ISSN 1817-7077

FAO JECFA Monographs 17

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

80th meeting 2015

Food and Agriculture Organization of the United Nations

World Health Organization

ISSN 1817-7077

FAO JECFA Monographs

17

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

80th Meeting 2015

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

Rome, 2015

The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) or of the World Health Organization (WHO) concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these are or have been endorsed or recommended by FAO or WHO in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters. All reasonable precautions have been taken by FAO and WHO to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall FAO and WHO be liable for damages arising from its use.

The views expressed herein are those of the authors and do not necessarily represent those of FAO or WHO.

ISBN 978-92-5-108929-3

© FAO and WHO, 2015

FAO and WHO encourage the use, reproduction and dissemination of material in this information product. Except where otherwise indicated, material may be copied, downloaded and printed for private study, research and teaching purposes, provided that appropriate acknowledgement of FAO and WHO as the source and copyright holder is given and that FAO and WHO's endorsement of users' views, products or services is not implied in any way.

All requests for translation and adaptation rights, and for resale and other commercial use rights should be made via www.fao.org/contact-us/licence-request or addressed to copyright@fao.org.

FAO information products are available on the FAO website (www.fao.org/publications) and can be purchased through publications-sales@fao.org

SPECIAL NOTE

While the greatest care has been exercised in the preparation of this information, FAO expressly disclaims any liability to users of these procedures for consequential damages of any kind arising out of, or connected with, their use.

TABLE OF CONTENTS

LIST OF PARTICIPANTS[1]

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES, 80th MEETING
Rome, 16 – 25 June, 2015

Members

Dr D. Benford, Risk Assessment Unit, Science Evidence and Research Division, Food Standards Agency, London, England, United Kingdom

Dr M. DiNovi, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, MD, USA

Dr M. Feeley, Bureau of Chemical Safety, Food Directorate, Health Canada, Ottawa, Ontario, Canada

Dr D. Folmer, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, MD, USA

Dr Y. Kawamura, Division of Food Additives, National Institute of Health Sciences, Tokyo Japan

Dr A. Mattia, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, MD, USA (Vice-Chairperson)

Mrs I. Meyland, Birkerød, Denmark (Chairperson)

Dr U. Mueller, Food Standards Australia New Zealand, Barton, ACT, Australia (Joint Rapporteur)

Dr G. Pascal, Le Breuil, Saint Alyre d'Arlanc, France

Dr J. Schlatter, Zurich, Switzerland

Dr M. Veerabhadra Rao, Quality Control Department, Department of the President's Affairs, Al Ain, United Arab Emirates

Mrs H. Wallin, Helsinki, Finland (Joint Rapporteur)

Professor G.M. Williams, Department of Pathology, New York Medical College, Valhalla, NY, USA

Secretariat

Dr A. Agudo, Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Institut Català d'Oncologia, L'Hospitalet de Llobregat, Spain (WHO Expert)

Dr B. Amzal*, LASER, United Kingdom Headquarters, London, England, United Kingdom (WHO Expert)

Dr J.H. Andersen, National Food Institute, Technical University of Denmark, Søborg, Denmark (WHO Expert)

Dr S. Barlow, Brighton, East Sussex, England, United Kingdom (WHO Expert)

Dr A. Bruno, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (Codex Secretariat)

Ms A.S. Bulder, Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (WHO Expert)

Dr S. Cahill, Food Safety and Quality Unit, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (FAO Secretariat)

Dr R. Cantrill, AOCS, Urbana, IL, USA (FAO Expert)

Mr P. Cressey, ESR (Institute of Environmental Science and Research Ltd), Christchurch, New Zealand (FAO Expert)

Dr M. De Nijs, RIKILT Wageningen UR, Wageningen, the Netherlands (FAO Expert)

Dr E. Dessipri, General Chemical State Laboratory, Athens, Greece (FAO Expert)

Dr J.A. Edgar, CSIRO Food and Nutritional Sciences, North Ryde, NSW, Australia (FAO Expert)

Dr V. Fattori, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (FAO Joint Secretary)

[1] Participants marked with an asterisk (*) did not attend the entire meeting.

Dr H. Håkansson, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden (WHO Expert)

Ms T. Hambridge, Food Data Analysis Section, Food Standards Australia New Zealand, Barton, ACT, Australia (WHO Expert)

Dr S.M.F. Jeurissen, Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (WHO Expert)

Professor F. Kayama, Department of Environmental & Preventive Medicine, School of Medicine, Jichi Medical University, Yakushiji, Shimotsuke-shi, Tochigi-ken, Japan (WHO Expert)

Mr J. Kim, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (WHO Secretariat)

Dr J.C. Leblanc, Food Safety and Quality Unit, Agriculture and Consumer Protection Department, Food and Agriculture Organization of the United Nations, Rome, Italy (FAO Secretariat)

Dr T. Rawn, Food Research Division, Health Canada, Ottawa, Ontario, Canada (FAO Expert)

Dr K. Schneider, Forschungs- und Beratungsinstitut Gefahrstoffe GmbH (FoBiG), Freiburg, Germany (WHO Expert)

Ms M. Sheffer, Orleans, Ontario, Canada (WHO Technical Editor)

Dr J.R. Srinivasan, Division of Biotech and GRAS Notice Review, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, MD, USA (FAO Expert)

Professor I. Stankovic, Department of Bromatology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia (FAO Expert)

Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (WHO Joint Secretary)

Dr T. Umemura, Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan (WHO Expert)

Professor Dr M. Van den Berg*, Toxicology and Veterinary Pharmacology Division, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands (WHO Expert)

Dr Y. Wu, China National Center for Food Safety Risk Assessment, Beijing, China (FAO Expert)

Dr X. Yang, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, Guangdong Province, China (WHO Expert)

Dr H.J. Yoon, Food Contaminants Division, Food Safety Evaluation Department, Korea Food and Drug Administration, Cheongwon-gun, Chungcheongbuk-do, Republic of Korea (FAO Expert)

Dr Y. Zang, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, MD, USA (WHO Expert)

INTRODUCTION

This volume of FAO JECFA Monographs contains specifications of identity and purity prepared at the 80th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Rome on 16 - 25 June 2015. The specifications monographs are one of the outputs of JECFA's risk assessment of food additives, and should be read in conjunction with the safety evaluation, reference to which is made in the section at the head of each specifications monograph. Further information on the meeting discussions can be found in the summary report of the meeting (see Annex 1), and in the full report which will be published in the WHO Technical Report series. Toxicological monographs of the substances considered at the meeting will be published in the WHO Food Additive Series.

Specifications monographs prepared by JECFA up to the 65th meeting, other than specifications for flavouring agents, have been published in consolidated form in the Combined Compendium of Food Additive Specifications which is the first publication in the series FAO JECFA Monographs. This publication consists of four volumes, the first three of which contain the specifications monographs on the identity and purity of the food additives and the fourth volume contains the analytical methods, test procedures and laboratory solutions required and referenced in the specifications monographs. FAO maintains an on-line searchable database of all JECFA specifications monographs from the FAO JECFA Monographs, which is available at: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/. The specifications for flavourings evaluated by JECFA, and previously published in FAO Food and Nutrition Paper 52 and subsequent Addenda, are included in a database for flavourings specifications. All specifications for flavourings that have been evaluated by JECFA since its 44th meeting, including the 79th meeting, are available in the online searchable database at the JECFA website at FAO: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-flav/en/. The databases have query pages and background information in English, French, Spanish, Arabic and Chinese. Information about analytical methods referred to in the specifications is available in the Combined Compendium of Food Additive Specifications (Volume 4), which can be accessed from the query pages.

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

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

Contact and Feedback

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

jecfa@fao.org

SPECIFICATIONS FOR CERTAIN FOOD ADDITIVES

New and revised specifications

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

Advantame (R)
Annatto extracts (solvent-extracted bixin) (R)
Annatto extracts (solvent-extracted norbixin) (R)
Calcium silicate (R)
Lipase from Fusarium heterosporum expressed in Ogataea polymorpha (N)
Magnesium stearate (N)
Maltotetraohydrolase from Pseudomonas stutzeri expressed in Bacillus licheniformis (N)
Mixed β-glucanase and xylanase from Disporotrichum dimorphosporum (N, T)
Mixed β-glucanase, cellulase and xylanase from Rasamsonia emersonii (N, T)
Polyvinyl alcohol (PVA)-polyethylene glycol (PEG) graft co-polymer (N)
Silicon dioxide, amorphous (R, T)
Sodium aluminium silicate (R, T)

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

Change of an additive name

Sodium aluminosilicate (INS 554) was renamed as *sodium aluminium silicate* to ensure consistency with the other silicate additives (e.g. potassium aluminium silicate).

Withdrawal of specifications

Tentative specification monographs were withdrawn for several additives since requested information had not been received:

Aluminium silicate
Calcium aluminium silicate
Glycerol ester of gum rosin

The monograph for glycerol ester of gum rosin will be immediately removed from the online edition of the Compendium. The specifications for aluminium silicate (INS 559) and calcium aluminium silicate (INS 556) were adopted as Codex specifications. Their possible revocation will be discussed by the 48th Codex Committee on Food Additives. Once the Codex Alimentarius Commission decides to revoke them, they will be removed as well from the online edition.

Modified starches

The 79th meeting of JECFA recommended that the specifications monograph for the modified starches be split into 16 individual specifications monographs. The Committee, as noted at its seventy-sixth meeting, considered that it would also be necessary to revise the specifications for all the modified starches, including test methods, at future meetings.

The Committee was informed of the steps taken in response to this recommendation. As a first step, the 16 specifications have been separated into stand-alone documents based on the current content of the adopted monograph, without adding, deleting or modifying any information. Some of the resulting single draft specifications monographs are incomplete; in some cases, essential information is missing, in particular information that would normally be needed to serve the purpose of a specification to unambiguously

characterize the additive. Therefore, a revision of at least some of these individual draft specifications monographs is required.

In order to facilitate the submission of comments by interested parties, the draft specifications monographs have been made available on the FAO JECFA webpage http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/

Editorial changes to specifications

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

Specifications monographs	***INS***	***Description of changes***
Microcrystalline wax	905c(i)	Under SYNONYMS the INS number was changed to be consistent with CAC/GL 36-1989
Mineral oil (high viscosity)	905d	Under SYNONYMS the INS number was changed to be consistent with CAC/GL 36-1989
Polyoxyethylene (20) sorbitan monostearate	435	Last sentence of the introduction note (in italic) was changed due to an editorial error.
Diammonium hydrogen phosphate	342(ii)	CAS number changed from 7783-54-0 to 7783-28-0
1-hydroxyethylidene-1,1-diphosphonic acid	-	Under SYNONYMS the entry *editronic acid* was changed to the correct name *etidronic acid*

Acknowledgement

Analytical methods for polyvinyl alcohol (PVA)-polyethylene glycol (PEG) graft co-polymer (INS 1209) were partially adapted from methods published by the United States Pharmacopoeia and the European Pharmacopoeia. The generous permission by EDQM and the USP Convention to refer to them is acknowledged and thanked for.

ADVANTAME

Prepared at the 80th JECFA (2015), published in FAO JECFA Monographs 17 (2015), superseding tentative specifications prepared at 77th JECFA (2013). An ADI of 0-5 mg/kg body weight was established at the 77th JECFA (2013).

SYNONYMS INS No. 969

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

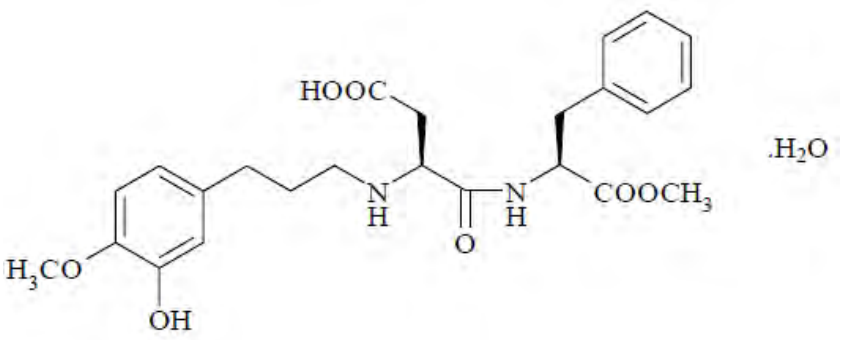
Only the following solvents may be used for the production: methanol and ethyl acetate.

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

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

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

Structural formula



Formula weight 476.52

Assay Not less than 97.0% and not more than 102.0% on the anhydrous basis

DESCRIPTION White to yellow powder

FUNCTIONAL USES Sweetener, flavour enhancer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Very slightly soluble in water, sparingly soluble in ethanol

Infrared spectrum	The infrared spectrum of a potassium bromide dispersion of the sample corresponds to the standard infrared spectrum in Appendix A.

PURITY

Water (Vol. 4)	Not more than 5% (Karl Fischer)
Residue on ignition (Vol. 4)	Not more than 0.2% (use 5 g of the sample)
N-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenyl-alanine (acid of advantame)	Not more than 1% See description under TESTS
Other related substances	Not more than 1.5% (expressed as acid of advantame) See description under TESTS
Specific rotation (Vol. 4)	$[\alpha]_D^{20}$: Between -46° and -39° (0.2% solution in ethanol, on an anhydrous basis)
Residual solvents	Methanol: Not more than 500 mg/kg Ethyl acetate: Not more than 500 mg/kg See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

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

Principle

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

Mobile phase

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

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

Standard solution

Dissolve the acid of advantame reference standard (available from Wako Pure Chemicals, Osaka, Japan) in a mixture of water and acetonitrile (7:3 v/v) to concentrations of 15, 10, 5, 2 and 0.2 µg/ml.

Preparation of Sample solution: Dissolve the sample in a mixture of water

and acetonitrile (7:3 v/v) to a concentration of 1 mg/ml.

System suitability solution
Prepare a solution containing 10 µg/mL of advantame reference standard and 10 µg/ml of acid of advantame reference standard (both available from Wako Pure Chemicals, Osaka, Japan) in a mixture of water and acetonitrile (7:3 v/v).

HPLC conditions:

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

Gradient program:

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

System suitability requirement
The resolution between the advantame and acid of advantame peaks is not less than 3.0 in the chromatogram of the System suitability solution. (Note: The approximate retention times for acid of advantame and advantame are 29.6 min and 56.0, respectively).

Analysis
Separately inject the Standard solution and the Sample solution into the chromatograph, record the chromatograms, and determine the peak area responses for the peaks in the resulting chromatograms.

Calculation
Calculate the percentage of acid of advantame in the sample using the following formula:

$$\text{Acid of advantame (\%)} = (r_U/r_S) \times (C_S/C_U) \times 100$$

where

r_U is the peak area response of advantame acid obtained from the chromatogram of the sample solution
r_S is the peak area response of advantame acid from the chromatogram of the standard solution
C_S is the concentration of the standard solution (µg/ml)
C_U is the concentration of the sample solution (µg/ml)

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

Other related substances

Calculation

Calculate the total percentage of other related substances from the results of the Test for *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl]-α-aspartyl]-L-phenylalanine using the following formula:

$$\text{Total content of other related substances (\%)} = (r_T/r_S) \times (C_S/C_U) \times 100$$

where

r_T is the total peak response of all peaks, except those of advantame and advantame acid obtained from the chromatogram of the sample solution (disregard any peak areas less than 0.02%)
r_S is the peak area response of advantame acid from the chromatogram of the standard solution
C_S is the concentration of the standard solution (µg/ml)
C_U is the concentration of the sample solution (µg/ml)

Residual solvents

Principle

Proceed as directed in Residual Solvents by Headspace Gas Chromatography (Vol. 4) using the following:

Sample solution

Accurately weigh about 0.08 g of advantame to an appropriate headspace vial, and add 2 ml of DMF, apply the stopper, cap, and mix.

Standard Solution

Accurately weigh 0.1 g methanol, and add DMF to make exactly 10 ml (stock solution 1). Accurately weigh 0.1 g of ethyl acetate, and add DMF to make exactly 20 ml (stock solution 2). Transfer 1 ml of stock solution 1 and 1 ml of stock solution 2 into a 10-ml volumetric flask, and add DMF to make exactly 10 ml. Transfer 1 ml of this solution into a 10 ml volumetric flask, and add DMF to make exactly 10 ml (mixture stock solution). Transfer 1 ml of mixture stock solution into a 25 ml volumetric flask, and add DMF to make exactly 25ml. Transfer 2 ml of this solution to an appropriate headspace vial, apply the stopper, cap, and mix.

Procedure

Analyse using the analytical conditions for Residual Solvents by Headspace Gas Chromatography as described in Vol. 4.

