COMpendium of food additive specifications

Volume 1

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Combined Specifications from 1st through the 37th Meetings 1956 - 1990

Food and Agriculture Organization of the United Nations
Rome, 1992
CORRIGENDUM

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Joint FAO/WHO Expert Committee on Food Additives (JECFA)

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INTRODUCTION

A Joint FAO/WHO Conference on Food Additives met in Geneva, Switzerland, in September 1955 and recommended that the two international organizations collect and disseminate information on food additives. Since that time, over 500 substances have been evaluated and provided with specifications for purity and identity by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

JECFA specifications of food additives are intended to serve as a guide for manufacturers and users of the additives, as well as the basis for new or revised national legislation or regulations of member countries of FAO and WHO. The specifications were published variously in FAO Nutrition Meetings Reports Series (NMRS), within WHO Technical Report Series (TRS) or as FAO Food and Nutrition Papers (FNP) comprising 35 separate volumes, published over many years and most of them now out of print.

During its more than 30 years of activity, the Committee has reevaluated many food additives in light of changing requirements or new technical and scientific information. Some have been reevaluated more than once. As a result, the specifications for 50 substances were withdrawn over the years because the use of these additives was no longer technically justified or because of safety considerations based on newer scientific information.

This present Compendium was prepared in order to consolidate in one reference source, all of the JECFA food additive specifications which are currently applicable. This includes specifications from all JECFA meetings dealing with food additives up to and including the 37th meeting in 1990. The Compendium consists of general notices; remarks applying to the standards, tests and assays; individual specifications listed in alphabetical order by substance title; and seven annexes plus an index.

There are many general tests for identity and purity which are applicable to a wide range of substances in the Compendium. In 1978, these were assembled along with other general reference material and were published as FNP number 5, "Guide to Specifications - JECFA". FNP 5 was revised in 1983 and a second revision was published in 1991 to coincide with and complement this Compendium. The test procedures and other information from FNP 5, revision 2, are referenced in the specifications throughout the Compendium.

Users of this Compendium are encouraged to submit their comments or suggestions to the Chief, Food Quality and Standards Service, Food Policy and Nutrition Division, FAO, via delle Terme di Caracalla, 00100 Rome, Italy.
ACKNOWLEDGEMENTS

This Compendium was prepared by Dr. Kenji Ishii of the Japan Food Additives Association (JFAA) over a period of 18 months at the FAO Headquarters in Rome, Italy. Dr. Ishii was assisted in this massive undertaking by several colleagues in Japan. These were Dr. Kunitoshi Yoshihira and Dr. Toshio Itoh, both of the National Institute of Hygienic Science, as well as the following scientists from the JFAA: Mr. Koh Murai, Dr. Takashi Akiyama, Mr. Rikio Goto, Mr. Nobuyoshi Nosaka, Dr. Tetsuya Kato and Dr. Izumi Kumashiro. FAO gratefully acknowledges the considerable effort of all these experts and especially the outstanding work done by Dr. Ishii. FAO further expresses its appreciation to Mr. Saburosuke Suzuki, Chairman, and Dr. Teruo Shiro, Senior Managing Director, of the Japan Food Additives Association and to the National Institute of Hygienic Science of Japan, for their generous support in providing the services of Dr. Ishii and of the other experts to prepare this document.

The final draft of this Compendium was reviewed and edited by Mr. D.F. Dodgen, Review Chemist, Division of Food Chemistry and Technology, Centre for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, D.C., USA, and Dr. Juhani Paakkanen, Senior Advisor, Ministry of Trade and Industry, Division for Food Affairs, Helsinki, Finland. Their review was invaluable.

FAO acknowledges with gratitude the generous contribution of the International Life Sciences Institute, Washington, D.C., USA, to the publication of this Compendium.
SCOPE OF THIS COMPENDIUM

This Compendium presents in a consolidated and up-dated form, all of the specifications for identity and purity of over 500 food additives, prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and published in about 35 separate volumes since the first session of the Committee in 1956. Each volume previously published represented the results of a given meeting.

The format of individual additive specifications varied from meeting to meeting and ranged from relatively uncomplicated earlier specifications to the more complex recent issuances. The consolidation and up-dating for this Compendium necessitated a uniform format of presentation for the specifications. This format was agreed to by JECFA experts after being proposed by the consultant preparing the draft Compendium (Dr. Kenji Ishii - see Acknowledgements). Early specifications tended to be quite simple in presentation. These were edited to generally conform to the agreed format without changing the substance or meaning of the specification requirements. Typographical errors were also corrected where found.

Many of the older specifications do not meet modern requirements for identity and purity. These specifications will be reviewed at future JECFA meetings and revised accordingly.

The user of this Compendium will need to become acquainted with both the index and the various annexes. To assist in this, the following summary and explanation of each is offered:

- Annex 1 details the General Specifications for Enzyme Preparations Used in Food Processing, with two appendices. Appendix A is "Determination of Antibiotic Activity" and Appendix B is "General Considerations and Specifications for Enzymes from Genetically Manipulated Microorganisms". All enzyme substances must meet the requirements detailed in Annex 1, in addition to the individual specification for the enzyme. Enzymes from microbial sources must also meet the requirements regarding antibiotic activity in Appendix A. Lastly, any enzyme from a genetically manipulated microorganism should further include specification requirements as summarized in Appendix B.

- Annex 2 was included to provide general guidance on specifications for colour lakes prepared by the reaction of alumina and appropriate colouring agents.

- Annex 3 is designed to clarify the nomenclature to be used in describing chlorophyll and its derivatives.

- Annex 4 provides a listing of former titles of specifications which have been changed in the present Compendium, along with a listing of their current titles. This permits cross-checking of older JECFA references, if desired.

- Annex 5 is a cross-index of Codex functional classes and JECFA defined functional uses.

- Annex 6 represents a listing of the individual substances by JECFA functional uses.

- Annex 7 identifies those substances which previously had specifications, but which are not included in this Compendium, for reasons stated in the Annex.

- The Index at the end of this Compendium will assist the user in finding specifications as it cross references the various synonyms used for each substance.
SPECIAL NOTE

The methods and analytical procedures described in this Compendium are designed to be carried out by properly trained personnel in a suitably equipped laboratory. In common with many laboratory procedures, the methods quoted frequently involve hazardous materials.

For the correct and safe execution of these methods it is essential that laboratory personnel follow standard safety procedures for the handling of hazardous materials.

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.
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ANNEXES

1. General Specifications for Enzyme Preparations used in Food Processing
   Appendix A: Determination of Antibiotic Activity
   Appendix B: General Considerations and Specifications for Enzymes from Genetically Manipulated Microorganisms

2. General Specifications for Aluminum Lakes of Colouring Matters

3. Nomenclature of Chlorophyll and Chlorophyll Derived Products

4. Former and Current Titles of JECFA Specifications

5. Cross Index of Codex Functional Classes and JECFA Defined Uses

6. Substances Listed by JECFA Functional Uses

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NOTICES


Function of General Notices

These General Notices provide in summary form, a commentary on how the specifications of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) are to be interpreted. Where, occasionally, exceptions to the General Notices are necessary, the language in the individual Specifications or in the General Methods of the Guide to JECFA Specifications, FAO Food and Nutrition Paper (FNP) 5, revision 2, 1991, takes precedence and specifically indicates the directions or intent. Otherwise, these General Notices apply.

Component Parts of Individual Specifications

1. Substances other than enzyme preparations

The specifications for each food additive or group of additives other than enzyme preparations generally consist of the following major sections: Title, Synonym(s), Definition, Description, Functional Use(s), Characteristics, Tests and Method of Assay. These sections are discussed below in the order in which they occur in the specifications.

TITLE

The title selected for individual specifications is the name of the additive or group of additives which, in the view of JECFA, most appropriately identifies the substance or substances defined by the specifications. The name used for the title is not necessarily the chemical name of the additive, nor the name used by the International Union of Pure and Applied Chemistry (IUPAC). Furthermore, the titles of the specifications in this document may differ from the titles used in the toxicological monograph, which sometimes apply to large groups of additives (e.g. acetates, phosphates) for which specifications are established separately. See also the Guidelines for Designating Titles for Specifications Monographs, part 2.3.4, report of the 33rd meeting of JECFA, WHO Technical Report Series No. 776, 1989.

