 10 mg/kg See description under TESTS
Methyl ethyl ketone	Not more than 10 mg/kg See description under TESTS
Lead (Vol.4)	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

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: 280º
- column: Hold for 1 min at 100º, then 100-300º at 12°/min, hold for 45 min at 300º
- detector: 320º

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:

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

Dimethylformamide

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

Standard solutions

Prepare a stock solution containing 1.00 mg/ml of dimethylformamide in tetrahydrofuran. Prepare a range of standard solutions containing 0.05, 0.1 and 0.2 μg/ml of dimethylformamide by diluting the stock solution with tetrahydrofuran.

Chromatography conditions

Column: Polyethylene glycol (30 m x 0.32 mm i.d. with a 0.5 μm film)
Carrier gas: Helium
Pressure: 150 kPa (constant pressure)
Detector: Nitrogen/phosphorus detector or thermionic specific detector)
Temperatures:

- injection: 180º
- column: Hold for 2 min at 40º, then 40-160º at 20º/min, hold for 2 min at 160º
- detector: 325º

Injection method: Splitless injection of 1.0 μl with auto-injector, followed by start of purge after 1.0 min.
The retention time of dimethylformamide measured under the above conditions is approx. 6.4 min.

Procedure
Weigh accurately 2 g of sample into a 20-ml volumetric flask, add 10 ml of tetrahydrofuran to dissolve the sample, add tetrahydrofuran to the mark, and mix the solution well. Inject 1.0 μl of the sample solution into the chromatograph.

Standard curve
Prepare daily by injecting 1.0 μl of each of the standard solutions into the chromatograph.

Calculate the concentration of dimethylformamide in mg/kg (C_{DFA}) from:

C_{DFA} (mg/kg) = C x 20 / W

where
C is dimethylformamide concentration determined (μg/ml)
W is weight of sample (g)

Note: The nitrogen/phosphorus detector is insensitive to components that do not contain nitrogen or phosphorus. As a consequence, the capillary column can become obstructed with compounds of low volatility, although the baseline of the chromatogram is stable. Accordingly, the column must be reconditioned frequently. Overnight reconditioning (flow carrier gas in the reverse direction at 180º) is required after about every 15 samples.

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 180º at a rate of 10º/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: 210º
- column: 160º

The retention time of dimethyl sulfoxide measured under the above conditions is approx. 3.4 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_{DMSO} (mg/kg) = C x 25 / W

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

Propylene glycol

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

Internal standard solution
Prepare a 500 μg/ml solution of ethylene glycol in tetrahydrofuran.

Standard solutions
Prepare a range of standard solutions containing 1, 5, 10, 25 and 50 μg/ml of propylene glycol with 5 μg/ml of ethylene glycol in tetrahydrofuran.

Chromatography conditions
Column: Polydimethylsiloxane (30 m x 0.32 mm i.d. with 0.25 μm film)
Carrier gas: Helium
Flow rate: 1.5 ml/min (Constant flow)
Detector: FID
Temperatures:
- injection: 230º
- column: Hold for 3 min at 40º, then 40-250º at 20º/min, hold for 5 min at 250º
- detector: 270º

The retention times of ethylene glycol and propylene glycol derivatives are approx. 7.6 min and 7.8 min, respectively.

Procedure
Weigh accurately 1 g of the sample into a 10-ml volumetric flask, and add 100 μl of the internal standard solution. Dissolve and make to volume with tetrahydrofuran. Take 0.5 ml of the sample solution in a centrifugation tube, and add 0.25 ml of 1,1,1,3,3,3-hexamethyldisilazane and 0.1 ml of trimethylchlorosilane. After sealing the tube, shake it vigorously, let stand for 30 min at room temperature, then centrifuge. Inject 1.0 μl of this centrifugal supernatant into the chromatograph.

Standard curve
Prepare following the same procedure using 0.5 ml of the standard solutions in place of the sample solution.

Calculate the concentration of propylene glycol in mg/kg (C_{PG}) from:

C_{PG} (mg/kg) = C x 10 / W

where
C = propylene glycol concentration determined (μg/ml)
W = weight of sample (g)

Methanol, isopropanol, isobutanol, ethyl acetate 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, isopropanol, isobutanol, ethyl acetate 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: 110º
- column: 40º
- detector: 110º

Head space sampler:
- sample heat insulating temperature: 80º
- sample heat insulating period: 40 min
- syringe temperature: 85º
- 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 (mg/kg)= w_i / W

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

W is weight of sample (g)

METHOD OF ASSAY Determine by HPLC using the following conditions:

Procedure

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

Chromatography conditions

Column: Styrene-divinylbenzene copolymer for gel permeation chromatography (TSK-GEL G2000HXL (Tosoh) x 4 column in series or equivalent)
Mobile phase: HPLC-grade degassed tetrahydrofuran
Flow rate: 1.0 ml/min
Detector: Refractive index
Temperatures:
- Column: 40º
- Detector: 40º

Record the chromatogram for about 30 min.

Calculate the percentage of sucrose ester content in the sample from:

$$\% \text{ sucrose ester} = 100\ A/T$$

where

A is the sum of peak areas for the three main components, the mono-, di- and tri-esters, eluting at about 24.9, 23.6 and 22.8 min, respectively

T is the sum of all peak areas eluting within 30 min

WITHDRAWAL OF SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

Anisyl acetone and furfural

The food additives specifications monographs for anisyl acetone and furfural were withdrawn, as the Committee concluded that these substances only have flavouring functions.

Zeaxanthin rich extract from Tagetes erecta

The tentative specifications for zeaxanthin rich extract from *Tagetes erecta* were withdrawn as insufficient information was received to allow removal of the tentative designation.