Calculation

Calculate the content (mg/kg) of each residual solvent using the following formulae:

$$\text{Content of methanol (mg/kg)} = W_{SA} / W_T \times A_{TA} / A_{SA} \times 80$$
$$\text{Content of ethyl acetate (mg/kg)} = W_{SB} / W_T \times A_{TB} / A_{SB} \times 40$$

where

A_{TA} is the peak area of methanol from the Sample solution;
A_{TB} is the peak area of ethyl acetate from the Sample solution
A_{SA} is the peak area of methanol from the Standard solution;
A_{SB} is the peak area of ethyl acetate from the Standard solution;
W_T is the weight (g) of Advantame in the Sample solution;
W_{SA} is the weight (g) of methanol in the Standard solution; and
W_{SB} is the weight (g) of ethyl acetate in the Standard solution.

METHOD OF ASSAY

Principle

Determine by HPLC using the following conditions:

Mobile phase

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

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

Iinternal standard

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

Standard stock solution

Accurately weigh about 40 mg of advantame reference standard (available from Wako Pure Chemical Industries, Osaka, Japan), dissolve in a mixture of water and acetonitrile (7:3 v/v) to make 50 ml.

Standard solution

Pipet 8, 9 10, 11, 12 ml of standard stock solution into five volumetric flasks. Add 5 ml of the Internal standard solution to each volumetric flask, and add a mixture of water and acetonitrile (7:3 v/v) to make exactly 50 ml.

Sample solution

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

HPLC conditions

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

Gradient program:

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

System suitability

Suitability requirement 1: The resolution between the benzoic acid and advantame peaks is not less than 10 in the chromatogram of the standard solution having the concentration of advantame reference standard closest to 160 µg/ml. (Note: The elution order must be benzoic acid then advantame).

Suitability requirement 2: When injected six consecutive times, the relative standard deviation for the retention time of the advantame peak is not more than 1.0% for the standard solution having the concentration of advantame reference standard closest to 160 µg/ml.

Analysis

Separately inject the Standard solutions into the chromatograph (including the stock solution), record the chromatograms, and determine the peak area responses for the major peaks in the resulting chromatographs (Note: The approximate retention time for advantame is 16.5 min). For each standard solution, calculate the ratio of the peak area response of the advantame peak to that of the benzoic acid internal standard peak. Create a standard curve by plotting the resulting peak area response ratios versus the concentrations of the standard solutions. Inject the Sample solution into the chromatograph, record the chromatogram, and determine the peak area responses for the major peaks in the resulting chromatogram. Calculate the ratio of the peak area response of the advantame peak to that of the benzoic acid internal standard peak. Using the standard curve, determine the concentration of advantame (C_A) in the sample solution, in µg/m.

Calculation

Calculate the percentage of advantame ($C_{24}H_{30}N_2O_7$) in the sample taken:

$$\text{Advantame (\%)} = (C_A/C_U) \times 100$$

where

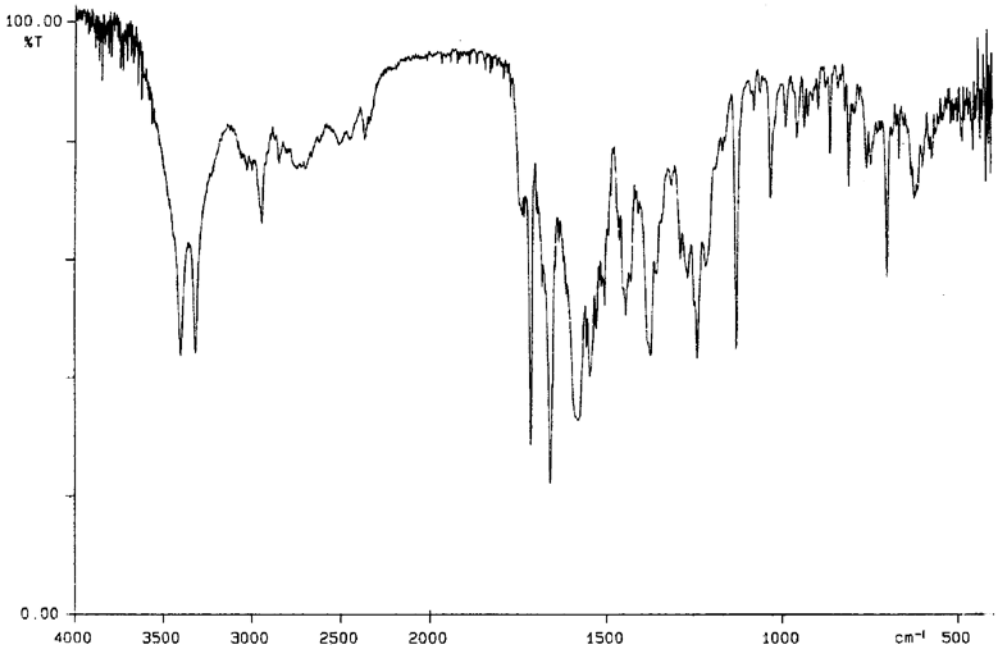
C_A is the concentration of advantame in the sample solution as determined from the standard curve (µg/mL)

C_U is the concentration of the sample solution (µg/mL)

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

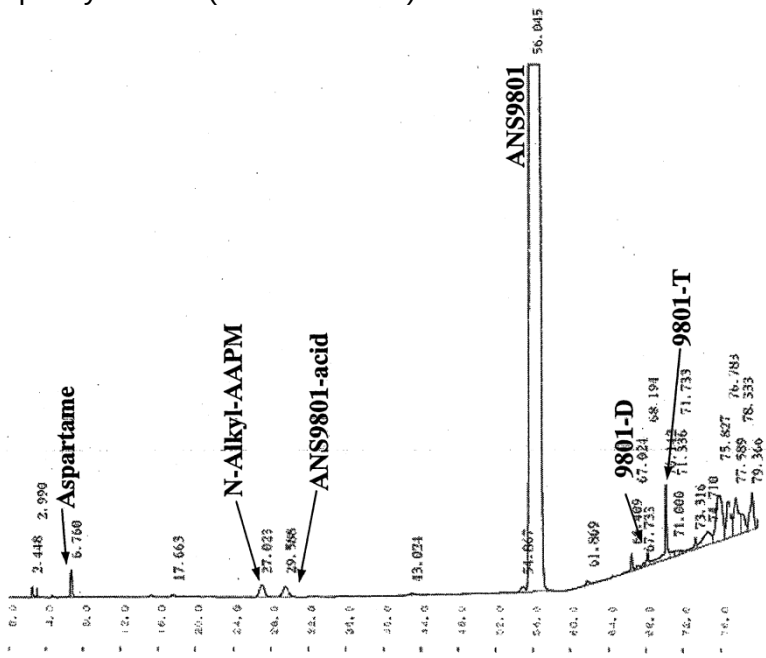
Appendix A

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



Appendix B

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

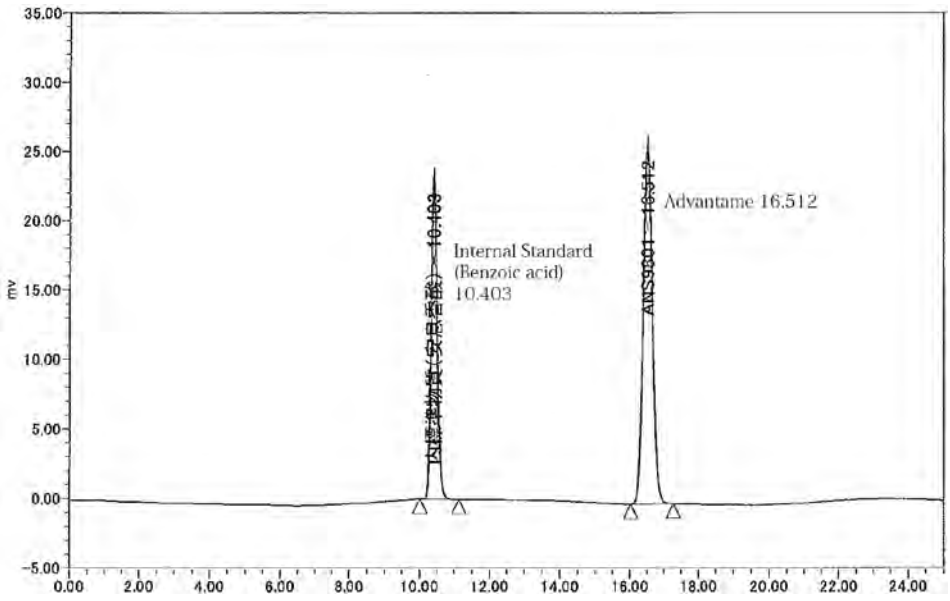


Other identified compounds:

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

Appendix C

Representative chromatogram for Advantame using the Method of Assay at 280 nm



ANNATTO EXTRACTS (SOLVENT-EXTRACTED BIXIN)

Prepared at the 80th JECFA and published in FAO JECFA Monographs 17 (2015) superseding specifications prepared at the 67th JECFA (2006) published in FAO JECFA Monographs 3 (2006). An ADI for bixin of 0 – 12 mg/kg bw was established at the 67th JECFA (2006).

SYNONYMS Annatto B, Orlean, Terre orellana, L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b(i)

DEFINITION Solvent-extracted bixin is obtained by the removal of the outer coating of the seeds of the annatto tree (*Bixa orellana* L) with one or more of the following food grade solvents: acetone, methanol, hexane, ethanol, isopropyl alcohol, ethyl acetate, alkaline alcohol or supercritical carbon dioxide. The resulting preparation may be acidified, followed by the removal of the solvent, drying and milling.

Solvent-extracted bixin contains several coloured components; the major colouring principle is *cis*-bixin, a minor colouring principle is *trans*-bixin; thermal degradation products of bixin may also be present as a result of processing.

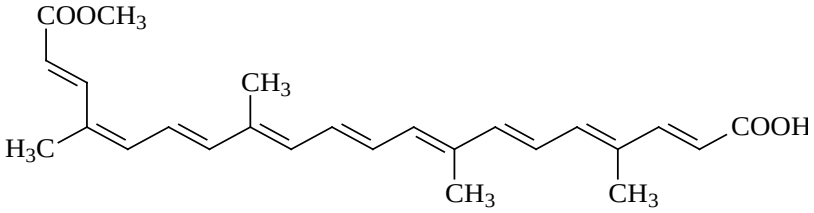
Products supplied to the food industry may be formulated with appropriate carriers of food grade quality.

Chemical name *cis*-Bixin: Methyl (9-*cis*)-hydrogen-6,6'-diapo-Ψ,Ψ-carotenedioate

C.A.S. number *cis*-Bixin: 6983-79-5

Chemical formula *cis*-Bixin: $C_{25}H_{30}O_4$

Structural formula



cis-Bixin

Formula weight 394.5

Assay Not less than 85 % colouring matter (expressed as bixin)

DESCRIPTION Dark red-brown to red-purple powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

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

UV/VIS absorption (Vol. 4) The sample in acetone shows absorbance maxima at about 425, 457 and 487 nm

Thin Layer Chromatography

Activate a TLC plate (e.g. LK6D SILICA GEL 60 A (layer thickness: 250 μm, size: 5 x 20 cm)) for 1 h at 110°. Prepare a 5% solution of the sample in 95% ethanol and apply 10 µl to the plate. Allow to dry and develop using a mixture of n-butanol, methyl ethyl ketone and 10% aqueous ammonia (3:2:2 by volume) until the solvent front has ascended about 10 cm. Allow to dry. Bixin and norbixin appear as yellow spots with R_f values of about 0.50 to 0.45, respectively. Spray with 5% sodium nitrite solution and then with 0.5 mol/l sulphuric acid and the spots immediately decolourise.

PURITY

Norbixin (Vol. 4)

Not more than 2.5 % of total colouring matters.

Residual Solvents

Acetone:	Not more than 30 mg/kg
Methanol:	Not more than 50 mg/kg
Hexane:	Not more than 25 mg/kg
Ethanol: Isopropyl alcohol: Ethyl acetate:	Not more than 50 mg/kg, singly or in combination

See description under TESTS

Arsenic (Vol. 4)

Not more than 3 mg/kg
Determine using an AAS (Hydride generation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

Lead (Vol. 4)

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

Mercury (Vol. 4)

Not more than 1 mg/kg
Determine using AAS (Cold vapour generation technique). The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY

Proceed as directed in Food Colours, Colouring Matters Content by Spectrophotometry (Vol. 4), procedure 2, using 10 ml tetrahydrofuran to dissolve the sample and acetone in place of cyclohexane. Measure the absorbance at the A_{max} of about 487 nm. The specific absorbance ($A^{1\%}_{1\ cm}$) is 3090.

TESTS

Residual solvents

Proceed as directed in Residual Solvents by Headspace Gas Chromatography (Vol. 4) using the following:

Stock standard solutions
Add 10 ml dimethylformamide to a 20 ml volumetric flask. Accurately weigh, to within 0.01 mg, each flask. Pipet 250 µl each of chromatography grade methanol, ethanol, isopropanol, and ethyl acetate, and 150 µl each

of acetone and hexane into each of the flask. Reweigh accurately and then fill the flask with dimethylformamide. Mix well.

Standard mixture solution A: Pipet each 3.0 ml of stock standard solution into a 20 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution B: Pipet 4.0 ml solution A into a 10 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution C: Pipet 2.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution D: Pipet 1.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Samples
Weigh accurately 0.2 g sample into a 20 ml injection vial. Add 2.5 ml dimethylformamide and seal.

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

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

Procedure
Place the sample solution in the sample tray on head-space gas chromatograph. Heat vials at 60° for 20 min with continuous agitation.

Analyze using the analytical conditions for Residual Solvents by Headspace Gas Chromatography as described in Vol. 4.

Measure the peak area for each solvent and obtain the concentration of each solvent (C, mg/ml) from the standard curves.

Calculation
Calculate the concentration of each residual solvent in samples from;

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

where:

W is weight of sample (g).

ANNATTO EXTRACTS (SOLVENT-EXTRACTED NORBIXIN)

Prepared at the 80th JECFA and published in FAO JECFA Monographs 17 (2015) superseding specifications prepared at the 67th JECFA (2006) published in FAO JECFA Monographs 3 (2006). A group ADI for norbixin and its disodium and dipotassium salts of 0 – 0.6 mg/kg bw expressed as norbixin was established at the 67th JECFA (2006).

SYNONYMS Annatto B, Orlean, Terre orellana, L. Orange, CI (1975) 75120 (Natural Orange 4), INS 160b(ii)

DEFINITION Solvent-extracted norbixin is obtained from the outer coating of the seeds of the annatto tree (Bixa orellana L.) by washing with one or more of the following food grade solvents: acetone, methanol, hexane, ethanol, isopropyl alcohol, ethyl acetate, alkaline alcohol or supercritical carbon dioxide followed by solvent removal, crystallization and drying. Aqueous alkali is added to the resultant powder, which is then heated to hydrolyse the colouring matter and cooled. The aqueous solution is filtered, and acidified to precipitate the norbixin. The precipitate is filtered, washed, dried and milled, to give a granular powder.

Solvent-extracted norbixin contains several coloured components; the major colouring principle is *cis*-norbixin, a minor colouring principle is *trans*-norbixin; thermal degradation products of norbixin may also be present as a result of processing.

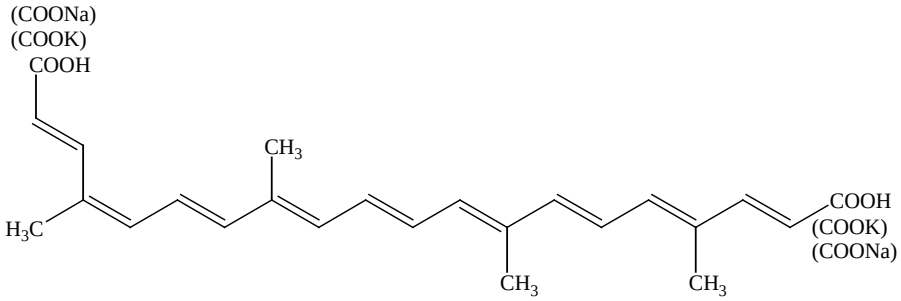
Products supplied to the food industry may be formulated with appropriate carriers of food grade quality.