SYNONYM(S)

Listed in this section are names, acronyms, and abbreviations under which a given additive is widely known, other than those used for the Title or Chemical name (see below). The International Numbering System (INS) number which was adopted by the Codex Alimentarius Commission, the European Economic Community (EEC) number and the USA FD & C number (for colours) are also listed here where applicable. Common or trivial names may be included. However, registered trade names are not used as titles or synonyms.

DEFINITION

This section defines the additive. The Chemical name, Chemical formula, Structural formula, Molecular or Formula weight and Assay are usually given. Sometimes, where appropriate, items such as, the Chemical Abstracts Service (CAS) number, Class name and/or Color Index (C.I.) number for colours are also given. For some substances, such as those of natural origin or those with mixed components, detailed descriptions, are given as necessary to define the additives.
Where an IUPAC or IUB (the International Union of Biochemistry) name exists for an additive (whether systematic name or recommended common name), it is listed first among the chemical names. Other chemical names may also be provided.

These are provided where known or where generally accepted, for the additive itself, or for the active component(s) of the additive.

The term "Molecular weight" is used in preference to the IUPAC recommendation of "Relative molecular mass" for molecular substances. The term "Formula weight" will be applicable in general to any substance having a chemical formula - compound or element - regardless of the nature of its constituent particles - atoms, ions, molecules, or extended arrays of covalently linked atoms. Molecular or Formula weights (as well as gravimetric factors specified in analytical procedures) are calculated from values given in the 1987 IUPAC Table of Standard Atomic Weights, which are based on the carbon-12 scale.

A quantitative assay requirement is provided where applicable to indicate the minimum acceptable content, or acceptable range, of the principal chemical component(s) of an additive. When an upper limit is not given, the assay should show the equivalent of not more than 100.5%. Sometimes the minimum acceptable content or the range of the constituent relating to the content of the component of the additive is given.

Information pertaining to physical appearance and other properties such as stability, odour and taste is provided in this section, as well as special conditions in usage and/or storage. Such information should not be interpreted as rigidly as measurable characteristics and does not constitute standards or tests of identity or purity.

Functional uses are provided to indicate the primary and secondary recognized technological application(s) of the additive in foods or in food processing. The statement, however, is not intended to indicate that the additive has no other utility than the functional use(s) listed.

Identification tests for a substance are not all-inclusive and do not, of themselves, establish absolute proof of identity. They serve, however, to generally identify the substance and if the substance fails these tests, it can be assumed that the substance is not what it purports to be.

Tests for trace impurities as well as for other parameters, such as physical properties, are based on current knowledge of the manufacturing process at the time the specifications are prepared. Limits for such constituents are provided at levels that are consistent with current good manufacturing practice and are deemed to be safe and otherwise unobjectionable under conditions in which the additive is customarily used (including consideration of the Acceptable Daily Intake established for the additive by JECFA).

Procedures and specific conditions of Identification Tests and Purity Tests are presented in detail in this subsection whenever there is no General Methods reference. However, in cases where the procedure can be given in brief, it may be presented in the section on Characteristics.
ASSAY

Assay methods which are given include detailing the principle involved, listing the apparatus and reagents, detailing the analytical procedure and the method of calculating results. Where possible, however, assay methods refer to specific procedures in the General Methods.

2. Enzyme preparations

Enzyme preparations used in food processing whether from animal, vegetable or microbial sources have to meet the general specification, "General Specifications for Enzyme Preparations used in Food Processing" (see Annex 1). In addition, enzyme preparations have to meet individual specifications which generally consist of the following sections discussed below in the order in which they occur:

TITLE

The name of the active principle(s) which most accurately characterizes the preparation defined by the specification. The name used for the title is not necessarily the systematic name recommended by the Nomenclature Committee of the IUB. Where appropriate, the sources appear as a component of the title.

SYNONYM(S)

Listed in this section are names and abbreviations under which the preparation is widely known, other than those used for the Title or Systematic Names (see below). The INS number is also listed where applicable.

SOURCE

Described in this section are animal tissues, plant material or microbial sources used. Also given are species, strains or variants, strain numbers and plasmid numbers if from recognized culture collections/depositories (e.g., ATCC) where appropriate. In the case where the source organism is derived from genetic manipulation, a description of the derivation is given. This includes the identity of the host organism and characterization of all introduced DNA.

ACTIVE PRINCIPLE(S)

Listed in this section are principle enzyme activities demonstrated by the preparation. IUB's recommended names are generally listed first. Other names may also be provided.

SYSTEMATIC NAME AND NUMBER

Where IUB's systematic name and enzyme number exist, they are listed for each active principle.

REACTION CATALYZED

Listed in this section are the specific substrates acted upon, the reactions catalyzed and the products formed by the active principles.

SECONDARY ENZYME ACTIVITY(S)

Listed here are minor enzyme activities which may be present in the enzyme preparations and influence the applications.

DESCRIPTION

Information pertaining to physical appearance, solubility in water and in organic solvents and other information such as manufacturing process, diluents, carriers, stabilizers, preservatives, immobilization agents is presented here.

FUNCTIONAL USE(S)

Listed in this section are principal and secondary technological applications of the enzyme preparation in food or in food processing.

GENERAL SPECIFICATIONS

A statement that all preparations have to conform to the "General Specifications for Enzyme Preparations used in Food Processing" is given here.
Assay methods for enzyme activities are provided here as a means of identifying the active principles. Where applicable, assay methods will refer to General Methods. Also listed are tests for trace impurities resulting from, for example, leakage of carriers and immobilization agents other than those noted by the General Specifications.

References


General Comments and Definition of Terms and Symbols

1. Substances endorsed for use in food by the Codex Alimentarius Commission (CAC) are identified by the symbol "CXAS/19XX" on the upper right corner of the monograph pages with the year of adoption indicated by the "19XX".

2. The term "Specifications" or "JECFA Specifications" refers to specifications of food additives prepared by JECFA.

3. The designation "Tentative" under the Title means the Specifications presented are considered to be incomplete. At its 23rd Session in 1979, JECFA decided to use the qualifier "Tentative" to describe those instances where the technical data was insufficient to adequately characterize the purity of a substance, or its identity, assay method or the minimum acceptable content or acceptable range of the principal ingredient. Such technical deficiencies may be indicated in the footnote of a Specification or in the JECFA meeting report (WHO Technical Report Series). Previous to the 23rd Session, JECFA had applied this designation also to specifications of substances for which the toxicological evaluation had resulted in a "temporary ADI" or "no ADI". This is no longer done.

4. International Numbering System numbers ("INS No. XXX") which are basically the expansion of European Economic Community numbers ("EEC No. XXX") were adopted by FAO/WHO at the 18th Session of CAC in July, 1989. Where these numbers are available, they are listed as synonyms to the title name of individual additives. The list of INS numbers is open and has been updated every two years by CAC. Therefore readers are expected to check the updated version of the list published by CAC when these numbers are used.

5. The EEC numbers are also listed where available. In referring to "EEC No." please note that numbers with an E prefix are those substances contained in existing EEC Directives. Those without an E have been allocated an official EEC number for labelling purposes, but are not necessarily authorized in all EEC member states.

6. For the definition and comments of following terms, refer to the General Notices section of the General Methods (Guide to JECFA Specifications, FNP 5/Rev.2, 1991):

- "Analytical Samples",
- "Analytical Standards",
- "Apparatus",
- "Blank Tests",
- "Constant Weight",
- "Dessicants and Desiccators",
- "Indicators",
- "Methods and Procedures",
- "Odourless",
- "Reagents",
- "Significant Figures",
- "Solubilities",
- "Solutions",
- "Temperatures",
- "Turbidity",
- "Vacuum",
- "Water",
- "Water-bath".
9. Weights and Measures