Chemical name *cis*-Norbixin: 6,6'-Diapo-Ψ,Ψ-carotenedioic acid
cis-Norbixin dipotassium salt: Dipotassium 6,6'-diapo-Ψ,Ψ-carotenedioate
cis-Norbixin disodium salt: Disodium 6,6'-diapo-Ψ,Ψ-carotenedioate

C.A.S. number *cis*-Norbixin: 542-40-5
cis-Norbixin dipotassium salt: 33261-80-2
cis-Norbixin disodium salt: 33261-81-3

Chemical formula *cis*-Norbixin: $C_{24}H_{28}O_4$, *cis*-Norbixin dipotassium salt: $C_{24}H_{26}K_2O_4$, *cis*-Norbixin disodium salt: $C_{24}H_{26}Na_2O_4$

Structural formula



cis-Norbixin

Formula weight 380.5 (acid), 456.7 (dipotassium salt), 424.5 (disodium salt)

Assay Not less than 85 % colouring matter (expressed as norbixin)

DESCRIPTION Dark red-brown to red-purple powder

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in alkaline water, slightly soluble in ethanol

UV/VIS absorption (Vol. 4) The sample in 0.5% potassium hydroxide solution shows absorbance maxima at about 453 nm and 482 nm.

Thin Layer Chromatography Activate a TLC plate (e.g. LK6D SILICA GEL 60 A (layer thickness: 250 μm, size: 5 x 20 cm)) for 1 h at 110°. Prepare a 5% solution of the sample in 95% ethanol and apply 10 µl to the plate. Allow to dry and develop using a mixture of n-butanol, methyl ethyl ketone and 10% aqueous ammonia (3:2:2 by volume) until the solvent front has ascended about 10 cm. Allow to dry. Bixin and norbixin appear as yellow spots with R_f values of about 0.50 to 0.45, respectively. Spray with 5% sodium nitrite solution and then with 0.5 mol/l sulfuric acid and the spots immediately decolourise.

PURITY

Residual Solvents

Acetone:	Not more than 30 mg/kg
Methanol:	Not more than 50 mg/kg
Hexane:	Not more than 25 mg/kg
Ethanol: Isopropyl alcohol: Ethyl acetate:	Not more than 50 mg/kg, singly or in combination

See Description under TEST

Arsenic (Vol. 4) Not more than 3 mg/kg
Determine using an AAS (Hydride generation technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

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

Mercury (Vol. 4) Not more than 1 mg/kg
Determine using AAS (Cold vapour generation technique). The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under “General Methods, Metallic Impurities”).

METHOD OF ASSAY Proceed as directed in Food Colours, Colouring Matters Content by Spectrophotometry (Vol. 4), procedure 1, using 0.5 % potassium

hydroxide as solvent. Measure the absorbance at the A_{max} of about 482 nm. The specific absorbance ($A_{1cm}^{1\%}$) is 2870.

TESTS

Residual solvents

Proceed as directed in Residual Solvents by Headspace Gas Chromatography (Vol. 4) using the following:

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

Standard mixture solution A: Pipet each 3.0 ml of stock standard solution into a 20 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution B: Pipet 4.0 ml solution A into a 10 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution C: Pipet 2.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.
Standard mixture solution D: Pipet 1.0 ml solution A into a 20 ml volumetric flask and fill the flask with dimethylformamide.

Samples
Weigh accurately 0.2 g sample into a 20 ml injection vial. Add 2.5 ml dimethylformamide and seal.

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

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

Procedure
Place the sample solution in the sample tray on head-space gas chromatograph. Heat vials at 60° for 20 min with continuous agitation. Analyze using the analytical conditions for Residual Solvents by Headspace Gas Chromatography as described in Vol. 4.
Measure the peak area for each solvent and obtain the concentration of each solvent (C, mg/ml) from the standard curves.

Calculation
Calculate the concentration of each residual solvent in samples from;

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

Where:
W is weight of sample (g).

CALCIUM SILICATE

Specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs 17 (2015), superseding tentative specifications prepared at the 77th JECFA (2013), published in FAO JECFA Monographs 14 (2013). An ADI 'not specified' for silicon dioxide and certain silicates including calcium silicate was established at the 29th JECFA (1985)

SYNONYMS Silicic acid, calcium salt; calcium silicon oxide; INS No. 552

DEFINITION Calcium silicate is an inorganic substance that is a hydrous or anhydrous substance with varying proportions of calcium as calcium oxide, and silicon as silicon dioxide. It is prepared by various reactions between siliceous material (e.g. diatomaceous earth) and calcium compounds (e.g. lime, calcium hydroxide).

Chemical names Calcium silicate

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

Chemical formula $xCaO \cdot ySiO_2 \cdot zH_2O$

Assay Not less than 50% and not more than 95% of silicon dioxide (SiO_2) and not less than 3% and not more than 35% of calcium oxide (CaO), calculated on the ignited basis.

DESCRIPTION Very fine, white or off-white powder with low bulk density and high physical water absorption

FUNCTIONAL USES Anticaking agent, processing aid (filtering aid)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water and ethanol

pH (Vol. 4) 8.4-12.5 (5% slurry)

Test for calcium Passes test
See description under TESTS

Test for silicon Passes test
See description under TESTS

PURITY

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

Loss on ignition (Vol. 4)	5.0–14.0% on the dried basis (1000°, constant weight)
Fluoride (Vol. 4)	Not more than 50 mg/kg Weigh 1 g of the sample to the nearest mg, and proceed as directed in the Fluoride Limit Test (Method II).
Impurities soluble in 0.5 M hydrochloric acid	Lead : Not more than 5 mg/kg Arsenic: Not more than 3 mg/kg See description under TESTS

TESTS

IDENTIFICATION TESTS

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

PURITY TESTS

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

METHOD OF ASSAY

Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid. Mix and melt completely using a torch burner and allow to stand at room temperature. Place the reaction product along with crucible into 150 ml hot deionized water in a 250-ml PTFE beaker and dissolve residue by agitation. Wash the crucible with hot deionized water and remove it. Add 50 ml hydrochloric acid and transfer the contents into a 250-ml polypropylene volumetric flask. Wash the beaker three times with hot deionized water, transfer the washings to the volumetric flask and make up to volume (Solution A). Prepare the test solution by diluting Solution A with 2% hydrochloric acid. Analyze calcium and silicon in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer. Use analytical lines for Ca (393.366 nm) and Si (251.611 nm). Read the concentration of Ca and Si in sample solution (as µg/ml) from respective standard curves. Calculate the calcium oxide and silicon dioxide content of the sample on the anhydrous basis using the formula:

$$\mathrm{CaO}\ (\%) = \frac{1.399 \times \mathrm{C} \times 250 \times \mathrm{DF}}{\mathrm{W} \times 10^{6}} \times 100$$

$$\mathrm{SiO_2}\ (\%) = \frac{2.139 \times \mathrm{C} \times 250 \times \mathrm{DF}}{\mathrm{W} \times 10^6} \times 100$$

where

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

LIPASE FROM *FUSARIUM HETEROSPORUM* EXPRESSED IN *OGATAEA POLYMORPHA*

New specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs17 (2015). An ADI "not specified" was established at the 80th JECFA (2015).

SYNONYMS Triglyceride lipase; tributyrase; butyrinase; glycerol ester hydrolase; tributyrinase; triacylglycerol ester hydrolase

SOURCES Produced by submerged straight-batch or fed-batch fermentation of a genetically modified non-pathogenic, non-toxigenic strain of *Ogataea polymorpha* which contains a synthetic gene coding for the lipase from *Fusarium heterosporum.* The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cell mass along with the solid waste slurry carrying the residual microorganism from the enzyme by centrifugation and/or filtration. The liquid enzyme filtrate is concentrated by ultrafiltration followed by polish filtration. Food-grade preservatives are added to the liquid enzyme concentrate before spray drying or agglomeration and the product is formulated to the desired activity with food-grade ingredients.

Active principles Triacylglycerol lipase

Systematic names and numbers Triacylglycerol acylhydrolase; EC 3.1.1.3; CAS No. 9001-62-1

Reactions catalyzed Hydrolysis of ester bonds, primarily the 1 and 3 position of triglycerides (yielding di- or monoglycerides plus free fatty acids)
Hydrolysis of SN-1 ester bonds of diacyl-phospholipids and diacyl-galactolipids (yielding monoacyl-phospholipids or monoacyl-galactolipids and fatty acids, respectively)

Secondary enzyme activities No significant levels of secondary enzyme activities

DESCRIPTION Off-white to light yellow powder

FUNCTIONAL USES Enzyme preparation
Used as a processing aid in the manufacture of bakery products, pasta and noodles, in egg yolk and in oil degumming

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

CHARACTERISTICS

IDENTIFICATION

Lipase activity The sample shows lipase activity.
See description under TESTS.

TESTS

Lipase activity

Principle

Lipase activity is determined by measuring the rate of release of free fatty acid that results from the hydrolysis of lecithin, used as a substrate. Continuous titration of the liberated free fatty acid with 0.05 M sodium hydroxide enables the determination of the lipase activity from the consumption of base as a function of time. Lipase of known activity is used as control sample.

The lipase activity is expressed in Titratable Phospholipase Units (TIPU). One TIPU is defined as the amount of enzyme which liberates 1 µmol free fatty acid per minute at the specified conditions.

Apparatus

Water bath with external circulation at 37.0°
Thermostable holder (stand) with the corresponding glass beakers
Homogeniser (Ultra Turrax or equivalent)
pH-stat titrator (Radiometer Phm 290 or equivalent)

Reagents and solutions

Stock solution $CaCl_2$ 0.6 M: Dissolve 8.8 g $CaCl_2$. 2 H_2O in demineralised water and make up to 100 ml.

Substrate

4% lecithin, 4% Triton X-100, and 6 mM $CaCl_2$:
Disperse 12 g lecithin (Sigma product P3644 - Phosphatidylcholine content ≥ 30%) and 12 g Triton X-100 in approx. 200 ml demineralised water with a magnetic stirrer. Add 3.0 ml of 0.6 M $CaCl_2$. Adjust the volume to 300 ml with demineralised water and homogenise the emulsion with the homogeniser (20,000 rpm, 20 sec). Prepare the substrate fresh every day.

Sample preparation

Prepare an enzyme solution to give a slope on the titration curve between 0.06 and 0.18 ml/min, with an addition of 300 µL enzyme solution. Weigh an amount of enzyme equal to (1800/expected activity enzyme)g in order to prepare 100 ml of enzyme solution. Dissolve the enzyme in 50 ml of demineralised water in a beaker and stir the solution for approx. 15 min. Transfer to a 100 mL volumetric flask and make up to the final volume.

Control sample

Prepare a solution of a control enzyme sample of known activity in demineralised water.

Procedure (carry out in duplicate):

1. Add 25.0 ml substrate to the thermostable glass beaker and thermostat to 37.0°. Thermostating takes ca. 10 min.

2. Adjust the pH of the substrate to 7.0 with 0.05 M NaOH. Stir the solution continuously. Start the pH-stat titrator and add 300 µl enzyme solution.

3. After 8 min stop the titration and calculate the slope (α) of the titration curve between 5 and 7 min.

Measure a control sample of known activity prepared in the same way as the sample.

The detection limit is 3 TIPU/ml enzyme solution.

Calculations

Calculate the lipase activity, expressed in TIPU/g enzyme:

$$\text{TIPU/g} = \frac{\alpha \cdot N \cdot 10^3 \cdot V_1}{m \cdot V_2}$$

where

α is the slope of the titration curve between 5 and 7 min of reaction time (ml/min)
N is the normality of the NaOH used (mol/l)
V_1 is the volume in which the enzyme is dissolved (ml)
m is the amount of enzyme added to V1 (g)
V2 is the volume of enzyme solution added to the substrate (ml)

MAGNESIUM STEARATE

New specifications prepared at the 80th JECFA, published in FAO JECFA Monographs 17 (2015). An ADI "not specified'' was established at the 80th JECFA (2015).

SYNONYMS Magnesium distearate, dibasic magnesium stearate, INS No. 470(iii)

DEFINITION Magnesium stearate is a mixture of magnesium salts of fatty acids obtained from edible fats and oils. The product consists mainly of magnesium stearate and palmitate in varying proportions. It is manufactured by one of the two following processes: a) direct process wherein fatty acids are directly reacted with a magnesium source, such as magnesium oxide to form magnesium salts of the fatty acids; b) indirect process where a sodium soap is produced by the reaction of fatty acids with sodium hydroxide in water and the product is precipitated by adding magnesium salts to the soap.

Chemical names Magnesium stearate, magnesium octadecanoate, fatty acids C_{16}-C_{18} magnesium salts

C.A.S number 557-04-0 (magnesium stearate),
91031-63-9 (fatty acids C16-18 magnesium salts)

Chemical formula $Mg(C_{18}H_{35}O_2)_2$ (magnesium distearate)

Formula weight 591.27 (magnesium distearate)

Assay Magnesium: Not less than 4.0% and not more than 5.0%, on dried basis
Fatty acids: Not less than 40.0% stearic acid in the fatty acid fraction; and not less than 90.0% as the sum of stearic acid and palmitic acid in the fatty acid fraction.

DESCRIPTION Off-white to white, very fine powder; greasy to the touch

FUNCTIONAL USES Anticaking agent, emulsifier, binder

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Practically insoluble in water

Magnesium Using the Method of Assay, identify presence of magnesium in the sample

Fatty acid composition Using the Method of Assay, identify the individual fatty acids

PURITY

Loss on drying (Vol. 4) Not more than 6% (105°, constant weight, use 1 g of sample)

Acidity or alkalinity Passes test
See description under TESTS

Unsaponifiable matter — Not more than 2%
See description under TESTS

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

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

Nickel (Vol. 4) — Not more than 3 mg/kg
Determine using an 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”). Use analytical line (emission wavelength): 231.60 nm, curve type: linear, and calibration range: 0.10 – 10.0 µg/ml

TESTS

IDENTIFICATION TEST

PURITY TESTS

Acidity or alkalinity — To 1.0 g sample add 20 ml of freshly prepared deionized water (carbon dioxide free) and boil for 1 min with continuous shaking. Cool and filter. To 10 ml of the filtrate add 0.05 ml of bromothymol blue solution (prepared by dissolving 100 mg of bromothymol blue in a mixture of equal volumes of ethanol (96%) and water and dilute to 100 ml with the same mixture, filter if necessary). Not more than 0.05 ml of 0.1 M hydrochloric acid or 0.1 M sodium hydroxide is required to change the colour of the indicator.

Unsaponifiable matter — Weigh about 5 g, nearest to 0.01 g, well-mixed sample into a 250 ml round-bottom flask. Add approximately 50 ml of 0.5N potassium hydroxide solution and some pumice, attach a reflux condenser, and boil gently for 1 h. Stop heating. Add 100 ml of distilled water through the top of the condenser and swirl.

After cooling, transfer the solution to a separatory funnel. Rinse the flask and the pumice several times with diethyl ether (100 ml in all) and pour this solvent into the separatory funnel. Stopper and shake vigorously for 1 min, periodically releasing pressure by inverting the separating funnel and opening the stopcock.

Allow to stand until there is complete separation of the two phases. Then draw off the soap solution as completely as possible into a second separating funnel. Extract the solution twice more, each time in the same way with 100 ml of diethyl ether. Combine the three ether extracts in one separating funnel containing 40 ml of water.
Gently rotate the separating funnel containing the combined extracts

and the 40 ml water. Violent agitation at this stage may result in troublesome emulsions. Allow the layers to separate completely and draw off the lower aqueous layer. Wash the ether layer twice more with 40 ml portions of water, shaking vigorously each time and discarding the lower aqueous layers after separation. Draw off each washing solution up to 2 ml, then rotate the separating funnel around its axis, wait some min to give the last remainders the opportunity for collection and draw off the collected remainders, close stopcock when ether starts to pass the bore of the stopcock.

Wash the ether layer successively with 40 ml of 0.5 N potassium hydroxide solution, 40 ml of water, and again with 40 ml of potassium hydroxide solution, then at least twice more with 40 ml of water. Continue to wash with water until the wash-water no longer gives a pink colour on the addition of a drop of phenolphthalein solution.

Transfer the ether layer quantitatively a little at a time through the top of the separating funnel into a flask previously dried and weighed to the nearest 0.0001 g.

Evaporate the solvent by distillation on a boiling-water bath. Add 5 ml of acetone and remove the volatile solvent completely in a gentle current of air, holding the flask obliquely while turning it in a boiling-water bath.

Dry the residue at 103±2° for 30 min, placing the flask in an almost horizontal position. Cool in a desiccator and weigh to the nearest 0.0001 g (m_1. Repeat the drying for successive 15 min periods until the loss of weight between two successive weighings is less than 0.002 g.

After weighing the residue dissolve it in 4 ml of diethyl ether and then add 20 ml of ethanol previously neutralized to a faint pink colour, using phenolphthalein TS as indicator. Titrate with standard 0.1N ethanolic potassium hydroxide solution (prepared by dissolving 6 g of potassium hydroxide in about 5 ml of water and making up to 1 liter with ethanol) to the same final colour.