The metric system of weights and measures is used. The units and abbreviations commonly used are as follows:

\[
\begin{align*}
\text{m} & = \text{meter} \\
\text{cm} & = \text{centimeter} \ (10^{-2} \text{ m}) \\
\text{mm} & = \text{millimeter} \ (10^{-3} \text{ m}) \\
\mu\text{m} & = \text{micrometer} \ (10^{-6} \text{ m}), \\
\text{nm} & = \text{nanometer} \ (10^{-9} \text{ m}), \\
\text{g} & = \text{gram} \\
\text{kg} & = \text{kilogram} \ (10^{-3} \text{ g}) \\
\text{mg} & = \text{milligram} \ (10^{-3} \text{ g}) \\
\mu\text{g} & = \text{microgram} \ (10^{-6} \text{ g}) \\
\text{ng} & = \text{nanogram} \ (10^{-9} \text{ g}) \\
\text{L} & = \text{liter} \\
\text{ml} & = \text{milliliter} \ (10^{-3} \text{ L}) \\
\mu\text{l} & = \text{microliter} \ (10^{-6} \text{ L}) \\
\text{h} & = \text{hour(s)} \\
\text{min} & = \text{minute(s)} \\
\text{sec} & = \text{second(s)} \\
\text{°} & = \text{degrees Celsius (centigrade)} \\
\text{N} & = \text{normality (gram equivalent per liter)} \\
\text{M} & = \text{molarity (mole per liter)} \\
\text{cm}^2 & = \text{wave number} \\
\text{mmHg} & = \text{mm of mercury, unit of pressure} \\
\text{rpm} & = \text{revolution per minute} \\
\text{bar} & = \text{unit of pressure (kgm}^{-1}\text{sec}^{-2}\text{)}
\end{align*}
\]

Abbreviations commonly used in this publication are as follows:

AD\text{I} = \text{Acceptable Daily Intake} \\
AO\text{AC} = \text{Association of Official Analytical Chemists} \\
AST\text{M} = \text{American Society for Testing Materials} \\
AT\text{CC} = \text{American Type Culture Collection} \\
CA\text{C} = \text{Codex Alimentarius Commission} \\
CA\text{S} = \text{Chemical Abstracts System} \\
C.I. = \text{Colour Index} \\
CX\text{AS} = \text{Codex Advisory Specifications} \\
EC = \text{Enzyme Commission of IUB} \\
E\text{EC} = \text{European Economic Community} \\
FD&\text{C} = \text{Food, Drug and Cosmetic} \\
FN\text{P} = \text{FAO Food and Nutrition Paper} \\
FN\text{S} = \text{FAO Food and Nutrition Series} \\
NM\text{RS} = \text{FAO Nutrition Meeting Report Series} \\
FC\text{C} = \text{Food Chemicals Codex (USA)} \\
IN\text{S} = \text{International Numbering System (for food additives)} \\
ISO = \text{International Organization of Standardization} \\
IUB = \text{International Union of Biochemistry} \\
IUP\text{AC} = \text{International Union of Pure and Applied Chemistry} \\
JEC\text{FA} = \text{Joint FAO/WHO Expert Committee on Food Additives} \\
i.d. = \text{internal diameter} \\
o.d. = \text{outer diameter} \\
IR = \text{Infrared} \\
GC = \text{Gas chromatography} \\
GL\text{C} = \text{Gas liquid chromatography} \\
H\text{PLC} = \text{High Performance Liquid Chromatography} \\
LC = \text{Liquid chromatography} \\
meq = \text{milli equivalent} \\
MW = \text{Molecular weight} \\
soln = \text{solution} \\
TLC = \text{Thin Layer Chromatography} \\
TR\text{S} = \text{WHO Technical Report Series} \\
UV = \text{Ultraviolet}

10. Remarks on Specifications

1. The present publication is a compilation of the most recent versions of existing JEC\text{FA} Specifications for individual substances. The 542 substances covered are mostly food additives but include food processing aids and a few substances usually considered to be foods, but with food additive uses (e.g. Gelatin, Saffron and Turmeric). Exceptions to the inclusions are as follows:
a. Substances evaluated as "not to be used" by JECFA because of safety concerns.

b. Substances whose Specifications have been withdrawn by JECFA because of lack of certain information, for example information on actual usage.

Substances for which there were previous specifications, but which are not included in the present compendium, are listed in Annex 7.

2. The present compendium also includes the following two general specifications and one tentative general specification:

a. General Specifications for Enzyme Preparations Used in Food Processing (Annex 1)

b. General Considerations and Specifications for Enzymes from Genetically Manipulated Microorganisms (Tentative, see Appendix B to Annex 1)

c. General Specifications for Aluminium Lakes of Colouring Matters (Annex 2)

3. In preparing this publication each of the Specifications were edited for uniform presentation taking into account the provisions in the revised General Methods (Guide to JECFA Specifications, FNP 5/Rev.2, 1991), while maintaining the content of the original Specifications. Typographical errors in the previous monographs on JECFA Specifications were also corrected when these were found.

4. Information on identification, purity and assay for some additives (i.e., Acetone Peroxide, Ammonium Persulfate) discussed at the 9th Session of JECFA in 1969 are considered insufficient and therefore these Specifications are designated as "Tentative".

5. Monographs of JECFA Specifications are listed in the alphabetical order of the Title name without considering prefixes such as α-, β-, p-.

6. Title names of some substances that have been changed by JECFA in recent years or those which were editorially modified for this publication are listed in Annex 4. When changes or modifications were made, the former names are generally listed as Synonyms.

7. Infrared spectrums that appeared collectively in the annexes of previous specifications publications (Specifications for Identity and Purity of Certain Food Additives, FAO Food and Nutrition Paper Series) are appended to individual Specifications in this Compendium.

8. The index lists each substance title name (in bold letters) as well as the synonym(s) in alphabetical order. Additional synonyms listing the chemical group name first, are also listed for ease in referring to specifications of structurally related compounds.

For example:

<table>
<thead>
<tr>
<th>Title name</th>
<th>Additional name used in the index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium Acetate</td>
<td>Acetic Acid Calcium Salt</td>
</tr>
<tr>
<td>Sodium Acetate</td>
<td>Acetic Acid Sodium Salt</td>
</tr>
</tbody>
</table>

III. Remarks on Test Methods

1. In describing procedures or conditions of identification tests, purity tests and methods of assay, the Specifications refer to the General Methods where appropriate.

2. In instances where a test method is used for a group of substances, but does not appear in the General Methods, the complete text will appear only in the first substance specification and will be referred to in the specifications for the other substances.
3. Examples of new entries in the revised General Methods are:

"pH Determination", "Metallic Impurities/General Instrumental Methods", "General Methods for Food Colours" (14 items), "General Methods for Enzyme Preparations" (8 items), "General Methods for Microorganisms" (4 items).

4. Examples of replaced or revised General Methods are:

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic Limit Test</td>
<td>Indication of Method II (Colorimetric Method) as the default method.</td>
</tr>
<tr>
<td>Heavy Metals Limit Test</td>
<td>Indication of the use of 20μg lead (Pb) in the control.</td>
</tr>
<tr>
<td>Residual Solvent</td>
<td>Former methods have been replaced by the method annexed in FNP 31/1, 1984, and FNP 37, 1986.</td>
</tr>
<tr>
<td>Gum Constituents</td>
<td>Former methods have been replaced by the method annexed in FNP 37, 1986.</td>
</tr>
</tbody>
</table>
ACESULFAME POTASSIUM

SYNONYMS
Acesulfame K, INS No. 950

DEFINITION
Chemical names
Potassium salt of 6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide, potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide
C.A.S. number
55589-62-3
Chemical formula
C₉H₈KNO₅S
Structural formula

\[
\begin{align*}
\text{O} & \quad \text{H} \\
\text{N} & \quad \text{SO}_2 \\
\text{K}^+ & \\
\end{align*}
\]

Molecular weight
201.24
Assay
Content not less than 99% and not more than 101% of C₉H₈KNO₅S on the dried basis

DESCRIPTION
Odourless, white crystalline powder having an intensely sweet taste

FUNCTIONAL USE
Sweetening agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water
Very slightly soluble in ethanol

B. Ultraviolet absorption
Dissolve 0.01 g of the sample in 1,000 ml of water. The solution shows an absorption maximum at 227 ± 2 nm

C. Positive test for potassium
Passes test
Test the residue obtained by igniting 2 g of the sample

D. Precipitation test
Add a few drops of a 10% solution of sodium hexanitrocobaltate (III) to a solution of 0.2 g of the sample in 2 ml of acetic acid TS and 2 ml of water. A yellow precipitate is produced.

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

PURITY TESTS

* Loss on drying
  Not more than 1% (105°, 2 h)

* Fluoride
  Not more than 30 mg/kg (Method III)

* Arsenic
  Not more than 3 mg/kg (Method II)

* Selenium
  Not more than 30 mg/kg (Method II)

* Heavy metals
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in the Limit Test (Method II)

METHOD OF ASSAY

Dissolve about 0.15 g of the sample, previously dried and accurately weighed, in 50.0 ml glacial acetic acid and titrate potentiometrically with 0.1 N perchloric acid or add two drops of crystal violet TS and titrate with 0.1 N perchloric acid, to a blue-green end-point which persists for at least 30 sec. Perform a blank determination and make any necessary correction. 1 ml of 0.1 N perchloric acid is equivalent to 20.12 mg of C₂H₅KNO₃S.

ACETIC ACID, GLACIAL*

SYNONYMS
INS No.260, EEC No.E260

DEFINITION
Chemical names Acetic acid, ethanoic acid
C.A.S. number 64-19-7
Chemical formula C₂H₄O₂
Structural formula CH₃COOH
Molecular weight 60.05
Assay Glacial acetic acid contains not less than 99.0% of C₂H₄O₂

DESCRIPTION
Colourless liquid or crystalline solid, having a pungent characteristic odour

FUNCTIONAL USES
Acidifier, flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility Miscible with water, ethanol or ether
** B. Boiling point About 118°
** C. Positive test for acid Passes test: A 1 in 3 solution is acid
** D. Positive test for acetate Apply a 1 in 3 solution of the sample

PURITY TESTS
** Solidification point Not lower than 14.5°
** Non-volatile residue Not more than 0.01% after evaporation of 20 g of the sample and holding at 100° for 2 h.
Readily oxidizable substances Dilute 2 ml of the sample in a glass-stoppered container with 10 ml of water and add 0.1 ml of 0.1 N potassium permanganate. The pink colour does not change to brown within 30 min.

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).
PURITY TESTS (continued)

* **Arsenic**

Not more than 3 mg/kg
A solution of 1 g of the sample in 35 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

**Heavy metals**

Not more than 10 mg/kg
See description under TESTS

TESTS

PURITY TESTS

* **Heavy metals**

To the residue obtained in the test for Non-volatile residue add 8 ml of 0.1 N hydrochloric acid, warm gently until solution is complete, and dilute to 100 ml with water. A 10-ml portion of this solution diluted to 25 ml with water meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Measure about 2 ml of the sample into a tared, glass-stoppered flask, and weigh accurately. Add 40 ml of water, then add phenolphthalein TS and titrate with 1 N sodium hydroxide. Each ml of 1 N sodium hydroxide is equivalent to 60.05 mg of C₂H₄O₂.

**ACETIC AND FATTY ACID ESTERS OF GLYCEROL**

**SYNONYMS**
Acetic acid esters of mono- and diglycerides, acetoglycerides, acetylated mono- and diglycerides

**DEFINITION**
The product consists of mixed glycerol esters of acetic acid and fatty acids of food fats. It contains mono- and diesters of fatty acids with glycerol which is itself partially acetylated; it may also contain free glycerol and free fatty acids.

**Structural formula**

\[
\begin{array}{c}
\text{CH}_2 - \text{OR}_1 \\
\text{CH} - \text{OR}_2 \\
\text{CH}_2 - \text{OR}_3 \\
\end{array}
\]

where \( R_1, R_2 \) and \( R_3 \) each may be a fatty acid moiety, - COCH\(_3\) or H.

**DESCRIPTION**
The product varies from liquid to solid in consistency and is white to pale yellow in colour. It may have the odour of acetic acid and conforms to the following specifications.
The article of commerce may be further specified as the saponification value, acid value, free fatty acid content, solidification point of the free fatty acids, Reichert-Meissl value, iodine value and free glycerol content.

**FUNCTIONAL USE**
Emulsifier

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

** A. Solubility**
Insoluble in water. Soluble in ethanol

** B. Positive test for fatty acids**
Passes test

** C. Positive test for acetic acids**
Passes test

** D. Positive test for glycerol**
Passes test

---

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Arsenic

Not more than 3 mg/kg (Method II)

* Heavy metals

Not more than 10 mg/kg
Test 2.0 g of the sample as directed in Method II under the Limit Test for Heavy Metals

Acids

Acids other than acetic and fatty acids shall not be detectable

ACETONE*

SYNONYMS
Dimethylketone, propanone

DEFINITION

Chemical names 
Acetone, 2-propanone

C.A.S. number 
67-64-1

Chemical formula 
C₃H₆O

Structural formula 
CH₃COCH₃

Molecular weight 
58.08

Assay 
Not less than 99.5% (w/w) of C₃H₆O

DESCRIPTION
Clear, colorless, volatile, highly flammable liquid with a characteristic odour. Free from sediment and suspended matter.

FUNCTIONAL USES
Extraction solvent (for fats and oils, including essential oils), precipitation agent (for the purification of starches, sugars and their derivatives).

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility 
Miscible in all proportions with water and with ethanol.

** B. Specific gravity 
$\rho_2^0 : 0.790 - 0.793$

** C. Refractive index 
$n^0_d : 1.358 - 1.360$

PURITY TESTS

** Distillation range 
55.5° - 57.0°

Non-volatile residue 
Not more than 0.001% (w/w) 
See description under TESTS

Acidity 
Not more than 0.002% (w/w) (calculated as acetic acid). 
See description under TESTS

Phenol 
Not more than 0.001% (w/w) 
See description under TESTS

---

* This specification was prepared at the 14th session of JECFA (1970) and published in NMRS 48B (1971).

PURITY TESTS (continued)

Readily oxidizable substances

Passes test

See description under TESTS

TESTS

PURITY TESTS

Non-volatile residue

**Apparatus**
A weighed basin of platinum, silica or borosilicate glass; a boiling water bath; an oven at a temperature of 100° ± 2°; a desiccator; balance.

**Procedure**
Evaporate 100 ml of the sample to dryness in a weighed basin on a boiling water bath. Dry the residue for 30 minutes in an oven at a temperature of 100° ± 2°. Cool in a desiccator and weigh.

**Calculation and results**
Non-volatile residue, per cent w/w = \( \frac{W}{d} \)

where:
- \( W \) = weight in grammes of residue and
- \( d \) = specific gravity of the sample.

Acidity

**Principle**
Determine the acidity by titration with sodium hydroxide.

**Apparatus**
Microburette, 500 ml-conical flask, stopper with soda-lime tube.

**Reagents**
The reagents used should be of a recognized analytical reagent quality. Distilled water, or water of at least equal purity, should be used throughout.

- Sodium hydroxide, 0.1 N solution
- Phenolphthalein indicator, dissolve 0.5 g phenolphthalein in 100 ml ethanol (95%) and make faintly pink by the addition of dilute sodium hydroxide solution.

**Procedure**
Place 100 ml of freshly boiled and cooled distilled water (neutralized to phenolphthalein indicator) and a few clean antibumping granules into a 500 ml conical flask of boro-silicate glass and boil gently for 5 min to eliminate carbon dioxide. Cool slightly and add 100 ml of the sample. Boil gently for a further 5 min. At the end of this period close the neck of the flask with a stopper carrying a soda-lime tube and allow to cool. When cold remove the stopper, add 0.5 ml of the phenolphthalein indicator, and examine for alkalinity: if not alkaline titrate with the sodium hydroxide solution using a micro-burette.
Acidity (continued)

**Calculation and results**

The acidity calculated as acetic acid, $\text{CH}_3\text{COOH}$ in percent w/w =

$$\frac{6 \times T}{d \times 1,000}$$

where:

- $T$ = volume, in ml, of 0.1 N sodium hydroxide solution used and
- $d$ = specific gravity of the sample.

**Phenol**

Place 3 ml of acetone in a crucible and evaporate to dryness at 60°, add 3 drops of a solution of 0.1 g sodium nitrite dissolved in 5 ml of sulfuric acid and allow to stand for 2-3 min; carefully add 3 ml of 2 N sodium hydroxide. No colour is produced.

**Readily oxidizable substances**

30 ml does not discolor 0.1 ml of 3% w/v freshly prepared aqueous potassium permanganate solution when shaken and allowed to stand at 20° for 15 min.

**METHOD OF ASSAY**

Weigh accurately about 1 g of acetone in a flask containing 20 ml of water, and add water to 1,000 ml. Place 10 ml of the solution in a glass stoppered flask, add 25 ml of 1 N sodium hydroxide, and allow to stand for 5 min. Add 25 ml of 0.1 N iodine, stopper, allow to stand in a cold and dark place for 10 min, and add 30 ml of 1 N sulfuric acid. Titrate the excess iodine with 0.1 N sodium thiosulfate, using starch TS as the indicator. Perform a blank test in the same manner as the sample to make any necessary correction.