Correct the weight of the residue for the free acidity content of the blank. Calculate the per cent unsaponifiable matter using the formula:

$$\frac{100 \times (m_1 \times T \times V)}{m}$$

where

m is the mass, in g, of the test portion
m_1 is the mass, in g, of the residue
V is the number of ml of the standardized potassium hydroxide solution used
T is the exact normality of the potassium hydroxide solution used

METHOD OF ASSAY

Magnesium (Vol. 4) Determine using an 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”).

Fatty Acid Composition

Principle:
Fatty acids in the sample are esterified using boron trifluoride and alkaline methanol and the fatty acid methyl esters are determined by gas chromatography. Relative percentage of fatty acids in the fatty acid portion of sample are determined by normalization technique.

Sample solution
In a conical flask fitted with a reflux condenser, dissolve 0.10 g of the sample in 5 ml of boron trifluoride-methanol solution (prepared by dissolving 140 g of boron trifluoride in anhydrous methanol). Boil under a reflux for 10 min. Add 4 ml of heptane through the condenser and boil again under reflux for 10 min. Allow it to cool. Add 20 ml of saturated sodium chloride solution, shake and allow the layers to separate. Dry the organic layer over 0.1 g of anhydrous sodium sulfate (previously washed with heptane).

Reference solution
Prepare the reference solution in the same manner as the test solution using 50.0 mg of palmitic acid (96% pure) and 50.0 mg of stearic acid (96% pure).

Gas chromatography (Vol. 4)

GC column:	Polyethylene glycol 20000, 30 m x 0.32 mm id x 0.5 µm film thickness (Macrogol 20000 R or equiv.)
Carrier gas:	Helium (> 99.995 % pure); flow rate: 1 ml/min.
Column temperature:	180^{o} isothermal conditions.
Injector:	250^{o}
Detector:	Flame Ionization, 250^{o}

System suitability
Resolution between the peaks of methyl palmitate and methyl stearate in the reference solution shall be >5.0
Relative standard deviation, determined on areas of 6 injections using reference solution, shall be <3.0% for methyl palmitate and <1.0% for methyl stearate.

Procedure
Condition the gas chromatograph using above conditions; inject 1µl of reference solution and record retention times for the constituent fatty acid methylesters. Using area normalization technique determine the relative percentages of palmitic and stearic acid esters in the reference solution. Inject 1 µl of test solution and determine the relative percentages of fatty acids in the sample solution.

MALTOTETRAOHYDROLASE FROM *PSEUDOMONAS STUTZERI* EXPRESSED IN *BACILLUS LICHENIFORMIS*

New specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs 17 (2015). An ADI "not specified" was established at the 80th JECFA (2015).

SYNONYMS Exo-maltotetraohydrolase; 1,4-alpha-D-glucan maltotetraohydrolase

SOURCES Produced by straight-batch or fed-batch fermentation of a genetically modified non-pathogenic, non-toxigenic strain of *Bacillus licheniformis* containing the gene from *Pseudomonas stutzeri*. The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cell mass along with the solid waste slurry carrying the residual microorganism from the enzyme by centrifugation and/or filtration. The liquid enzyme filtrate is concentrated by ultrafiltration followed by polish filtration. The final product is standardized with food-grade materials to the desired activity.

Active principles Maltotetraohydrolase

Systematic names and numbers 4-alpha-D-glucan maltotetraohydrolase; EC 3.2.1.60

Reactions catalyzed Hydrolysis of (1→4)-alpha-D-glucosidic linkages in amylaceous polysaccharides, to remove successive maltotetraose residues from the non-reducing chain ends, using amylaceous polysaccharides like starch as a substrate.

Secondary enzyme activities No significant levels of secondary enzyme activities

DESCRIPTION Amber liquid or off-white granulate

FUNCTIONAL USES Enzyme preparation.
Used in the manufacture of baked goods, and in starch processing.

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

CHARACTERISTICS

IDENTIFICATION

Maltotetraohydrolase activity The sample shows maltotetraohydrolase activity. See description under TESTS.

TESTS

Enzyme Activity

Principle

Maltotetraohydrolase activity is measured by a method using end-blocked *p*-nitrophenyl-maltoheptaoside substrate in the presence of 2 exo-acting enzymes, namely amyloglucosidase and α -glucosidase (maltase). When the oligosaccharide is hydrolyzed by maltotetraohydrolase, the non-blocked nitrophenyl-linked maltooligosaccharide that is produced is hydrolysed to glucose and free *p*-nitrophenol by the combined action of excess amyloglucosidase and α-glucosidase present in the reaction mixture. The amyloglucosidase hydrolyses p-nitrophenyl maltooligosaccharide to glucose and *p*-nitrophenyl α-D-glucoside, and the α -glucosidase hydrolyses the *p*-nitrophenyl α -D-glucoside to glucose and yellow coloured *p*-nitrophenol. The reaction is terminated by the addition of an alkaline solution which assists in optimal colour development.

The *p*-nitrophenol release is proportional to maltotetraohydrolase activity, and is monitored at 410 nm.

One BMU refers to an internal standard with a defined activity of the enzyme. One BMU is defined as the activity degrading 0.0351 mmole per min of blocked *p*-nitrophenyl-α-D-maltoheptaoside in the presence of amyloglucosidase and α-glucosidase at 25°, in a specific assay mix for 5 min.

Apparatus

Spectrophotometer
Water bath with thermostatic control
Positive displacement pipettes

Reagents and solutions

MilliQ water, or equivalent
Blocked *p*-Nitrophenyl-α-d-Maltoheptaoside (BPNPG7), (Sekisui Diagnostics, US# 70-3685-01/International# BLMN-70-3685 or equivalent)
α-Glucosidase (maltase), (Sekisui Diagnostics, US# 70-1235-01/International # MALT-70-1235 or equivalent)
Glucoamylase, (Sekisui Diagnostics: # GLUC-70-6881 or equivalent)
DL-malic acid, (Sigma # M0875 or equivalent)
Sodium Chloride, reagent grade, (Sigma # S9888 or equivalent)
Calcium Chloride, anhydrous, (Sigma # C1016 or equivalent)
Bovine Serum Albumin, (Sigma # A3294 or equivalent)
1N Sodium Hydroxide TS (from J.T. Baker #3728-01 or equivalent)
Boric Acid, (Sigma # B0394 or equivalent)
1,2-Propanediol (Propylene glycol), (Sigma # P4347 or equivalent)
Enzyme standard (standard solution with certified activity expressed in BMU/ml, available from DuPont (Danisco US Inc.), Rochester, NY, USA).

50 mM Malate buffer, pH 5.6 (For Assay buffer)

Dissolve 6.7 g DL-malic acid in 800 ml MilliQ water. Add 2.92 g NaCl and 0.29 g $CaCl_2$ and dissolve. Adjust pH to 5.6 with 1N NaOH. Make up to 1.0 l with MilliQ water.

Assay buffer
1% Bovine Serum Albumin (BSA) in Malate Buffer, pH 5.6:
Weigh an amount of BSA and dissolve in the appropriate volume of 50 mM Malate Buffer, pH 5.6, in order to obtain a 1% (w/v) solution. (Example: 1.0 g BSA dissolved in 100 ml 50 mM Malate Buffer, pH 5.6)

Stop solution (200mM Borate solution)
Dissolve 6.18 g Boric Acid in 400 ml MilliQ water. Adjust pH to 10.2 with 1N NaOH TS. Make up to 500 ml with MilliQ water.

Working substrate solution
Add 54.5 mg BPNPG7, 300 Units α-Glucosidase (maltase), and 120 Units glucoamylase in 10 ml Assay Buffer. Label as Working Substrate Solution.

Standard solutions
Weigh out an amount of enzyme standard, and dilute with Assay buffer to make three dilutions to set up a three point standard curve with a linear range of ~0.2 to 1.6 BMU/ml, at 410 nm. Label accordingly.

Prepare a blank by pipetting 50 µl Assay buffer into a test tube. Label accordingly.
Store at room temperature.

Samples
Liquid sample: Heat liquid samples to 37 - 40° in a water bath for 15 - 30 min to dissolve any precipitate. Dilute the samples with Assay buffer to obtain a final absorbance within the linear range of the assay. Heat the final dilutions of samples to 37 - 40° in a water bath for 15 - 30 min before assaying. Store diluted samples at room temperature.

Granular sample: Weigh out duplicate granular samples (0.5 g-10 g) in a 100 ml beaker. Add approximately 80 ml of assay buffer to each. Stir on a magnetic plate for 20 min. Transfer to a 100 ml volumetric flask, and adjust to volume with Assay buffer. Prepare additional dilutions if necessary, using Assay Buffer, to obtain a final absorbance within the linear range of the standard curve, at 410 nm. Store diluted samples at room temperature.

Procedure
Preheat water bath to 30º.

Prepare tubes, in duplicates, on a rack. Label as Working Standard, Sample and Blank. Using a positive displacement pipette, dispense 50 µl standards and working sample dilutions to each labeled tube. Place the rack in a water bath at 25°. Also place the Working Substrate Solution in the water bath to equilibrate for 5 min.

Using a positive displacement pipette, dispense 400 µl aliquots of Working Substrate Solution into the labelled test tubes, at timed intervals. Incubate all test tubes at 25° for exactly 5 min, in a water bath. Add 600 µl Stop Solution to each test tube and vortex. Transfer the content of each tube to a plastic cuvette. Measure the absorbance at 410 nm, after appropriately zeroing with blank.

Prepare the standard curve using linear regression. The correlation coefficient must be ≥ 0.99. Determine the maltotetraohydrolase

concentration of each enzyme standard and sample from the standard curve.

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

Calculations

Calculate the activity for each sample in BMU/g as follows:

$$\text{Maltotetraohydrolase Activity, BMU per gram} = \frac{\text{C} \times \text{DF}}{\text{W}}$$

Where

C is concentration of maltotetraohydrolase from the standard curve in BMU/ml
DF is Dilution Factor of sample
W is sample density in g/ml

MIXED β-GLUCANASE AND XYLANASE FROM *DISPOROTRICHUM DIMORPHOSPORUM* (TENTATIVE)

New tentative specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs 17 (2015). An ADI "not specified" was established at the 80th JECFA (2015).

Information required:

- *method to determine the identity for β-glucanase, including data from a minimum of five batches, using the method described;*
- *a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories and data from a minimum of five batches, using the method described.*

SOURCES

The mixed β-glucanase and xylanase enzyme preparation is produced by submerged fermentation of a non-pathogenic and non-toxigenic strain of *Disporotrichum dimorphosporum*. The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cell mass by vacuum drum filtration or centrifugation. The enzyme is concentrated by ultrafiltration and/or evaporation. 40-45% glycerol is added to the polished liquid enzyme concentrate, to standardise to desired activity. Sodium benzoate is added as a stabilising agent, and the liquid enzyme preparation is filtered again prior to packaging.

Active principles: β-glucanase and xylanase

Systematic names and numbers:

β-glucanase:	3-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase
EC No.:	3.2.1.6
CAS No.:	62213-14-3
Xylanase:	1,4-beta-D-xylan xylanohydrolase
EC No.:	3.2.1.8
CAS No.:	9025-57-4

Reactions catalyzed: β-glucanase: endohydrolysis of (1→3) or (1→4) linkages in β-D-glucans
Xylanase: endohydrolysis of 1,4-beta-D-xylosidic linkages in xylans

DESCRIPTION

Light brown to dark brown liquid.

FUNCTIONAL USES

Enzyme preparation.
The mixed β-glucanase and xylanase-enzyme preparation is used as a processing aid in brewing and in potable alcohol production to degrade beta-D-glucans and xylans to reduce viscosity and improve grist stability. The enzyme preparation is also used in grain processing to degrade cell wall components, in order to improve mechanical treatments.

GENERAL SPECIFICATIONS

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

CHARACTERISTICS

IDENTIFICATION

β-Glucanase activity — The sample shows β-glucanase activity.
Method information required.

Xylanase activity — The sample shows xylanase activity.
Method information required.

TESTS

β-Glucanase activity

Principle

Enzyme activity is measured by a method based on the reduction of viscosity of a beta-glucan substrate at pH 5.60 and 45°, in the presence of β-glucanase. This is measured using a calibrated Ubbelhode viscosimeter. Activity is expressed in Beta-Glucanase Fungique (BGF) units. One BGF unit is the amount of enzyme per milliliter (ml) of reaction mixture (15 ml substrate and 2 ml enzyme solution) that causes a change in viscosity of the substrate with a speed giving a slope of 0.147 per min under the conditions of the test, when the reaction time is plotted against the inverse of the falling time.

Apparatus

Ubbelhode No. 1C Viscosimeter, with a circulation flow constant of approximately 0.03, or equivalent
Analytical balance, accuracy to within 0.001 g
Waterbath

Description of Ubbelhode Viscosimeter:

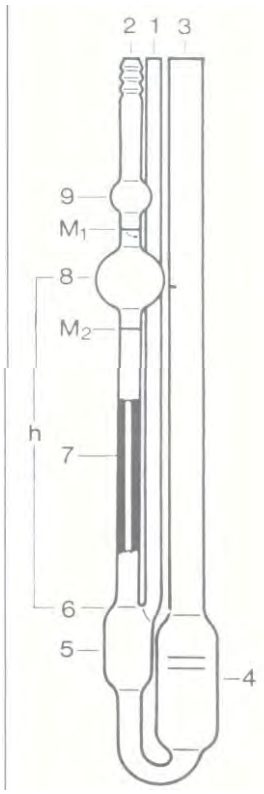


Figure legend:

1. Ventilating tube
2. Capillary tube
3. Filling tube
4. Reservoir
5. Reference level vessel
6. Dome-shaped top part
7. Capillary
8. Measuring sphere
9. Pre-run sphere

M1. Upper timing mark
M2. Lower timing mark

Reagents and solutions

Potassium dihydrogen phosphate (KH_2PO_4)
Phosphoric acid, 85%, P-BGBM beta-glucan from barleyv viscosity 20–30 cst. Megazyme
Sodium Hydroxide, 1 mol/l
Ultrahigh quality water, resistance >18.2 mega Ohm/cm, TOC < 500 µg/l.

Phosphoric acid 1.0 mol/l:
Slowly and while stirring continuously add 67 ml phosphoric acid 85% to approximately 500 ml water in a 1 l volumetric flask. Make up to volume and mix. This solution may be kept for 2 months at room temperature.

Phosphate Buffer, 1.0 mol/l, pH 5.00:
Dissolve 13.6 g potassium dihydrogen phosphate in approximately 80 ml water in a 100 ml volumetric flask. If necessary heat while dissolving and allow cooling to ambient temperature. Adjust the pH to 5.00 by adding phosphoric acid solution (1 mol/l) or sodium hydroxide solution (1 mol/l). Make up to volume with water and mix. This solution may be kept for 2 months.

Beta-glucan substrate solution:
Use a reserved batch of beta-glucan, with a known substrate batch factor. (This is the factor used to bridge new substrates to the existing substrate used for calibration).

Dissolve 1.0 g beta-glucan in approximately 30 ml water in a 100 ml Erlen Meyer vial. Stir for approximately 1 h. Next place the solution in the bath with boiling water for 5 min and allow cooling to ambient temperature. Add
10.0 ml phosphate buffer 1 mol/l, pH 5.00 and quantitatively transfer to a 100 ml volumetric flask with water. Make up to volume with water and mix.
Check the pH of the solution. It must be 5.60 +/- 0.05. Prepare a new solution when pH is out of range. Only use a freshly prepared solution.

Control: A β-glucanase preparation of known activity is used as assay control.

Samples: Allow the sample to attain room temperature. Weigh, accurately to within 0.0001 gram, and in duplicate an amount of sample corresponding to 10 000 BGF in a 100 ml volumetric flask. Dissolve in water by stirring on a magnetic stirrer. Make up to volume with water and

mix. Dilute 0.25 ml of these solutions with 4.75 ml water in a centrifuge tube and mix. The activity of the sample should fall between 3.4 and 6.8 BGF/ml.

Procedure

Allow the Ubbelhode viscosimeter to equilibrate at 45° for at least 20 min.