1 ml of 0.1 N iodine = 0.9675 mg of $\text{C}_2\text{H}_4\text{O}$
ACETONE PEROXIDES*
(Tentative)

SYNONYM

INS No. 929

DEFINITION

Chemical name
A mixture of monomeric and linear dimeric acetone peroxide (mainly 2,2-dihydroperoxy propane), with minor proportions of higher polymers

C.A.S. number
1336-17-0, 1073-91-2

Chemical formula
Monomer: C₄H₆O₄
Dimer: C₆H₁₀O₄

Structural formula
(theoretical)

Monomer:

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{OOH} \\
\text{C} & \quad \text{H}_3\text{C} \\
& \quad \text{OOH}
\end{align*}
\]

Dimer:

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} - \text{O} & \quad \text{CH}_3 \\
\text{C} & \quad \text{C} \\
\text{H}_3\text{C} & \quad \text{O} - \text{O} & \quad \text{CH}_3
\end{align*}
\]

Assay
The product contains a minimum of 16 % of acetone peroxides

DESCRIPTION
Acetone peroxides occur mixed with an edible carrier such as cornstarch to give a fine, white, freeflowing powder. It has a sharp acrid odour.

Caution: Powerful oxidizing substance

FUNCTIONAL USES
Flour bleaching and strengthening agent

* These specifications were prepared at the 9th session of JECFA (1965) and published in NMBS 40ABC (1969).
ACTIVATED CARBON*

SYNONYM
Activated charcoal, decolourizing carbon

DEFINITION
A solid, porous, carbonaceous material prepared by carbonizing and activating organic substances. The raw materials, which include sawdust, peat, lignite, coal, cellulose residues, coconut shells, petroleum coke, etc., may be carbonized and activated at high temperature with or without the addition of inorganic salts in a stream of activating gases such as steam or carbon dioxide. Alternatively, carbonaceous matter may be treated with a chemical activating agent such as phosphoric acid or zinc chloride and the mixture carbonized at an elevated temperature, followed by removal of the chemical activating agent by water washing.

Chemical name
Carbon

Chemical formula
C

Formula weight
12.01

DESCRIPTION
Powder or granules, black, tasteless and odourless

FUNCTIONAL USES
Adsorbent, Decolourizing agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water and organic solvents and all usual solvents

B. Burning
When heated to redness it burns slowly without a flame

C. Adsorption
Pases test
See description under TESTS

D. Ignition
Pases test
See description under TESTS

PURITY TEST

Adsorption power
Within the range 90 to 110% of the declared value

** Loss on drying
Not more than 15% (120°, 4 h)

Sulfide compounds
Pases test
See description under TESTS

* These specifications were prepared at the 37th session of JECFA (1990) superseding the earlier Specifications for activated Carbon published in FNP 38 (1988).

PURITY TESTS (continued)

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TESTS

IDENTIFICATION TESTS

C. Adsorption

Place about 3 g of powdered sample in a glass-stoppered erlenmeyer flask containing 10 ml of dilute hydrochloric acid (5%), boil for 30 sec, and cool to room temperature. Add 100 ml of iodine TS, stopper, and shake vigorously for 30 sec. Filter through Whatman No 12 filter paper, or equivalent, discarding the first portion of filtrate. Compare 50 ml of the subsequent filtrate with a reference solution prepared by diluting 10 ml of iodine to 50 ml with water, but not treated with carbon. The colour of the carbon treated iodine solution is no darker than that of the reference solution, indicating the adsorptivity of the sample.

D. Ignition

Ignite a portion of the sample in air. Carbon monoxide and carbon dioxide are produced, and an ash remains.

PURITY TESTS

Adsonption power

To about 0.3 g of sample, accurately weighed, in a 100 ml ground-glass-stoppered conical flask add 25.0 ml of a freshly prepared solution of 0.5 g of phenazone in 50 ml of water. Shake thoroughly for 15 min. Filter and reject the first 5 ml of filtrate. To 10.0 ml of the filtrate add 1.0 g of potassium bromide and 20 ml of dilute hydrochloric acid. Using 0.1 ml of ethoxychrysoidine solution as indicator, titrate with 0.1 N potassium bromate until the colour changes from reddish-pink to yellowish-pink. Titrate slowly (1 drop every 15 sec) towards the end of the titration. Carry out a blank titration using 10.0 ml of the phenazone solution.

Calculate the quantity of phenazone adsorbed per 100 g of activated charcoal from the expression:

$$\frac{2.353 (a - b)}{m}$$

- **a** = Number of ml of 0.1 N potassium bromate use for the blank
- **b** = Number of ml of 0.1 N potassium bromate used for the test
- **m** = mass in grams of the substance to be examined

Not less than 90% and not more than 110% of the declared value of phenazone is adsorbed per 100 g of activated charcoal, calculated with reference to the dried substance.

Sulfides compounds

To 1.0 g in a conical flask add 5 ml of 1 N hydrochloric acid and 20 ml of water. Heat to boiling. The fumes released do not turn lead acetate paper brown. (Lead acetate paper is prepared by saturating filter paper with Lead acetate and drying the paper at 100°C).

Acid soluble substances

To about 1 g of sample, accurately weighed, add 25 ml of dilute nitric acid TS and boil for 5 min. Filter whilst hot through a sintered-glass filter (10) and wash with 10 ml of hot water. Evaporate the combined filtrate and washings to dryness on a water-bath, add to the residue 1 ml of hydrochloric acid, evaporate to dryness again and dry the residue to constant weight at 100°C to 105°C. The residue weighs not more than 3% of the sample weight.

Sulfated ash

Heat a silica or platinum crucible to redness for 30 min, allow to cool in a desiccator and weigh. Place the substance to be examined in the crucible and add 2 ml of sulfuric acid TS. Heat at first on a waterbath, then cautiously over a flame, then progressively to about 600°C. Continue the incineration until all black particles have disappeared and allow the crucible to cool. Add a few drops of dilute sulfuric acid TS, heat and incinerate as before and allow to cool. Add a few drops of ammonium carbonate solution (15.8 g in 100 ml). Evaporate and incinerate carefully, allow to cool, weigh, and repeat the ignition for 15 min to constant weight.

* Arsenic

Use a 20 ml portion of the filtrate obtained in the test for Water extractable substances diluted to 35 ml with water in the arsenic limit test (Method II).

* Lead

Use a 20 ml portion of the filtrate obtained in the test for Water extractable substances in the lead limit test using 10 μg of lead ions (Pb) in the control.

---

PURITY TESTS (continued)

* Heavy metals

Use a 10 ml portion of the filtrate obtained in the test for Water extractable substances in the Heavy metals test, using 20 µg of lead ion (Pb) in the control (solution A).

* Zinc

Accurately weigh about 2 g of the sample into a conical flask, add 50 ml of hydrochloric acid, 2 N and boil gently under reflux for 1 h, filter and wash the filter with hydrochloric acid, 2 N. Evaporate the combined filtrate and washings to dryness on a water bath, dissolve the residue in hydrochloric acid, 0.1 N and dilute to 50.0 ml with the same acid. Determine the content of Zinc in the solution by the use of Atomic Absorption Spectrophotometry.

Water extractable substances

Transfer about 5 g of sample, accurately weighed, into a 250 ml flask provided a 250 ml flask provided with a reflux condenser and a Bunsen valve. Add 100 ml of water and several glass beads, and reflux for 1 h. Cool slightly, and filter through Whatman No 12 or equivalent filter paper, discarding the first 10 ml of filtrate. Cool the subsequent filtrate to room temperature, and pipet 25.0 ml into a tared crystallization dish. (note: retain the remainder of the filtrate for the Arsenic, Heavy metals, and Lead tests). Evaporate the filtrate in the dish to incipient dryness on a hot plate never allowing the solution to boil. Dry for 1 h at 100 °C in a vacuum oven, cool and weigh. Calculate the percentage of water extractables in the filtrate, based on the sample weight and fraction of sample taken for gravimetric measurement.

Alkali soluble coloured substances

To 0.25 g add 10 ml of 2 N sodium hydroxide solution and boil for 1 min. Cool, filter and dilute the filtrate to 10 ml with water. The solution is not more intensely coloured than reference solution GY₄.