1. Place a reagent tube containing 15.0 ml of beta-glucan substrate in the 45° water bath and allow it to equilibrate for at least 20 min.
2. Start a stopwatch (Stopwatch 1) set to T= 0 min, and leave this running throughout the assay to record incubation time. Add 2.0 ml of sample solution to the equilibrated substrate at T = 0 min. Mix on a tube shaker.
3. Fill the reservoir of the viscosimeter through Filling Tube to a level between the two marks on the reservoir (refer to the Description of Ubbelhode Viscosimeter, if necessary).
4. Measure the viscosity of this solution at every three min for a 15 min period, by following step 5 - 9. This will result in 5 measurements in total.
5. At T = ~ 2.5 min close Ventilating Tube (with finger) and execute suction on Capillary Tube to fill the Capillary Tube up to the Pre-run sphere (refer to the Description of Ubbelhode Viscosimeter, if necessary).
6. Stop suction, remove finger from Ventilating Tube and start Stopwatch 2 when the liquid reaches the Upper Timing mark (M1).
7. Read the time on Stopwatch 1 (do not stop stopwatch 1!). This will be Rt 1 for the calculations.
8. Allow the liquid to run down to the Lower Timing mark, M2. Stop Stopwatch 2 when the Lower Timing mark, M2 is reached. This will be Vt 1 in the calculation.
9. Repeat this procedure every three min. Record Rt 2 to Rt 5 and Vt 2 to Vt 5.
10. Repeat Steps 1-9 using a mixture of 15.0 ml substrate and 2.0 ml water as the substrate blank. Record the viscosity as Vtb.
11. Repeat Steps 1-9 using a mixture of 17.0 ml water as the water blank. Record the viscosity as Vtw.
12. Repeat Steps 1-9 if the enzyme activity is outside the linear range of the assay, with appropriate dilutions.

Calculations:

Calculate the Reaction Time (T1-T5), in sec, accurately to 0.01 sec using the equations:

$$T1 = \{Rt1 + (½\ Vt1)\}$$
$$T2 = \{Rt2 + (½\ Vt2)\}$$
$$T3 = \{Rt3 + (½\ Vt3)\}$$
$$T4 = \{Rt4 + (½\ Vt4)\}$$
$$T5 = \{Rt5 + (½\ Vt5)\}$$

1. Calculate the average time for drop in viscosity, for the five measuring points of the substrate blank in sec, using the equation:
   Vtbl = (Sum of Vt 1 to 5) / 5
2. Calculate the average time for drop in viscosity, for the five measuring points of the water blank in sec, using the equation:
   Vtw = (Sum of Vt 1 to 5) / 5
3. For each of the five measuring points of sample, calculate X using the equation:
   X = Vtbl / (Vt - Vtw).
4. For each of the measuring points, plot the T values (obtained in

Step 1, x axis) against the corresponding X (obtained in Step 4, y-axis). Calculate the slope according to the linear equation
$y = Px + b$
From this line calculate the slope, P

5. Calculate the enzyme concentration in the incubation mixture as follows:

$C = (W / 100) \times (0.25 / 5.00)$

Where
C is Sample Concentration in g/ml
W is Sample weight, g
0.25/5.00 is Dilution Factor
100 is Volume of Dilution, ml

6. Calculate the enzyme activity of the sample as follows:

$\beta\text{-glucanase Activity, BGF/g} = (P \times 60 / 0.147) \times (17/2) \times (1/C) \times Sf$

Where
P is Slope from Step 5
60 is Conversion of sec to min
0.147 is Conversion Factor from definition of Enzyme Activity
17/2 is Correction Factor for incubation mixture
C is Sample Concentration from Step 6

Xylanase activity

The sample shows xylanase activity.
Method information required.

MIXED β-GLUCANASE, CELLULASE AND XYLANASE FROM RASAMSONIA EMERSONII (TENTATIVE)

New tentative specifications prepared at the 80th JECFA (2015) and published in FAO JECFA Monographs 17 (2015). An ADI "not specified" was established at the 80th JECFA (2015).

Information required:

- *method to determine the identity for β-glucanase, including data from a minimum of five batches, using the method described;*
- *method to determine the identity for cellulase, including data from a minimum of five batches, using the method described;*
- *a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories and data from a minimum of five batches, using the method described.*

SOURCES

The mixed β-glucanase, cellulase and xylanase enzyme preparation is produced by submerged fermentation of a non-pathogenic and non-toxigenic strain of Rasamsonia emersonii. The enzyme is recovered from the fermentation broth. The recovery process includes the separation of cell mass by vacuum drum filtration or centrifugation. The enzyme is concentrated by ultrafiltration and/or evaporation. The mixed enzyme concentrate is then formulated with glycerol, and potassium sorbate, added to standardise and stabilise the preparation, respectively.

Active principles: β-Glucanase, cellulase, and xylanase

Systematic names and numbers:

β-Glucanase:	3-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase
EC No.:	3.2.1.6
CAS No.:	62213-14-3
Cellulase:	4-(1,3;1,4)- β-D-glucan 4-glucanohydrolase
EC No.:	3.2.1.4
CAS No.:	9012-54-8
Xylanase:	1,4-β-D-xylan xylanohydrolase
EC No.:	3.2.1.8
CAS No.:	9025-57-4

Reactions catalysed:

β-glucanase:	Endohydrolysis of (1→3) or (1→4) linkages in β-D-glucans
Cellulase:	Endohydrolysis of (1→4)-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. Will also hydrolyse (1→4) linkages in β-D-glucans that also contain (1→3) linkages.
Xylanase:	Endohydrolysis of 1,4-β-D-xylosidic linkages in xylans

Secondary enzyme activities: Hemicellulase, pentosanase, arabinase, amylase, pectinase, protease

DESCRIPTION

Light brown to dark brown liquid.

FUNCTIONAL USES Enzyme preparation

The β–glucanase, cellulase, and xylanase enzyme preparation is used as a processing aid in brewing, grain processing and in the production of potable alcohol. The action of these enzymes allows for decreased viscosity, which in turn improves filterability, yield and consistency of product quality. The enzyme preparation is also used in grain processing to degrade cell wall components, in order to improve mechanical treatments.

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

CHARACTERISTICS

IDENTIFICATION

β-Glucanase activity — The sample shows β-glucanase activity.
Method information required

Cellulase activity — The sample shows cellulase activity.
Method information required

Xylanase activity — The sample shows xylanase activity.
Method information required

TESTS

β-glucanase and cellulase activity

Principle

Enzyme activity is measured by a method based on the reduction of viscosity of a beta-glucan substrate at pH 5.60 and 45°, in the presence of β-glucanase and cellulase. This is measured using a calibrated Ubbelhode viscosimeter. Activity is expressed in Beta-Glucanase Fungique (BGF) units. One BGF unit is the amount of enzyme per milliliter (ml) of reaction mixture (15 ml substrate and 2 ml enzyme solution) that causes a change in viscosity of the substrate with a speed giving a slope of 0.147 per min under the conditions of the test, when the reaction time is plotted against the inverse of the falling time.

Apparatus

Ubbelhode No. 1C Viscosimeter, with a circulation flow constant of approximately 0.03, or equivalent
Analytical balance, accuracy to within 0.001 g
Waterbath

Description of Ubbelhode Viscosimeter:

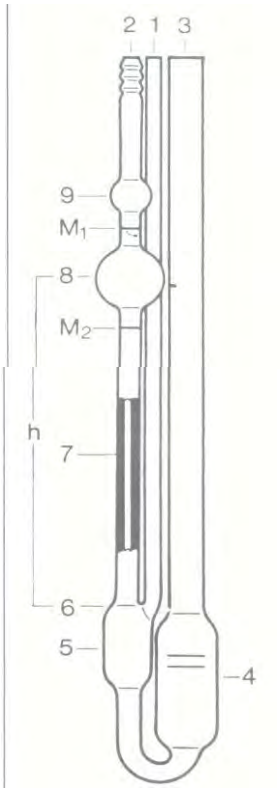


Figure legend:

1. Ventilating tube
2. Capillary tube
3. Filling tube
4. Reservoir
5. Reference level vessel
6. Dome-shaped top part
7. Capillary
8. Measuring sphere
9. Pre-run sphere

M1. Upper timing mark
M2. Lower timing mark

Reagents and solutions

Potassium dihydrogen phosphate (KH_2PO_4)

Phosphoric acid, 85%, P-BGBM beta-glucan from barley; viscosity 20–30 cst. Megazyme

Sodium Hydroxide, 1 mol/l

Ultrahigh quality water, resistance >18.2 mega Ohm/cm, TOC < 500 µg/l.

Phosphoric acid 1.0 mol/l

Slowly and while stirring continuously add 67 ml phosphoric acid 85% to approximately 500 ml water in a 1 l volumetric flask. Make up to volume and mix. This solution may be kept for 2 months at room temperature.

Phosphate Buffer, 1.0 mol/l, pH 5.00

Dissolve 13.6 g potassium dihydrogen phosphate in approximately 80 ml water in a 100 ml volumetric flask. If necessary heat while dissolving and allow cooling to ambient temperature. Adjust the pH to 5.00 by adding phosphoric acid solution (1 mol/l) or sodium hydroxide solution (1 mol/l). Make up to volume with water and mix. This solution may be kept for 2 months.

Beta-glucan substrate solution

Use a reserved batch of beta-glucan, with a known substrate batch factor, Sf. (This is the factor used to bridge new substrates to the existing substrate used for calibration).

Dissolve 1.0 g beta-glucan in approximately 30 ml water in a 100 ml glass Erlen Meyer flask. Stir for approximately 1 h. Next place the solution in the bath with boiling water for 5 min and allow cooling to ambient temperature. Add 10.0 ml phosphate buffer 1 mol/l, pH 5.00 and quantitatively transfer to a 100 ml volumetric flask with water. Make up to volume with water and mix.

Check the pH of the solution. It must be 5.60 +/- 0.05. Prepare a new solution when pH is out of range. Only use a freshly prepared solution.

Control

β-glucanase and cellulase of known activities are used as assay controls.

Samples

Allow the sample to attain room temperature. Weigh, accurately to within 0.0001 g, and in duplicate an amount of sample corresponding to 10 000 BGF in a 100 ml volumetric flask. Dissolve in water by stirring on a magnetic stirrer. Make up to volume with water and mix. Dilute 0.25 ml of these solutions with 4.75 ml water in a centrifuge tube and mix. The activity of the sample should fall between 3.4 and 6.8 BGF/ml.

Procedure

Allow the Ubbelhode viscosimeter to equilibrate at 45° for at least 20 min.

Place a reagent tube containing 15.0 ml of beta-glucan substrate in the 45° water bath and allow it to equilibrate for at least 20 min.

Start a stopwatch (Stopwatch 1) set to T= 0 min, and leave this running throughout the assay to record incubation time. Add 2.0 ml of sample solution to the equilibrated substrate at T = 0 min. Mix on a tube shaker.

Fill the reservoir of the viscosimeter through Filling Tube to a level between the two marks on the reservoir (refer to the Description of Ubbelhode Viscosimeter, if necessary).

Measure the viscosity of this solution at every three min for a 15 min period, by following step 5 - 9. This will result in 5 measurements in total.

At T = ~ 2.5 min close Ventilating Tube (with finger) and execute suction on Capillary Tube to fill the Capillary Tube up to the Pre-run sphere (refer to the Description of Ubbelhode Viscosimeter, if necessary).

Stop suction, remove finger from Ventilating Tube and start Stopwatch 2 when the liquid reaches the Upper Timing mark (M1).

Read the time on Stopwatch 1 (do not stop stopwatch 1!). This will be Rt1 for the calculations.

Allow the liquid to run down to the Lower Timing mark, M2. Stop Stopwatch 2 when the Lower Timing mark, M2 is reached. This will be Vt1 in the calculation.

Repeat this procedure every three min. Record Rt2 to Rt5 and Vt2 to Vt5.

Repeat Steps 1-9 using a mixture of 15.0 ml substrate and 2.0 ml water as the substrate blank. Record the viscosity as Vtb.

Repeat Steps 1-9 using a mixture of 17.0 ml water as the water blank. Record the viscosity as Vtw.

Repeat Steps 1-9 if the enzyme activity is outside the linear range of the assay, with appropriate dilutions.

Calculations

Calculate the Reaction Time (T1-T5), in sec, accurately to 0.01 sec using the equations:

T1 = {Rt1 + (½ Vt1)}
T2 = {Rt2 + (½ Vt2)}
T3 = {Rt3 + (½ Vt3)}
T4 = {Rt4 + (½ Vt4)}
T5 = {Rt5 + (½ Vt5)}

Calculate the average time for drop in viscosity, for the five measuring points of the substrate blank in sec, using the equation:

Vtbl = (Sum of Vt 1 to 5) / 5

Calculate the average time for drop in viscosity, for the five measuring points of the water blank in sec, using the equation:

Vtw = (Sum of Vt 1 to 5) / 5

For each of the five measuring points of sample, calculate X using the equation:

X = Vtbl / (Vt - Vtw).

For each of the measuring points, plot the T values (obtained in Step 1, x axis) against the corresponding X (obtained in Step 4, y-axis). Calculate the slope according to the linear equation

y = Px + b

From this line calculate the slope, P.

Calculate the enzyme concentration in the incubation mixture as follows:

C = (W / 100) x (0.25 / 5.00)

where

C is Sample Concentration in g/ml
W is Sample weight, g
0.25/5.00 is Dilution Factor
100 is Volume of Dilution, ml

Calculate the enzyme activity of the sample as follows:

β-Glucanase Activity, BGF/g = (P x 60 /0.147) x (17/2) x (1/C) x Sf

where

P is Slope from Step 5
60 is Conversion of sec to min
0.147 is Conversion Factor from definition of Enzyme Activity
17/2 is Correction Factor for incubation mixture
C is Sample Concentration from Step 6

Xylanase activity — The sample shows xylanase activity.
Method information required

POLYVINYL ALCOHOL (PVA)-POLYETHYLENE GLYCOL (PEG) GRAFT CO-POLYMER

New specifications prepared at the 80th JECFA, and published in FAO JECFA Monographs 17 (2015). The 80th JECFA (2015) considered the additive to be of no safety concern for use in food supplements for the functional uses listed.

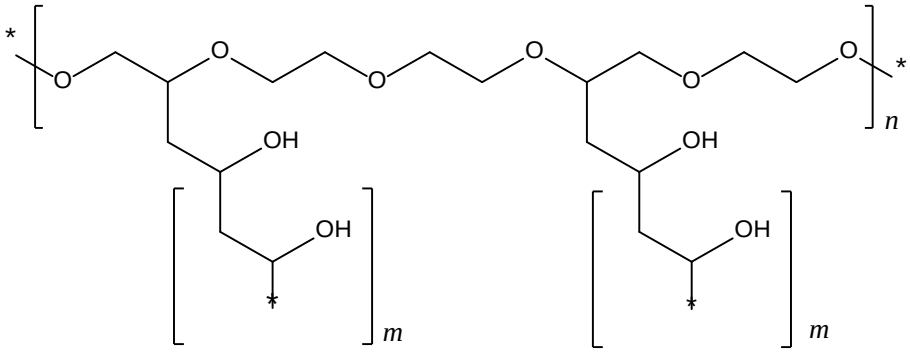
SYNONYMS Macrogol poly(vinyl alcohol) grafted co-polymer; Ethylene glycol and vinyl alcohol graft copolymer; INS No. 1209

DEFINITION Polyvinyl alcohol (PVA)-polyethylene glycol (PEG) graft co-polymer is a graft copolymer of ethylene glycol and vinyl alcohol consisting of approximately 75% vinyl alcohol units and 25% ethylene glycol units. The copolymer is produced by grafting polyvinyl acetate onto a backbone of polyethylene glycol followed by hydrolysis of the polyvinyl acetate side chains to form polyvinyl alcohol grafted side chains. The copolymer may contain colloidal silica at levels of 0.3 to 0.5% to improve flow properties.

Chemical names Polyvinyl alcohol-polyethylene glycol-graft-co-polymer; Poly(ethylene glycol)-graft-poly(vinyl alcohol)

C.A.S number 96734-39-3, 121786-16-1

Structural formula



Molecular weight 40,000 to 50,000 Daltons (weight-average)

DESCRIPTION White to pale yellow powder

FUNCTIONAL USES Glazing agent, binder for tablets, stabilizer

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water, dilute acids, and dilute solutions of alkali hydroxides; practically insoluble in ethanol, acetic acid, and acetone.

pH (Vol. 4) 5.0 to 8.0 (20% soln)

Infrared spectrum (Vol. 4) Dissolve 0.2 g in 20 ml of water, spread a few drops of the solution on a thallium bromoiodide plate and evaporate the solvent at 110° for 30 min. The infrared absorption spectrum of the sample corresponds to that of the reference spectrum in Appendix.

Film formation	Dissolve 0.4 g in 2 ml of water. Place 1 ml of the solution on a glass plate and allow to dry. A transparent film is formed.
Viscosity – Rotational method	Less than 250 mPa·s Determine using a 20% solution (m/m), use a viscometer at 25° with a Brookfield RV2 spindle, and a rotation speed of 100 rpm.

PURITY

Loss on drying (Vol. 4)	Not more than 5%. (1.0 g sample, vacuum (<20 mmHg) at 140°, 1 h)
Sulfated ash (Vol. 4)	Not more than 2% (5 g, 650°)
Ester value	Not less than 10 and not more than 75 mg KOH/g See description under TESTS
Vinyl acetate	Not more than 20 mg/kg See description under TESTS
Acetate	Not more than 1.5% See description under TESTS
1,4-Dioxane	Not more than 10 mg/kg See description under TESTS
Ethylene oxide	Not more than 0.2 mg/kg See description under TESTS
Ethylene glycol and diethylene glycol	Not more than 400 mg/kg (singly or in combination) See description under TESTS
Lead (Vol. 4)	Not more than 1 mg/kg Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4 (under “General Methods, Metallic Impurities”).

TESTS

PURITY TESTS

Ester value

Determine the acid value (I_A) as follows. Dissolve 5.00 g of sample in 100 ml of distilled water while stirring with a magnetic stirrer. Titrate with 0.01 M alcoholic potassium hydroxide, determining the end-point potentiometrically. Carry out a blank determination under the same conditions.

$$I_A = \frac{0.561(n_1 - n_2)}{m}$$

Where

n_1 is the volume of titrant used in the test, ml
n_2 is the volume of titrant used in the blank, ml
m is the mass of the sample, g

Determine the saponification value (I_S) as follows. Place 5.00 g of sample in a 250 ml borosilicate glass flask fitted with a reflux condenser. Add 50.0 ml of 0.5 M alcoholic potassium hydroxide and stir vigorously with a magnetic stirrer. Attach the condenser and heat under reflux for 30 min. Add 1 ml of phenolphthalein solution (0.1% (w/w) Phenolphthalein in ethanol) and titrate the excess potassium hydroxide immediately (while still hot) with 0.5 M hydrochloric acid. Carry out a blank determination under the same conditions.

$$I_S = \frac{28.05(n_1 - n_2)}{m}$$

Where

n_1 is the volume of titrant used in the test, ml
n_2 is the volume of titrant used in the blank, ml
m is the mass of the sample, g

The ester value (I_E) is calculated from the saponification value (I_S) and the acid value (I_A):

$$I_E = I_S - I_A$$

Vinyl Acetate

Principle

Determine by liquid chromatography using 1-vinylpyrrolidine-2-one as an internal standard.

Equipment

High performance liquid chromatograph equipped with gradient valve and UV detector
Column: Size: 4.6 mm x 25 cm
Stationary phase: end-capped octadecylsilyl silica gel for chromatography with embedded polar groups (5 µm)
Column temperature: 30 °
A precolumn containing octadecylsilyl silica gel for chromatography (5 µm) may be used if a matrix effect is observed.

Reagents

Vinyl acetate (99.9% purity, Fluka Part No. 46060, or equiv.)
1-vinylpyrrolidin-2-one (>99%, Sigma-Aldrich Part No. V3409, or equiv.)
Acetonitrile, methanol and deionized water (HPLC grade)

Procedure

Preparation of internal standard solution:

Accurately weigh about 5 mg of 1-vinylpyrrolidin-2-one, add 10 ml of methanol and dilute to 50 ml with water in a volumetric flask.

Preparation of standard solution

Accurately weigh about 5.0 mg of vinyl acetate, quantitatively transfer into a 50 ml volumetric flask using 5 ml methanol, add 10 ml of internal standard solution and dilute to volume with water.

Preparation of sample solution

Accurately weigh about 250 mg of sample, transfer to a 10-ml volumetric flask with about 2 ml of methanol, and ultrasonicate, if necessary. After cooling to ambient temperature, dilute with water to volume, and mix. Pass through a 0.2-micron filter.

Mobile phase
Mobile phase A: acetonitrile, methanol, water (5:5:90 V/V/V);
Mobile phase B: methanol, acetonitrile, water (5:45:50 V/V/V);

Time (min)	Solution A (% v/v)	Solution B (% v/v)
0-2	100	0
2-40	100 → 85	0 → 15
40-42	85 → 0	15 → 100

Flow rate: 1.0 ml/min
Detection: 205 nm
Injection: 10 µl
Retention time: Vinyl acetate = about 19 min; 1-vinylpyrrolidin-2-one = about 25 min.

System suitability
Resolution of about 5.0 between the peaks of vinyl acetate and 1-vinylpyrrolidin-2-one shall be obtained.

Analysis
Analyze standard solution and calculate the peak ratio between vinyl acetate and 1-vinylpyrrolidin-2-one peaks. Analyze the sample solution and calculate the peak area ratio between vinyl acetate and 1-vinylpyrrolidin-2-one peaks in the sample solution.

Obtain the concentration of vinyl acetate in the sample from the peak area ratios of sample & internal standard, standard & internal standard, concentration of standard, and weight of sample.

Acetate

Principle
Determine by liquid chromatography using citric acid (internal standard).

Equipment
High performance liquid chromatograph equipped with gradient valve and UV detector;
Column: Size: 4.6 mm x 25 cm
Stationary phase: end-capped octadecylsilyl silica gel for chromatography with embedded polar groups (5 µm)
Column temperature: ambient
A precolumn containing octadecylsilyl silica gel for chromatography (5 µm) may be used if a matrix effect is observed.

Reagents
Acetic acid standard (>99%)
Citric acid standard (>99%)
Deionized water (HPLC grade)

Procedure
Mobile phase
0.50 g/l solution of sulfuric acid

Preparation of internal standard solution
Accurately weigh about 30 mg of citric acid and dilute to 100 ml with mobile phase in a volumetric flask.
Preparation of standard solution

Accurately weigh about 30 mg of acetic acid, add 10 ml of internal standard solution and dilute to 50 ml in a volumetric flask with mobile phase.

Preparation of system suitability solution
Accurately weigh about 30 mg of citric acid, 30 mg of acetic acid, and dilute to 50 ml in a volumetric flask with mobile phase.

Preparation of sample solution
Accurately weigh about 200 mg of sample, add 2 ml internal standard solution and make up to 10 ml in a volumetric flask with mobile phase. Pass through a 0.2-micron filter.

Analysis
Chromatographic conditions
Flow rate: 1 ml/min
Detector: UV 205 nm
Injection: 20 µl
Retention time: acetate = about 5 min; citrate = about 7 min.
After each injection, rinse column with mixtures of equal volumes of acetonitrile and mobile phase.

System suitability
Resolution of about 2.0 between the peaks of acetate and citrate shall be obtained.

Inject standard solution and calculate the peak area ratio between acetate and citrate peaks. Inject sample solution and calculate the peak area ratio between acetate and citrate peaks in the sample solution.

Obtain the concentration of acetate in the sample from the peak area ratios of sample & internal standard, standard & internal standard, concentration of standard, and weight of sample.

Ethylene oxide

Principle
Determine by dynamic headspace (purge & trap) gas chromatography using acetaldehyde as internal standard

Equipment
Gas chromatograph equipped with flame ionization detector and suitable purge & trap system;
Detector temperature: 250°
Column: 0.32-mm × 30-m fused silica capillary column; 1.0-µm layer of dimethylpolysiloxane (e.g., DB-1 from J&W Scientific)

[**Caution—**Ethylene oxide is toxic and flammable. Prepare these solutions in a well-ventilated fume hood, using great care. Protect both hands and face by wearing polyethylene protective gloves and an appropriate face mask. Store all solutions in hermetic containers, and refrigerate at a temperature between 4° and 8°.]

Reagents
Ethylene oxide (99.8% purity, Messer Griesheim Part No. 1284, or equiv.)
Acetaldedhyde (99.9%, Fluka Part No. 00070, or equiv.)

Polyethylene glycol 200 (PEG 200, Fluka Part no. 81150 with a specific gravity of 1.127 g/cm^3 or equiv.)
Deionized water (HPLC grade)
Defoamer (Agitan 281, Münzing Chemie GmbH, Heilbronn)

Procedure

Preparation of ethylene oxide standard stock solution

Add about 25 ml of PEG 200 into a 50 ml volumetric flask and accurately weigh the flask. Pass about 100 mg of gaseous ethylene oxide through polyethylene glycol 200 (PEG 200) and weigh. Calculate the mass of ethylene oxide absorbed by PEG 200 from the difference in weight. Dilute to volume with PEG 200 and weigh the flask. Calculate the amount of ethylene oxide per gram of solution (approximately 1.8 mg/g)

Preparation of ethylene oxide working standard solution-1

Add about 25 ml of PEG 200 into a 50 ml volumetric flask and weigh the flask. Add about 1 g of ethylene oxide stock solution to the volumetric flask and weigh. Dilute to volume with PEG 200. Calculate the concentration of ethylene oxide in the working standard solution from the weight (approximately 32 µg/g).

Preparation of ethylene oxide working standard solution-2

Add about 25 ml of PEG 200 into a 50 ml volumetric flask and weigh the flask. Add about 1 g of ethylene oxide working standard solution 1 to the volumetric flask and weigh. Dilute to volume with PEG 200. Calculate the concentration of ethylene oxide in the working standard solution from the weight (approximately 0.57 µg/g).

Preparation of acetaldehyde internal standard solution

Accurately weigh about 40 mg of acetaldehyde (to the nearest 0.1 mg), dissolve in water and dilute to 100 ml with water. Pipet 5 ml into a 50 ml volumetric flask and dilute to volume with water (approximately 40 µg/ml).

Preparation of sample solution

Accurately weigh about 0.5 g sample into a headspace vial containing 4.0 ml water and add 50 µl of defoamer. Add 1 ml of internal standard solution. Seal the vial and mix thoroughly.

System suitability test solution

Add 1 ml of the acetaldehyde solution and 0.1 ml of the ethylene oxide working solution in a headspace vial and add 4 ml of water. Seal the vial and mix thoroughly.

Blank solution

Add 50 µl defoaming agent to 1.0 ml of internal standard solution, and 4 ml of water and seal the vial.

Standard solution

Accurately weigh 0.1 g of ethylene oxide working standard solution (approximately 0.057 µg ethylene oxide), add 1.0 ml of internal standard, 4.0 ml water and add 50 µl of defoamer. Seal the vial and mix thoroughly.

Procedure

Chromatographic conditions

Column temperature: See the temperature program table below.

Initial temperature (°)	Temperature ramp (°/min)	Final temperature (°)	Hold time at final temperature (min)
50	-	50	5
50	5	180	-
180	30	230	5

Carrier gas: Helium
Column head pressure: 0.8 ml/min
Injection type: Splitless mode
Injection port temperature: 250°

Dynamic Headspace autosampler
Follow manufacturer's recommended conditions

Analysis

Run blank solution. Ethylene oxide peak shall be either absent or below limit of detection. Run system suitability standard solution.

System suitability

Retention time: acetaldehyde = about 5.8 min; ethylene oxide = about 6.2 min.
Resolution: Not less than 1.5 between acetaldehyde and ethylene oxide
Signal-to-noise: Not less than 10 determined from the ethylene oxide peak

Analyze standard solution and calculate the peak ratio between ethylene oxide and acetaldehyde peaks. Analyze the sample solution and calculate the peak area ratio between ethylene oxide and acetaldehyde peaks in sample solution.

Obtain the concentration of ethylene oxide in the sample from the peak area ratios of sample & internal standard and standard & internal standard, amount of standard and weight of sample taken in the headspace vial.

1,4-Dioxane

[Caution: 1,4-dioxane is used as a solvent in the method for the determination of ethylene glycol and diethylene glycol. Take the necessary precautions to ensure that cross-contamination does not occur.]

Principle

Determine by headspace gas chromatography as directed in Residual Solvents by Headspace Gas Chromatography (Vol. 4) using the following:

Reagents

1, 4 dioxane (99.8% Sigma Aldrich Part No. 296309, or equiv.)
Deionized water (HPLC grade)
N,*N*-dimethylacetamide (≥99.9% Fluka Part No. 44901, or equiv.)
Defoamer (Agitan 281, Münzing Chemie GmbH, Heilbronn)

Preparation of stock standard solution (500 ug/ml): Accurately weigh about 25 mg of 1,4-Dioxane into a 50 ml volumetric flask and make up to volume with water.

Preparation of working standard solution (50 ug/ml): Pipet 5 ml stock standard solution into a 50 ml volumetric flask and make up to volume with water.

System suitability standard
Accurately weigh 0.5 g of sample to a 10-ml pressure headspace vial. Add 1.0 ml of Working standard solution and 1.0 ml of *N*,*N*-dimethylacetamide, seal the vial, and mix. Inject 1 ml.

Standard in headspace vial
Pipet 1.0 ml of 1,4-Dioxane working standard solution into a 10 ml headspace vial and add 1.0 ml of *N*,*N*-dimethylacetamide, seal the vial, and mix. Inject 1 ml.

Sample in headspace vial
Accurately weigh 0.5 g of sample to a 10-ml pressure headspace vial. Add 1.0 ml of *N*,*N*-dimethylacetamide and 1.0 ml of water, seal the vial, and mix. Inject 1 ml.

System suitability
Run system suitability standard.
The signal-to-noise shall not be less than 5 determined from the 1,4-Dioxane peak, and the relative standard deviation is not more than 15% (6 analyses).

Calculation
Analyze the standard and sample solutions using the analytical conditions for Residual Solvents by Headspace Gas Chromatography as described in Vol. 4. Calculate the concentration of 1,4-Dioxane in the sample from the peak areas of standard, sample, and amount of standard and sample in the headspace vials.

Ethylene glycol and diethylene glycol

Principle
Determine by gas chromatography after derivatization with N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA), and following the standard addition method.

Equipment
Capillary gas chromatograph with autosampler, split injector and flame ionization detector (FID)
Fused silica capillary column (0.25-mm × 30-m) coated with 0.25 µm layer of 14% cyanopropylphenyl-86%-dimethylpolysiloxane, (DB-1701, J&W Scientific, or equiv.)

Reagents
1,4- Dioxane (Purity 99.0 %, minimum J.T. Baker, part. no. 9231, or equiv.)
Ethylene glycol (EG) (Purity 99.5%, e.g. Fluka, part. no. 03750, or equiv.)
Propylene glycol (PG) (Purity >99.5% (GC), e.g. ABCR, part. no. AB207089, or equiv.)
Diethylene glycol (DEG) (Purity 99.9%, Sigma-Aldrich, part. no.

03128, or equiv.)
N-Methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) (Macherey & Nagel part. no. 701270.1100, or equiv.)

Procedure
[The method described involves the handling of hazardous substances. Attention is drawn to the handling of potentially dangerous materials]

Preparation of ethylene glycol and diethylene glycol standard solution
Accurately weigh about 50 mg each of ethylene glycol and diethylene glycol in a 25 ml volumetric flask and make to volume with 1,4-dioxane. Pipette 1 ml of solution into a 10 ml volumetric flask and make to volume with 1,4-dioxane. This yields a standard solution containing 0.2 mg/ml of ethylene glycol and 0.2 mg/ml diethylene glycol.

Preparation of ethylene glycol and propylene glycol standard solution
Accurately weigh about 100 mg each of ethylene glycol and propylene glycol in a 25 ml volumetric flask and make to volume with 1,4-dioxane. Pipette 1 ml of solution into a 10 ml volumetric flask and make to volume with 1,4-dioxane. This yields a standard solution containing 0.4 mg/ml of ethylene glycol and 0.4 mg/ml propylene glycol.

Preparation of stock sample solution
Accurately weigh about 500 mg of sample to the nearest 0.01 mg into a 10 ml volumetric flask, dissolve in 1,4-dioxane and make to volume.

Preparation of sample solution
Pipette 1 ml of stock sample solution into a reaction vial, add 1 ml of 1,4-dioxane, 2 ml of MSTFA and derivatize at 90° for 1 h. Inject and analyze 2 µl of the solution to determine an approximate amount of ethylene glycol and diethylene glycol present in the sample.

Preparation of system suitability solution A
Pipette 1 ml of ethylene glycol and diethylene glycol standard solution into a reaction vial, add 1 ml of 1,4-dioxane, 2 ml of MSTFA and derivatize at 90° for 1 h.

Preparation of system suitability solution B
Pipette 2 ml of ethylene glycol and propylene glycol standard solution into a reaction vial, add 2 ml of MSTFA and derivatize at 90° for 1 h.

Preparation of standard addition solutions
Pipette 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml and 0.6 ml of the ethylene glycol and diethylene glycol standard solution, respectively, into five separate reaction vials. Pipette 1 ml of stock sample solution into each reaction vial. Add 2 ml of MSTFA, and enough 1,4-dioxane to give a total volume of 4 ml in each reaction vial. Derivitize all solutions at 90° for 1 h.

Chromatographic system
Column temperature: See the temperature program table below.