Solution GY₄ is prepared as follows:

Reference solution GY₄ = 0.10 ml of Standard solution GY + 1.90 ml of 1% w/v hydrochloric acid

where

Standard solution GY = 9.6 ml of Ferric chloride TSC* + 0.2 ml of Cobaltous chloride TSC* + 0.2 ml of Cupric sulfate TSC*

Cyanogen compounds

Mix 5 g of sample with 50 ml of water and 2 g of tartaric acid. Distil the mixture, collecting 25 ml of distillate below the surface of a mixture of 2 ml of sodium hydroxide TS and 10 ml of water contained in a small flask placed in an ice bath. Dilute the distillate to 50 ml with water, and mix. Add 12 drops of ferrous sulfate TS to 25 ml of the diluted distillate, heat almost to boiling, cool, and add 1 ml of hydrochloric acid. No blue colour is produced.

Higher aromatic hydrocarbons

Extract 1 g of the sample with 12 ml of cyclohexane in a continuous-extraction apparatus for 2 h. Using matched Nessler tubes, the extract shows no more colour or fluorescence than does a solution of 100 µg of quinine sulfate in 1000 ml of 0.1 N sulfuric acid when observed in ultraviolet light.

Alcohol soluble substances

The filtrate obtained by boiling 2 g of the product with 20 ml N sodium hydroxide and filtering shall be colourless.

ADIPIC ACID*

SYNONYMS
INS No.355, EEC No.355

DEFINITION
Chemical names: Hexanedioic acid, 1,4-butanedicarboxylic acid
C.A.S. number: 124-04-9
Chemical formula: C₉H₁₀O₄
Structural formula: HOOC(CH₂)₂COOH
Molecular weight: 146.14
Assay: Adipic acid contains not less than 99.6% and not more than the equivalent of 101% of C₉H₁₀O₄

DESCRIPTION
White odourless crystals or crystalline powder, having an acid taste

FUNCTIONAL USES
Buffer, neutralizing agent

CHARACTERISTICS
IDENTIFICATION TESTS
** A. Solubility
Slightly soluble in water. Freely soluble in ethanol

** B. Melting range
Between 151.5° and 154.0°

PURITY TESTS
** Water-content
Not more than 0.2% (Karl Fischer Method)

** Sulfated ash
Not more than 20 mg/kg
See description under TESTS

** Arsenic
Not more than 3 mg/kg
A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* These specifications were prepared at the 19th session of JEFCA (1975) and published in NMRS 55B (1976).

PURITY TESTS (continued)

* **Heavy metals**

Not more than 10 mg/kg

Test 2 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 µg of lead ion (Pb) in the control (Solution A).

**TESTS**

PURITY TESTS

* **Sulfated ash**

Transfer 100 g of the sample to a tared 125-ml platinum dish which has been previously cleaned by fusing in it 5 g of potassium pyrosulfate or bisulfate, followed by boiling in dilute sulfuric acid TS and rinsing with water. Melt the sample completely over a gas burner, then ignite. After ignition starts, lower or remove the flame to prevent the sample from boiling and to keep it burning slowly until it is completely carbonized. Ignite at 850° in a muffle furnace for 30 min or until the carbon is completely removed, cool, and weigh.

**METHOD OF ASSAY**

Transfer about 3 g of the sample, accurately weighed, into a 250-ml conical flask, add 50 ml of methanol and dissolve the sample by warming gently on a steam bath. Cool, add phenolphthalein TS, and titrate with 1 N sodium hydroxide. Perform a blank determination and make any necessary correction. Each ml of 1 N sodium hydroxide is equivalent to 73.07 mg of C₆H₁₀O₆.

AGAR*

**SYNONYMS**

Agar-agar; gelose; Japan agar; Bengal, Ceylon, Chinese or Japanese isinglass; Layor Carang; INS No. 406, EEC No. E406

**DEFINITION**

Agar is the dried hydrophilic, colloidal substance extracted from certain marine algae of the class Rhodophyceae. It is a polygalactoside, about 90% of the galactose-molecules being of the D-form and 10% of the L-form. On about every tenth D-galactopyranose unit one of the CH₂OH- or CHOH-groups is esterified with one of the two hydrogen atoms of sulfuric acid. The other hydrogen atom of the sulfuric acid molecule is replaced by calcium, magnesium, potassium, or sodium.

**C.A.S. number**

9002-18-0

**Assay**

The threshold gel concentration should not be higher than 0.25%

**DESCRIPTION**

Agar is odourless or has a slight characteristic odour. Unground agar usually occurs in bundles consisting of thin, membranous, agglutinated strips, or in cut, flaked or granulated forms. It may be light yellowish orange, yellowish grey to pale yellow, or colourless. It is tough when damp, brittle when dry. Powdered agar is white to yellowish white or pale yellow. When examined in water under a microscope the agar appears granular and somewhat filamentous. A few fragments of the spicules of sponges and a few frustules of diatoms may be present. In chloral hydrate TS the powdered agar appears more transparent than in water, more or less granular, striated, angular and occasionally contains frustules of diatoms.

(To prepare the sample, place a few fragments of unground agar or some powder on a slide and add some drops of water or chloral hydrate TS.)

**FUNCTIONAL USES**

Thickening agent and stabilizer

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**

Insoluble in cold water, soluble in boiling water

**B. Gel formation with water**

Passes test

See description under TESTS

**C. Precipitate formation with ammonium sulfate solution**

Passes test

See description under TESTS

---

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION (continued)

D. Precipitate formation with lead acetate solution

PURITY TESTS

Water absorption

Passes test
See description under TESTS

* Loss on drying

Not more than 22% after drying at 105° until the difference between two weighings is less than 1 mg (about 5 h). Unground agar should be cut into pieces from 2 to 5 mm² before drying.

Total ash

Not more than 6.5% on the dried basis
See description under TESTS

Acid-insoluble ash

Not more than 0.5% on the dried basis
See description under TESTS

Foreign insoluble matter

Not more than 1%
See description under TESTS

* Arsenic

Not more than 3 mg/kg
A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic.

* Lead

Not more than 10 mg/kg
A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Lead.

* Heavy metals

Not more than 40 mg/kg
Test 0.5 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 µg of lead ion (Pb) in the control Solution A.

Starch and dextrines

Not detectable
See description under TESTS

Gelatin and other proteins

Not detectable
See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Gel formation with water

Prepare a 1.0% solution of the sample in boiling water in a flask and place the flask in water at 30° for 15 min. A firm, resistant gel is formed. Place the flask in water at 70° for 1 h. The gel is not molten.

C. Precipitate formation with ammonium sulfate solution

A warm (40°) 0.5% solution of the sample gives a precipitate with half its volume of a warm (40°) 40% ammonium sulfate solution. This test distinguishes agar from alginates, arabic gum, ghatti gum, karaya gum, pectin and tragacanth.

IDENTIFICATION TESTS (continued)

D. Precipitate formation with lead acetate solution

A warm 0.5% solution of the sample gives a precipitate with one fifth its volume of basic lead acetate TS. This test distinguishes agar from methyl cellulose.

PURITY TESTS

Water absorption

Place 5 g of the sample in a 100-ml graduated cylinder, fill to the mark with water, mix, and allow to stand at 25°C for 24 h. Pour the contents of the cylinder through moistened glass wool, allowing the water to drain into a second 100-ml graduated cylinder. Not more than 75 ml of water should be obtained.

* Total ash

Weigh 3 g of the sample to the nearest 0.1 mg in a tared crucible. Ignite at a low temperature (about 550°C), not to exceed a very dull redness, until free from carbon, cool in a desiccator, and weigh. If a carbon-free ash is not obtained, wet the charred mass with hot water, collect the insoluble residue on an ashless filter paper and ignite, in the crucible, the residue and filter paper until the ash is white or nearly so. Add the filtrate, evaporate to dryness and heat to a dull redness. If a carbon-free ash is still not obtained, cool the crucible, add 15 ml of ethanol, break up the ash with a glass rod, burn off the ethanol, again heat to a dull redness, cool and weigh. Calculate the percentage of total ash from the dry weight of the sample.

* Acid-insoluble ash

Boil the ash, obtained as directed under Total ash above, with 25 ml of dilute hydrochloric acid TS for 5 min. Collect the insoluble matter on a tared Gooch crucible or ashless filter, wash with hot water, ignite, cool and weigh. Calculate the percentage of acid-insoluble ash from the dry weight of the sample.