Initial temperature (°)	Tempera-ture ramp (°/min)	Final tempe-rature (°)	Hold time at final tem-perature (min)
100	-	100	5
100	5	125	-
125	30	300	5
300	-	300	15

Injector temperature: 300°
Detector temperature: 300°
Carrier gas: Helium
Column inlet pressure: 8.7 psi
Split: 10 ml/min (adapted to the sensitivity of the system as necessary)
Septum purge: 3 ml/min
Injection volume: 2 µl

Ethylene glycol has a retention time of approximately 5.6 min
Propylene glycol has a retention time of approximately 5.8 min
Diethylene glycol has a retention time of approximately 11.9 min

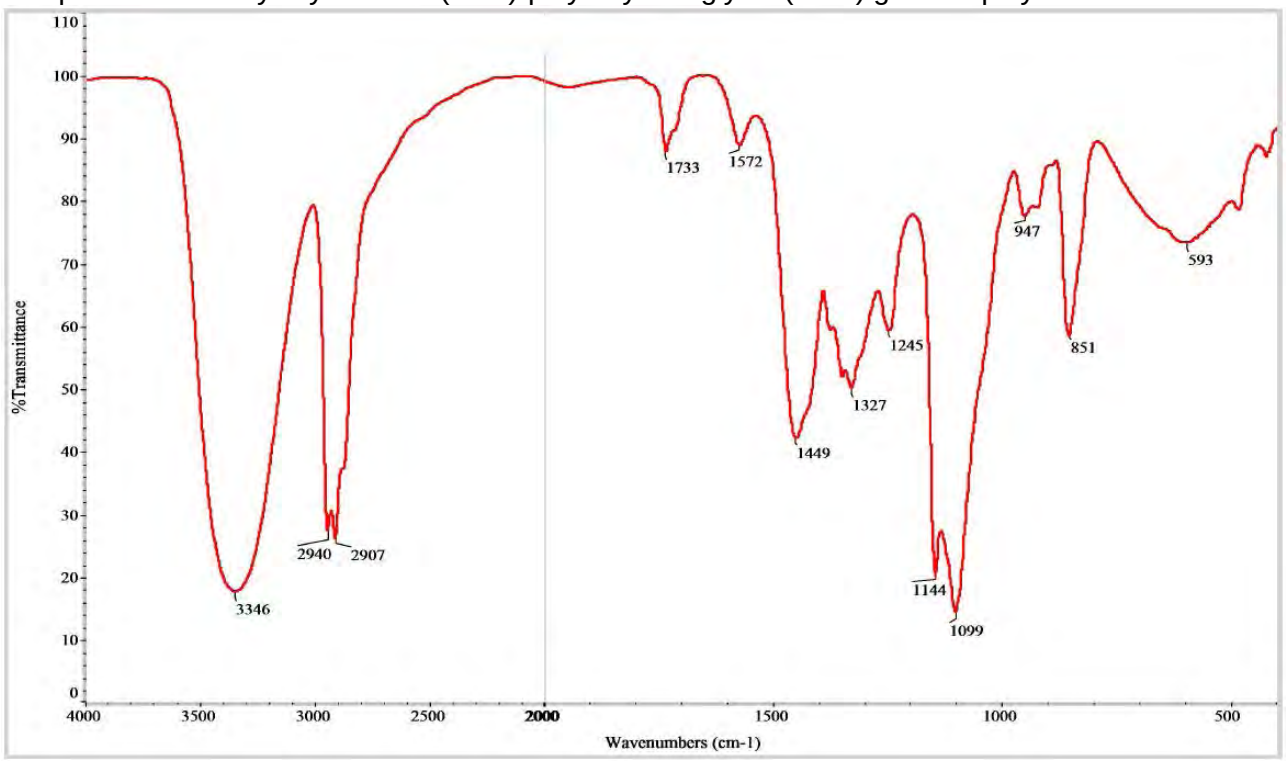
System suitability
Run Standard solution A. The system is suitable with a signal to noise ratio less than or equal to 10 for the signals of ethylene glycol and diethylene glycol

Run Standard solution B. The resolution shall not be less than 1.5 between the peaks of ethylene glycol and propylene glycol.

Analysis
Inject the derivatized standard addition solutions. Construct the standard curves by plotting the spiked mass (µg) of ethylene glycol or diethylene glycol on the x-axis versus the respective peak area on the y-axis, Deduce the amount of ethylene glycol or diethylene glycol in the sample (in the vial) from the x-intercept of the standard curve. Calculate the concentration in the sample from the amount of ethylene glycol or diethylene glycol determined from the standard curve, and the mass of sample in the vial.

Appendix

IR Spectrum for Polyvinyl alcohol (PVA)-polyethylene glycol (PEG) graft co-polymer



SILICON DIOXIDE, AMORPHOUS (TENTATIVE)

Prepared at the 80th JECFA and published in FAO JECFA Monographs 17 (2015), superseding tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI 'not specified' for silicon dioxide and certain silicates was established at the 29th JECFA (1985).

Information required on:

- *Raw materials used and methods of manufacture for different forms of silicon dioxide (pyrogenic silica, precipitated silica, hydrated silica, silica aerogel and colloidal silica)*
- *Identification methods allowing the differentiation between the above forms of silicon dioxide*
- *Functional uses of different forms, and information on the types of products in which it is used and the use levels in these products*
- *Data on solubility using the procedure documented in "Compendium of Food Additives Specifications, Vol.4, Analytical methods"*
- *Data on the impurities soluble in 0.5 M hydrochloric acid for all forms of silicon dioxide used as food additives, from a minimum of five batches. If a different extraction and determination method is used, provide data along with details of method and QC data.*
- *Suitability of the analytical method for the determination of aluminium, silicon and sodium using the proposed "Method of assay" along with data, from a minimum of five batches. If a different method is used, provide data along with details of the method and QC data.*
- *In addition to the above information, data on pH, loss on drying and loss on ignition for hydrated silica, silica aerogel and colloidal silica*

SYNONYMS

Silica; INS No. 551

DEFINITION

Silicon dioxide is an amorphous substance, which is produced synthetically by either a vapour-phase hydrolysis process, yielding pyrogenic (fumed) silica, or by a wet process, yielding precipitated silica (silica gel). Pyrogenic silica is produced in an anhydrous state, whereas the wet process products are obtained as hydrates or contain surface absorbed water.
(information required on hydrated silica, silica aerogel and colloidal silica)

Chemical names	Silicon dioxide	
C.A.S. number	7631-86-9	
Chemical formula	$(SiO_2)_x$	
Formula weight	60.08 (SiO_2)	
Assay	Pyrogenic (fumed) silica:	Not less than 99% of SiO_2 on the ignited basis
	Precipitated silica (silica gel):	Not less than 94% of SiO_2 on the ignited basis

	Hydrated silica:	*Information required*
	Silica aerogel:	*Information required*
	Colloidal silica:	*Information required*
DESCRIPTION	Pyrogenic silica:	a pyrogenic silicon dioxide occurring as a fine, white amorphous power or granules
	Precipitated silica (silica gel):	a precipitated, hydrated silicon dioxide occurring as a fine, white, amorphous powder, or as beads or granules
	Hydrated silica:	*Information required*
	Silica aerogel:	*Information required*
	Colloidal silica:	*Information required*
FUNCTIONAL USES	Anticaking agent (information on other functional uses required)	
CHARACTERISTICS		
IDENTIFICATION		
Identification of different forms	*Information required*	
Solubility (Vol. 4)	Insoluble in water and insoluble in ethanol (*Information required)*	
Test for silicon	Passes test See description under TESTS	
PURITY		
pH (Vol. 4)	Pyrogenic silica and precipitated silica: 3.5 – 7.5 (5% slurry) *Information required for other forms*	
Loss on drying (Vol. 4)	Pyrogenic silica:	Not more than 2.5% (105°, 2 h)
	Precipitated silica (silica gel):	Not more than 8% (105°, 2 h)
	Hydrated silica:	*Information required*
	Silica aerogel:	*Information required*
	Colloidal silica:	*Information required*
Loss on ignition (Vol. 4)	Pyrogenic silica:	Not more than 2.5% (1000°,1 h) on dried sample
	Precipitated silica, silica gel and hydrated silica:	Not more than 8.5% (1000°,1 h) on dried sample
	Hydrated silica:	*Information required*
	Silica aerogel:	*Information required*
	Colloidal silica:	*Information required*
Impurities soluble in 0.5 M hydrochloric acid	Lead : Not more than 5 mg/kg *(Information required)* Arsenic: Not more than 3 mg/kg *(Information required)* See description under TESTS	

TESTS

IDENTIFICATION TESTS

Identification tests for different forms — *Information required*

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

PURITY TESTS

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

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

METHOD OF ASSAY

Accurately weigh an appropriate quantity of the sample, depending on the moisture content) equivalent to about 0.5 g of dried sample, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, mix and melt completely using a torch burner and allow to stand at room temperature. Place the reaction product along with crucible into 150 ml hot deionized water in a 250-ml PTFE beaker and dissolve residue by agitation. Wash the crucible with hot deionized water and remove it. Add 50 ml hydrochloric acid and transfer the contents into a 250-ml polypropylene volumetric flask. Wash the beaker three times with hot deionized water. Transfer the washings to the volumetric flask and make up to volume (Solution A). Prepare the test solution by diluting Solution A with 2% hydrochloric acid, to get the readings within the standard curve range. Analyze silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use the analytical line for Si (251.611 nm) and construct standard curve using standard solutions 0.1 – 5.0 µg/ml. Read the concentration of Si in test solution (as µg/ml) and calculate the silicon dioxide content of the sample using the formula:

$$SiO_2\,(\%) = \frac{2.139 \times C \times 250 \times DF}{W \times 10^6} \times 100$$

Where

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

SODIUM ALUMINIUM SILICATE (TENTATIVE)

Prepared at the 80th JECFA and published in FAO JECFA Monographs 17 (2015), superseding tentative specifications prepared at the 77th JECFA (2013) and published in FAO JECFA Monographs 14 (2013). An ADI 'not specified' for silicon dioxide and certain silicates was established at the 29th JECFA (1985). A PTWI of 2 mg/kg bw for total aluminium was established at the 74th JECFA (2011). The PTWI applies to all aluminium compounds in food, including food additives.

Information required:

- *Functional uses other than anticaking agent, if any, and information on the types of products in which it is used and the use levels in these products*
- *Data on solubility using the procedure documented in the "Compendium of Food Additives Specifications, Vol. 4, Analytical methods"*
- *Data on the impurities soluble in 0.5 M hydrochloric acid, from a minimum of five batches. If a different extraction and determination method is used, provide data along with details of method and QC data.*
- *Suitability of the analytical method for the determination of aluminium, silicon and sodium using the proposed "Method of assay" along with data, from a minimum of five batches, using the proposed method. If a different method is used, provide data along with details of the method and QC data.*

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

DEFINITION Sodium aluminium silicate is a series of amorphous hydrated sodium aluminium silicates with varying proportions of Na_2O, Al_2O_3 and SiO_2. It is manufactured by, precipitation process, reacting aluminium sulphate and sodium silicate.

Chemical names Aluminium sodium silicate

C.A.S. number 1344-00-9

Chemical formula $xSiO_2 \cdot yAl_2O_3 \cdot zNa_2O$

Assay Not less than 66% and not more than 88% as silicon dioxide (SiO_2), not less than 5% and not more than 15% as aluminium oxide (Al_2O_3) and not less than 5% and not more than 8.5% as sodium oxide (Na_2O); on the ignited basis.

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

FUNCTIONAL USES Anticaking agent (information on other functional uses required)

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)	Information required
Test for sodium	Passes test See description under TESTS
Test for aluminium	Passes test See description under TESTS
Test for silicon	Passes test See description under TESTS

PURITY

pH (Vol. 4)	6.5 – 11.5 (5% slurry)
Loss on drying (Vol. 4)	Not more than 8.0% (105^{o}, 2h)
Loss on ignition (Vol. 4)	Not less than 5.0% and not more than 11.0% on the dried basis (1000^{o}, constant weight)
Impurities soluble in 0.5 M hydrochloric acid	Lead : Not more than 5 mg/kg *(information required)* Arsenic: Not more than 3 mg/kg *(information required)* See description under TESTS

TESTS

IDENTIFICATION TESTS

Test for aluminium, sodium and silicon	Prepare the test solution as shown under method of assay. Analyze aluminium and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer, use analytical lines for Al (396.15 nm), Na (589.52 nm) and Si (251.611 nm).

PURITY TESTS

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

METHOD OF ASSAY Weigh about 0.5 g of the sample to the nearest 0.1 mg, in a platinum or nickel crucible, add 5 g potassium hydroxide and 2 g boric acid, Mix and melt completely using a torch burner and allow to stand at room temperature. Place the reaction product along with crucible into 150 ml hot deionized water in a 250-ml PTFE beaker and dissolve residue by agitation. Wash the crucible with hot deionized water and remove it. Add 50 ml hydrochloric acid and transfer the contents into a 250-ml polypropylene volumetric flask. Wash the beaker three times with hot deionized water, transfer the washings to the volumetric flask and make up to volume. Dilute with 2% hydrochloric acid and prepare the test solution. Analyse aluminium and silica in the test solution by ICP-AES technique (Vol. 4). Set instrument parameters as specified by the instrument manufacturer. Use analytical lines for Al (396.152 nm), Si (251.611 nm) and Na (589.52 nm). Construct standard curve using standard solutions 0.2 – 5.0 µg/ml each. Read the concentration of Al and Si in sample solution (as µg/ml). Conduct as a blank determination following the above procedure. Calculate the content of aluminium oxide, sodium oxide and silicon dioxide in the sample using the formula:

$$Al_2O_3\,(\%) = \frac{1.889 \times (C - B) \times 250 \times DF}{W \times 10^6} \times 100$$

$$Na_2O\,(\%) = \frac{1.348 \times (C - B) \times 250 \times DF}{W \times 10^6} \times 100$$

$$SiO2\,(\%) = \frac{2.139 \times (C - B) \times 250 \times DF}{W \times 10^6} \times 100$$

Where:

C is concentration of Al or Na or Si in the test solution, µg/ml
B is concentration of Al or Na or Si in the blank solution, µg/ml
W is weight of sample on the ignited basis, g
DF is dilution factor

ANALYTICAL METHODS

The existing analytical method for determination of *Residual solvents* was revised and renamed by the Committee at its 80th meeting. This revised method *Residual solvents by headspace gas chromatography* replaces also the tentative method for determination of residual solvents in certain annatto extracts proposed by the Committee at its 77th meeting.

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

Residual solvents by headspace gas chromatography

The method describes the use of water or methanol as the solvent in which the sample is dissolved. Individual specifications may also recommend the use of other solvents for this determination such as dimethylformamide (DMF) or N,N-dimethylacetamide. In addition individual specifications may also recommend the use of different methods of preparation of the standard solutions. Acceptable conditions for the separation of residual solvents are listed below.

The solvents listed in the table below can be determined by this method based on headspace gas chromatography. The method may also be used for the determination of isobutyl acetate and methyl acetate. However, information on the approximate retention time for these two solvents is not available.

Solvent	Approximate retention time (min)
Ethanal	2.81
Methanol	2.93
Ethanol	4.09
Ethanenitrile	4.55
Propanone	4.76
2-Propanol	5.23
Ethoxyethane	5.67
2-Methyl-2-propanol	6.21
Dichloromethane	6.45
1-Propanol	7.78
Trimethylsilanol	8.41
2-Butanol	9.61

Solvent	Approximate retention time (min)
Ethylacetate	10.05
Chloroform	10.33
2-Methyl-1-propanol	11.05
1-Butanol	12.79
Hexamethyldisiloxane	14.42
Propylacetate	14.97
4-Methyl-2-pentanone	16.18
Pyridine	16.39
3-methyl-2-pentanone	16.90
Toluene	18.25
Butylacetate	20.61

Reagents:

Blank: sample with very low solvent content
Internal standard: 3-methyl-2-pentanone
Methanol
Demineralised water

Method I (Determination carried out in water)

Internal standard solution: Add 50.0 ml water to a 50 ml injection vial and seal. Accurately weigh and inject 15 µl 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

Blank solution: Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of water and 1.0 ml of the internal standard solution. Heat at 60° for 10 min and shake vigorously for 10 sec.

Samples: Weigh accurately 0.20 g sample into an injection vial. Add 5.0 ml water and add 1.0 ml of the internal standard solution. Heat at 60° for 10 min and shake vigorously for 10 sec.

Calibration solution: Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of the water and 1.0 ml of the internal standard solution. Weigh the vial accurately to within 0.01 mg. Inject a known volume of the component of interest through the septum and again reweigh the vial. Heat at 60° for 10 min and shake vigorously for 10 sec.