Foreign insoluble matter

Boil 5 g of the sample with 500 ml of water and 12 ml of sulfuric acid under a reflux condenser for 2 h. Allow to cool and filter through a tared, fine, sintered glass crucible. Wash flask and filter with 50 ml of water, dry at 105°C to constant weight and weigh. Calculate as percentage.

Starch and dextrins

To a warm (40°C) 0.5% solution of the sample, add 2 drops of iodine TS. Where the drops fall, a red-violet colour appears. After mixing, the solution should be golden brown and not blue or reddish.

Gelatin and other proteins

To a warm (40°C) 0.5% solution of the sample add 1 volume of warm (40°C) picric acid TS. No turbidity should appear within 10 min.

METHOD OF ASSAY

Threshold gel concentration

Prepare serial dilutions of the sample with known solids content (0.15%, 0.20%, 0.25%, etc.) and place in tubes, 150 mm long by 16 mm internal diameter, stoppered at both ends. Cool for 1 h at 20°C - 25°C. Allow cylinders of gel to slide from the tubes to a level surface. The lowest concentration of gel that resists gravity without rupture for 5-30 sec is the threshold concentration of the sample.

ALGINIC ACID*

SYNONYMS
INS No. 400, EEC No. E400

DEFINITION
Alginic Acid is a colloidal substance obtained from various species of brown seaweed (Phaeophyceae). It is a linear, high-polymer consisting mainly of β-(1→4) linked D-mannuronic acid in the pyranose ring form, with part of the mannuronic acid replaced by L-guluronic acid.

C.A.S. number
9005-32-7

Chemical formula
(C₆H₁₀O₅)ₙ

Structural formula

| mannuronic acid |

Molecular weight
Structural unit: 176.13 (theoretical)
Macromolecule: 200.00 (actual average)

Assay
Alginic Acid yields, on the dried basis, not less than 20.0% and not more than 23.0% of carbon dioxide (CO₂), equivalent to not less than 91.0% and not more than 104.5% of alginic acid (C₆H₁₀O₅)ₙ.

DESCRIPTION
Alginic Acid occurs in filamentous, grainy, granular and powdered forms. It is colourless or slightly yellow and may have a slight characteristic smell and taste.

FUNCTIONAL USES
Thickening agent and stabilizer.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water and organic solvents; slowly soluble in solutions of sodium carbonate, sodium hydroxide and trisodium monophosphate.

** B. Specific rotation
Clarify a 0.5% solution of the sample in sodium hydroxide TS with kieselguhr, and determine the rotation in a 2-cm tube. The specific rotation is not less than -0.8° at 20°

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

* C. pH 2.0 - 3.4 (3 in 100 suspension)

D. Precipitate formation with calcium chloride solution
   Passes test
   See description under TESTS

E. No precipitate formation with ammonium sulfate solution
   Passes test
   See description under TESTS

F. Colour reaction
   Dissolve as completely as possible 0.01 g of the sample by shaking with 0.15 ml of 0.1 N sodium hydroxide, and add 1 ml of acid ferric sulfate TS. Within 5 min, a cherry-red colour develops that finally becomes deep purple.

PURITY TESTS

* Loss on drying
  Not more than 15% (105°, 4 h)

* Total ash
  Not more than 4% on the dried basis
  See description under TESTS

Sodium hydroxide insoluble matter
  Not more than 1% on the dried basis
  See description under TESTS

* Arsenic
  Not more than 3 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic.

* Lead
  Not more than 10 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Lead.

* Heavy metals
  Not more than 40 mg/kg
  Test 0.5 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 μg of lead ion (Pb) in the control Solution A.

TESTS

IDENTIFICATION TESTS

D. Precipitate formation with calcium chloride solution
   To a 0.5% solution of the sample in sodium hydroxide TS add one-fifth of its volume of a 2.5% aqueous solution of calcium chloride. A voluminous, gelatinous precipitate is formed. This test distinguishes alginic acid from arabic gum, carboxymethyl cellulose, carboxymethyl starch, carrageenan, gelatine, ghatti gum, karaya gum, locust bean gum, methyl cellulose, pectin and tragacanth.

IDENTIFICATION TESTS (continued)

E. No precipitate formation with ammonium sulfate solution

To a 0.5% solution of the sample in sodium hydroxide TS add one-half its volume of a saturated solution of ammonium sulfate. No precipitate is formed. This test distinguishes alginic acid from agar, carboxymethyl cellulose, carrageenan, de-esterified pectin, gelatine, locust bean gum, methyl cellulose and starch.

PURITY TESTS

Total ash

Weigh 3 g of the sample to the nearest 0.1 mg in a tared crucible. Ignite at a low temperature (about 600°), not to exceed a very dull redness, until free from carbon, cool in a desiccator, and weigh. If a carbon-free ash is not obtained, wet the charred mass with hot water, collect the insoluble residue on an ashless filter paper, and ignite, in the crucible, the residue and filter paper until the ash is white or nearly so. Finally, add the filtrate, evaporate it to dryness, and heat the whole to a dull redness. If a carbon-free ash is still not obtained, cool the crucible, add 15 ml of ethanol, break up the ash with a glass rod, then burn off the ethanol, again heat the whole to a dull redness, cool and weigh. Calculate as percentage of the dry weight.

Sodium hydroxide insoluble matter

Weigh 1 g of the sample to the nearest mg, and dissolve in 100 ml of sodium hydroxide TS, centrifuge and decant. Wash the residue five times with water by mixing, centrifuging and decanting. Transfer the residue by means of water to a tared fine glass filter, dry for 1 h at 105°, cool and weigh. Calculate as percentage of the dry weight. (The weight of the residue should not exceed 10 mg).

METHOD OF ASSAY

Decarboxylation method

Proced as directed under Carbon Dioxide Determination by Decarboxylation in the General Methods.* Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO\textsubscript{2}) or 25 mg of alginic acid (equivalent weight 200.00).

Gravimetric method

Dissolve 0.500 g of the sample in 10 ml of sodium hydroxide TS, add 90 ml of water, and filter if necessary. Add 15 ml of 4 N hydrochloric acid and 100 ml of 90% v/v ethanol. Allow this mixture to stand for 2 h, decant the supernatant liquid as far as possible, and centrifuge. Decant the liquid and replace it by 90% v/v ethanol. Mix well, centrifuge and decant again. This washing is repeated until the hydrochloric acid is removed. Then transfer the precipitate by means of 90% v/v ethanol to a fine glass filter, wash with dry acetone, place the filter in a vacuum desiccator, and dry to constant weight at 100°.

Calculate the percent purity of the sample by the formula:

\[
\text{% Purity} = \frac{200 \times F \times W}{\% \text{ dry substance in sample}} \times 100
\]

in which \(F\) is a conversion factor specified as 1.000 and \(W\) is the weight (in grams) of the dry precipitate.

ALLURA RED AC

SYNONYMS

CI Food Red 17, FD&C Red No.40
INS No.129, EEC No.E129

DEFINITION

Allura Red AC consists essentially of disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalene-sulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Allura Red AC may be converted to the corresponding aluminium lake in which case only the General Specifications for Aluminium Lakes of Colouring Matters shall apply.

Class

Monoazo

Code numbers

CI (1975) No.16035
CAS No.25956-17-6

Chemical name

Disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalenesulfonate

Chemical formula

\( \text{C}_{14}\text{H}_{14}\text{N}_{2}\text{Na}_{4}\text{O}_{8}\text{S}_{2} \)

Structural formula

![Structural formula of Allura Red AC](image)

Molecular weight

496.43

Assay

Content not less than 85% total colouring matters

DESCRIPTION

Dark red powder or granules

FUNCTIONAL USE

Food colour

---

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** See Annex 2 at the end of this Compendium.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water. Insoluble in ethanol

** B. Identification of
colouring matters
   Passes test

PURITY TESTS

** Loss on drying at 135°
   } Not more than 15%

** Chloride and sulfate
   calculated as sodium
tsals

** Water insoluble matter
   Not more than 0.2%

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Proceed as directed in the Limit Test for Heavy Metals

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

Organic compounds other than colouring matters

Sodium 6-hydroxy-2-naphthalene-
sulfonate
   Not more than 0.3%
   See description under TESTS

4-Amino-5-methoxy-2-methylbenzene-
sulfonic acid
   Not more than 0.2%
   See description under TESTS

Disodium 6,6'-oxybis(2-naphthalene-
sulfonate)
   Not more than 1.0%
   See description under TESTS