Method II (Determination carried out in methanol)

Internal standard solution: Add 50.0 ml methanol to a 50 ml injection vial and seal. Accurately weigh and inject 15 µl 3-methyl-2-pentanone through the septum and reweigh to within 0.01 mg.

Blank solution: Weigh accurately 0.20 g of the blank into an injection vial. Add 5.0 ml of methanol and 1.0 ml of the internal standard solution. Heat at 60° for 10 min and shake vigorously for 10 sec.

Samples: Weigh accurately 0.20 g sample into an injection vial. Add 5.0 ml methanol and add 1.0 ml of the internal standard solution. Heat at 60° for 10 min and shake vigorously for 10 sec.

Calibration solution: Solution A: Add 50.0 ml methanol to a 50 ml vial and seal. Accurately weigh, to within 0.01 mg, the vial and inject 50 µl of the component of interest through the septum. Reweigh the vial. Mix well.

Weigh into an injection vial, a known amount of blank (0.20 g), add 4.9 ml of methanol and 1.0 ml internal standard solution. Introduce 0.1 ml of Solution A into the injection vial. Mix well and heat at 60° for 10 min and shake vigorously for 10 sec.

Procedure:

Place the sample, blank and calibration samples in the sample tray of the head-space gas chromatograph – FID system. Analyse using the following analytical conditions.

Column: Fused silica, length 0.8 m, i.d. 0.53mm, coated with DB-wax, film thickness 1 µm

Coupled with: Fused silica, length 30 m, i.d. 0.53 mm, coated with DB-1, film thickness 5 µm

Conditions:

Carrier gas: Helium
Flow rate: 208 kPa, 5 ml/min
Detector: FID
Temperatures
- Injector: 140°
- Oven conditions: 35° for 5 min, then 5°/min to 90°, then 6 min at 90°
- Detector: 300°

Head space sampler
Sample heating temperature: 60°
Sample heating period: 10 min
Syringe temperature: 70°
Transfer temperature: 80°
Sample gas injection: 1.0 ml in split mode

Calculation

$$A \times B \times C / 50 = \text{mg component per injection vial}$$

Where:

A = relative peak area of the component concerned
B = mg internal standard
C = calibration factor

Determination of calibration factors

Method 1:

$$C = D \times 50 / (E \times (F - G))$$

Method 2:

$$C = D / (E \times (F - G) \times 10)$$

Where:

D = mg component weighed
E = mg internal standard
F = relative peak area of component for the calibration solution
G = relative peak area of the same component for the blank solution

Annex I: Summary of recommendations from the 80th JECFA

Food additives considered for specifications only

Food additive	Specifications
Advantame	R[a]
Aluminium silicate	W[b]
Annatto extract (solvent-extracted bixin)	R[c]
Annatto extract (solvent-extracted norbixin)	R[c]
Calcium aluminium silicate	W[b]
Calcium silicate	R[d]
Glycerol ester of gum rosin	W[b]
Silicon dioxide, amorphous	R, T[e]
Sodium aluminium silicate	R, T[f]

R: existing specifications revised; T: tentative specifications; W: tentative specifications withdrawn

[a] The requested information was received, and the method of assay was revised. The tentative status of the specifications was removed.

[b] The requested information was not received.

[c] The specifications were revised to reflect the modification of the method for residual solvents by headspace gas chromatography and to include sample and standard preparation information.

[d] The requested information was received, and the specifications were revised to include information on functional uses, pH, loss on drying, loss on ignition, impurities soluble in 0.5 M hydrochloric acid and the assay. The tentative status of the specifications was removed

[e] Limited information was received. The specifications were revised to include information on pH, loss on drying, loss on ignition, impurities (lead and arsenic) soluble in 0.5 M hydrochloric acid and the assay for some forms of silicon dioxide. The tentative status of the specifications was maintained, and information was requested in order for the tentative specifications to be revised (see Annex 3).

[f] Limited information was received. The specifications were revised to include information on Chemical Abstracts Service number, chemical formula, pH, loss on drying, loss on ignition and limits on impurities (lead and arsenic) soluble in 0.5 M hydrochloric acid. The tentative status of the specifications was maintained, and information was requested in order for the tentative specifications to be revised (see Annex 3).

Food additives evaluated toxicologically and/or assessed for dietary exposure

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
Benzoates: dietary exposure assessment	NA	Based on the available data set, the Committee noted that there is consistency in the average typical range of concentration levels for benzoates reported to be used or analysed in non-alcoholic (“soft”) beverages (Codex General Standard for Food Additives [GSFA] food category 14.1). For example, typical reported concentration levels from industries ranged from 83 to 209 mg/L, and analytically quantified measurements ranged from 63 to 259 mg/L in GSFA food category 14.1.4; these levels are lower than national maximum limits (150–400 mg/L) or limits for GSFA food category 14.1.4 (600 mg/L). The Committee also noted that most of the reported estimates for mean and high percentile benzoate exposure were below the ADI of 0–5 mg/kg body weight (bw), expressed as benzoic acid, despite different methodologies and assumptions applied in the preparation of the exposure estimates. None of the mean exposure estimates for consumers of non-alcoholic (“soft”) beverages exceeded the upper bound of the ADI: 0.3–4.1 mg/kg bw per day for toddlers and young children, 0.2–2.7 mg/kg bw per day for other

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
		children including adolescents, and 0.1–1.7 mg/kg bw per day for adults. However, the Committee noted that the 95th percentile exposures for the consumers-only group exceeded the upper bound of the ADI in some cases: up to 10.9 mg/kg bw per day for toddlers and young children and up to 7.0 mg/kg bw per day for other children including adolescents. Additionally, the Committee noted that in some countries, the overall dietary exposure to benzoates for toddlers, young children and adolescents also exceeds the upper bound of the ADI at the high percentiles. Reduction of those exposures exceeding the upper bound of the ADI would require consideration of dietary patterns for both beverage and non-beverage foods containing benzoates and typical/allowed benzoate use levels in those countries.
Lipase from *Fusarium heterosporum* expressed in *Ogataea polymorpha*	N	No treatment-related adverse effects were seen at the highest dose tested (669 mg total organic solids [TOS]/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.5 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 669 mg TOS/kg bw per day results in a margin of exposure (MOE) of at least 1300. **The Committee established an ADI "not specified"[a] for lipase from *F. heterosporum* expressed in *O. polymorpha* when used in the applications specified and in accordance with good manufacturing practice.**
Magnesium stearate	N	The Committee estimated the potential total dietary exposure to magnesium stearate based on the proposed maximum use levels: 44 mg/kg bw per day for children and 83 mg/kg bw per day for adults, corresponding to 2 and 4 mg/kg bw per day, expressed as magnesium, respectively. These dietary exposures would contribute up to an additional 250 mg/day to the background exposure to magnesium from food of 180–480 mg/day. The Committee noted that the consumption of the food additive may lead to an additional dietary exposure to stearic and palmitic acids in the order of 5 g/day. An ADI "not specified"[a] has previously been established for a number of magnesium salts used as food additives. The Committee concluded that there are no differences in the evaluation of the toxicity of magnesium stearate compared with other magnesium salts. **The Committee confirmed the ADI "not specified" for magnesium salts of stearic and palmitic acids.** However, the Committee was concerned that the use of magnesium salts in many food additives may result in combined exposure that could lead to a laxative effect. Therefore, the Committee reiterated its previous recommendation to undertake an exposure assessment for magnesium from use of food additives.

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
Maltotetraohydrolase from *Pseudomonas stutzeri* expressed in *Bacillus licheniformis*	N	No treatment-related adverse effects were seen at the highest dose tested (93 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.1 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 93 mg TOS/kg bw per day results in an MOE of at least 900. **The Committee established an ADI "not specified"[a] for maltotetraohydrolase from *P. stutzeri* expressed in *B. licheniformis* when used in the applications specified and in accordance with good manufacturing practice.**
Mixed β-glucanase, cellulase and xylanase from *Rasamsonia emersonii*	N, T[b]	No treatment-related adverse effects were seen at the highest dose tested (84.8 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.08 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 84.8 mg TOS/kg bw per day results in an MOE of at least 1000. **The Committee established an ADI "not specified"[a] for the mixed β-glucanase, cellulase and xylanase enzyme preparation from *R. emersonii* when used in the applications specified and in accordance with good manufacturing practice.**
Mixed β-glucanase and xylanase from *Disporotrichum dimorphosporum*	N, T[b]	No treatment-related adverse effects were seen at the highest dose tested (199 mg TOS/kg bw per day) in a 13-week study of oral toxicity in rats. A comparison of the dietary exposure estimate of 0.7 mg TOS/kg bw per day (for a 60 kg individual) with the highest dose tested of 199 mg TOS/kg bw per day gives an MOE of at least 280. **The Committee established an ADI "not specified"[a] for the mixed β-glucanase and xylanase enzyme preparation from *D. dimorphosporum* when used in the applications specified and in accordance with good manufacturing practice.**
Polyvinyl alcohol (PVA) – polyethylene glycol (PEG) graft co-polymer	N	On the basis of the available studies, in which no treatment-related effects were seen at the highest doses tested, the Committee considered PVA-PEG graft co-polymer to be a substance of low oral toxicity in rats, rabbits and dogs. The bioavailability of PVA-PEG graft co-polymer in rats is negligible, and PVA-PEG graft co-polymer is unlikely to be genotoxic and is not associated with reproductive or developmental toxicity. Therefore, the Committee concluded that calculation of an MOE for PVA-PEG graft co-polymer would not be meaningful. Based on these data, the Committee would normally establish an ADI "not specified". However, the Committee decided not to establish an ADI "not specified" for PVA-PEG graft co-polymer in view of the impurities present, some of which may also be impurities in other food additives. The Committee had concerns that establishing an ADI "not specified" could lead to additional uses beyond those considered at the current meeting and consequently could increase exposure to the impurities. The use of PVA-PEG graft co-polymer that complies with the proposed specifications could lead to a dietary exposure to ethylene glycol and diethylene glycol from both food supplements and pharmaceutical products up to 0.016 mg/kg bw per day for children (high consumers).

Food additive	Specifications	Acceptable daily intakes (ADIs) and other toxicological or safety recommendations and dietary exposure information
		This is 3% of the tolerable daily intake (TDI) of 0.5 mg/kg bw per day derived by the Scientific Committee on Food of the European Union, and therefore the exposure to ethylene glycol and diethylene glycol from the use of PVA-PEG graft co-polymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified. The use of PVA-PEG graft co-polymer that complies with the proposed specifications could lead to a dietary exposure to vinyl acetate from both food supplements and pharmaceutical products up to 0.0008 mg/kg bw per day for children. This dietary exposure estimate is at least 62 500 times lower than the dose levels at which increases in tumour incidence are observed in oral studies of long-term toxicity and carcinogenicity in rats and mice. Therefore, the dietary exposure to vinyl acetate from the use of PVA-PEG graft co-polymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used in the applications specified. **The Committee concluded that the use of PVA-PEG graft co-polymer that complies with the specifications established at the current meeting is not of safety concern when the food additive is used as a glazing agent (aqueous film coating), stabilizer and binder for tablets in the preparation and formulation of food supplements and in accordance with good manufacturing practice.**

N: new specifications; NA: not applicable (dietary exposure assessment only); T: tentative specifications

[a] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice – i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

[b] Information is required in order for the tentative specifications to be revised (see Annex 3).

Annex 2. General information and further i nformation required or desired

Update on the draft specifications monographs for 16 modified starches

Following the recommendation made by the seventy-ninth meeting of the Committee, the 16 specifications for modified starches have been separated into stand-alone documents without adding, deleting or modifying any information. Some of the resulting single draft specifications monographs are incomplete; in some cases, essential information is missing, in particular information that would normally be needed to serve the purpose of a specification to unambiguously characterize the additive. Therefore, a revision of at least some of these individual draft specifications monographs is required. As the next step, the Committee recommended that the data and information necessary to complete and revise the 16 individual draft specifications monographs be requested through a call for data. In addition to the missing information (highlighted in the individual draft specifications monographs currently posted on the JECFA website at http://www.fao.org/fileadmin/ user_upload/agns/pdf/jecfa/2015_02_22_Modified_Starches.pdf), data relevant to the method of manufacture, detection methods, product characterization and levels of contaminants present (if any) should be requested as well.

HPLC method in the adopted specifications of cassia gum

The Committee recommended that the data to revise the high-performance liquid chromatography (HPLC) method for the determination of anthraquinones in cassia gum be requested through a call for data. Based on the information and data submitted, the Committee will consider revising the specifications as appropriate.

Mixed β-glucanase, cellulase and xylanase from Rasamsonia emersonii

New tentative specifications were prepared, with a request for the following information:

- a method to determine the identity for β-glucanase, including data from a minimum of five batches using the method described;
- a method to determine the identity for cellulase, including data from a minimum of five batches using the method described;
- a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories, and data from a minimum of five batches using the method described.

The above-requested information should be submitted by December 2016 in order for the tentative specifications to be revised; failure to provide this information may lead to a withdrawal of the specifications, with a possible impact on the ADI.

Mixed β-glucanase and xylanase from Disporotrichum dimorphosporum

New tentative specifications were prepared, with a request for the following information:

- a method to determine the identity for β-glucanase, including data from a minimum of five batches using the method described;
- a non-proprietary method to determine the identity and activity for xylanase that can be used by control laboratories, and data from a minimum of five batches using the method described.

The above-requested information should be submitted by December 2016 in order for the tentative specifications to be revised; failure to provide this information may lead to a withdrawal of the specifications, with a possible impact on the ADI.

Ethylene glycol and diethylene glycol impurities in food additives

The Committee noted that ethylene glycol and diethylene glycol, which are impurities in PVA-PEG graft co-polymer, may also be present as impurities in other food additives, such as polyethylene glycols and polysorbates, and the total exposure to these compounds from food additives may be higher than from PVA-PEG graft co-polymer alone. Currently, only the specifications monograph

for polyethylene glycols contains maximum limits for ethylene glycol and diethylene glycol (2500 mg/kg, singly or in combination). The Committee recommended setting and/or revising maximum limits for ethylene glycol and diethylene glycol that may occur as impurities in food additives at a future meeting.

Silicon dioxide, amorphous

The tentative status of the specifications was maintained, and the following information was requested:

- raw materials used and methods of manufacture for different forms of silicon dioxide (pyrogenic silica, precipitated silica, hydrated silica, silica aerogel and colloidal silica);
- identification methods allowing the differentiation between the above forms of silicon dioxide;
- functional uses of different forms, and information on the types of products in which it is used and the use levels in these products;
- data on solubility using the procedure documented in Volume 4 (Analytical methods) of the *Compendium of Food Additive Specifications*;
- data on the impurities soluble in 0.5 M hydrochloric acid for all forms of silicon dioxide used as food additives, from a minimum of five batches. If a different extraction and determination method is used, data should be provided along with details of the method and quality control (QC) data;
- suitability of the analytical method for the determination of aluminium, silicon and sodium using the proposed “Method of assay” along with data from a minimum of five batches. If a different method is used, data should be provided along with details of the method and QC data;
- in addition to the above information, data on pH, loss on drying and loss on ignition for hydrated silica, silica aerogel and colloidal silica.

The tentative specifications will be withdrawn unless the requested information is provided by December 2016.

Sodium aluminium silicate

The tentative status of the specifications was maintained, and the following information was requested:

- functional uses other than anticaking agent, if any, and information on the types of products in which it is used and the use levels in these products;
- data on solubility using the procedure documented in Volume 4 (Analytical methods) of the *Compendium of Food Additive Specifications*;
- data on the impurities soluble in 0.5 M hydrochloric acid, from a minimum of five batches. If a different extraction and determination method is used, data should be provided along with details of the method and QC data;
- suitability of the analytical method for the determination of aluminium, silicon and sodium using the proposed “Method of assay”, along with data, from a minimum of five batches, using the proposed method. If a different method is used, data should be provided along with details of the method and QC data.

The tentative specifications will be withdrawn unless the requested information is provided by December 2016.

COMPENDIUM OF FOOD ADDITIVE SPECIFICATIONS

Joint FAO/WHO Expert Committee on Food Additives

80th Meeting 2015

This document contains food additive specification monographs, analytical methods, and other information prepared at the seventy-ninth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome, Italy, from 16 – 25 June 2015. The specification monographs provide information on the identity and purity of food additives used directly in foods or in food production. The main three objectives of these specifications are to identify the food additive that has been subjected to testing for safety, to ensure that the additives are of the quality required for use in food or in processing and to reflect and encourage good manufacturing practice. This publication and other documents produced by JECFA contain information that is useful to all those who work with or are interested in food additives and their safe use in food.

ISBN 978-92-5-108929-3 ISSN 1817-7077

9 789251 089293

I5080E/1/10.15