** Un sulfonated primary aromatic amines
   Not more than 0.01% calculated as aniline

** Ether extractable
   matter
   Not more than 0.2%

---


TESTS

PURITY TESTS

* Subsidiary colouring matters
Use the following conditions:
Developing solvent: No. 4
Height of ascent of solvent front: approximately 17 cm

* Organic compounds other colouring matters
Use HPLC under the following conditions:
HPLC elution gradient: 0 to 18% at 1% per min (linear) then 18% to 62% at 7% per min (linear) followed by elution at 100%.
Flow rate: 0.6 ml per min

METHOD OF ASSAY

Determination of Total Colouring Matters by Titration with Titanous Chloride*

Use the following:

Amount to weigh: 0.5 - 0.6 g
Buffer: 15 g sodium hydrogen tartrate
Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N TiCl₃: 12.41 mg

ALLYL 3-CYCLOHEXYLPROPIONATE*  
(Tentative)**

SYNONYM
Allyl cyclohexanepropionate

DEFINITION

- **Chemical name(s)**: 2-Propenyl 3-cyclohexylpropionate
- **C.A.S. number**: 2705-87-5
- **Chemical formula**: \( \text{C}_{13}\text{H}_{20}\text{O}_2 \)
- **Structural formula**:

\[
\text{CH}_2\text{CH}_2\text{COOCH}_2\text{CH} = \text{CH}_2
\]

- **Molecular weight**: 196.29
- **Assay**: Content not less than 98% of \( \text{C}_{13}\text{H}_{20}\text{O}_2 \)

DESCRIPTION
Colourless or almost colourless liquid with a pineapple like odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Practically insoluble in water, propane-1,2-diol and glycerol. Miscible with higher alcohols and fatty oils.

*** B. Refractive index
\( \text{n}_D^\text{20} : 1.457 - 1.462 \)

*** C. Specific gravity
\( \text{d}_20^\text{20} : 0.948 - 0.953; \text{d}_20^\text{25} : 0.945 - 0.950 \)

D. Infrared spectrum
Information required**

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The reference to identity, purity and methods of analysis were felt to require further confirmation. Information on infrared spectrum is required. Information on levels and method for allyl alcohol are also required.

PURITY TESTS

* Solubility in ethanol
1 ml dissolves in 4 ml of 80% ethanol

* Acid value
Not more than 2

* Allyl alcohol content
Not more than 0.1%
Information on levels and method required

METHOD OF ASSAY

Method A
Weigh accurately about 1.3 g of the sample and proceed as directed under the method for Ester Determination in the General Methods*, using 98.15 as the equivalence factor (e) in the calculation.

Method B
Determine by gas-liquid chromatography, using the following conditions.

- Column length: 1.60 m
- Column diameter: 3.0 mm
- Column material: glass
- Column packing: S.P. 1000 4%
- Column support: chromosorb 100/120 mesh
- Carrier gas: Helium
- Flow rate: 50 ml/min
- Detector type: FID
- Detector temperature: 250°
- Temperature of injection port: 250°
- Column temperature:
  - Isothermal: 120°
  - Temperature programme: 5°/min up to 170° then 10°/min
  - Final temperature: isothermal at 250°

The above conditions are to be applied together with the information provided under the General Methods*.

Remark: Allow the chromatogram to develop until all compounds have been eluted.

ALLYL HEPTANOATE*
(Tentative)**

SYNONYMS
Allyl heptylate, allyl heptanoate, allyl enanthate
2-propenyl ester of heptanoic acid

DEFINITION

Chemical name
2-Propenyl heptanoate

Code number
C.A.S. number 142-19-8
FEMA number 2031
COE number 369

Chemical formula
C_{10}H_{16}O_2

Structural formula
\[
\begin{align*}
\text{CH}_2&=\text{CHCH}_2\text{OC(CH}_2)_2\text{CH}_3
\end{align*}
\]

Molecular weight
170.25

Assay
Content not less than 97% of C_{10}H_{16}O_2

DESCRIPTION
Colourless to yellow liquid having a sweet, fruity odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Information required

*** B. Refractive index
n_D^0 : 1.427 - 1.431

*** C. Specific gravity
d_D^0 : 0.881 - 0.884

D. Infrared spectrum
Information required

* New specifications prepared at the 37th session of JECFA 37th (1990).

** Information required under Identification Tests (solubility, infra-red spectrum) and Purity Tests (solubility in ethanol, method of determination of allyl alcohol).

PURITY TESTS

Solubility in ethanol  Information required

* Acid value  Not more than 1

* Allyl alcohol  Not more than 0.1%

METHOD OF ASSAY

Determination of Allyl heptanoate

Assay by titrimetric determination of esters
Proceed as directed under Ester Determination in the General Methods for Flavouring Substances using 1.3 g of the sample and an equivalence factor of 85.1.*

**ALLYL HEXANOATE**
(Tentative)**

**SYNONYMS**
Allyl caproate, propenyl hexanoate

**DEFINITION**

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>2-Propenyl hexanoate, 2-propenyl ester of hexanoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code number</td>
<td>C.A.S. number: 123-68-2</td>
</tr>
<tr>
<td></td>
<td>FEMA number: 2032</td>
</tr>
<tr>
<td></td>
<td>COE number: 2181</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₆H₁₀O₂</td>
</tr>
<tr>
<td>Structural formula</td>
<td>CH₃(CH₂)₄COOCH₂CH =CH₂</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>156.22</td>
</tr>
<tr>
<td>Assay</td>
<td>Content not less than 98% of C₆H₁₀O₂</td>
</tr>
</tbody>
</table>

**DESCRIPTION**
Colourless to pale yellow liquid having a strong, pineapple-like odour

**FUNCTIONAL USE**
Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

*** A. Solubility
Miscible with ethanol. Insoluble in propane-1,2-diol and in water.

*** B. Refractive index
nD: 1.422 - 1.426

*** C. Specific gravity
dD: 0.884 - 0.890

D. Infrared spectrum
See Appendix below

---

* These specifications were prepared at the 37th session of JECFA superseding the earlier specifications for Allyl hexanoate published in FNP 17 (1980)

** Information required under Purity Tests (content of allyl alcohol and its method of determination)

PURITY TESTS

* Solubility in ethanol 1 ml of the sample dissolves in 6 ml of 70% ethanol at 25\°

* Acid value Not more than 1

Allyl alcohol Information required

TESTS

PURITY TESTS

Allyl alcohol Information required

METHOD OF ASSAY

Determination of Allyl hexanoate

Assay by titrimetric determination of esters

Proceed as directed under Ester Determination in the General Methods for Flavouring Substances using 1 g of the sample and an equivalence factor of 78.12.*

APPENDIX

** Infrared Spectrum: Allyl Hexanoate

(Spectrum obtained on the sample as is, in a fixed volume sodium chloride cell or between salt plates).


ALLYL ISOVALERATE*  
(Tentative)**

SYNONYMS  Allyl isopentanoate, allyl isovalerianate, allyl 3-methylbutanoate, 2-propenyl ester of 3-methyl butanoic acid, 2-propenyl isovalerate, 2-propenyl isopentanoate

DEFINITION

Chemical name  2-Propenyl 3-methylbutanoate
Code number  
  C.A.S. number 2835-39-4  
  FEMA number 2045  
  COE number 209R
Chemical formula  C_{10}H_{16}O_{2}
Structural formula  \( \text{CH}_2 = \text{CHCH}_2\text{OCCH}_2\text{CH(CH}_3\text{)CH}_3 \)
Molecular weight  142.20
Assay  Content not less than 98% of \( \text{C}_{10}\text{H}_{16}\text{O}_2 \)

DESCRIPTION  Colourless liquid having a fermented, fruity, apple-like odour

FUNCTIONAL USE  Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility  Information required

*** B. Refractive index  \( n_\text{D}^\circ : 1.410 - 1.418 \)

*** C. Specific gravity  \( d_\text{D}^2 : 0.879 - 0.888 \)

*** D. Infrared spectrum  Information required

---

* New specifications prepared at the 37th session of JECFA (1990).

** Information required under Identification Tests (solubility, infrared spectrum), Purity Tests (solubility in ethanol, content of allyl alcohol and its method of determination) and Method of Assay.

*** See General Methods (Guide to JECFA Specifications), FNP 5/Rev.2 (1991)
PURITY TESTS

Solubility in ethanol  Information required

* Acid value  Not more than 1

Allyl alcohol  Information required

TESTS

PURITY TESTS

Allyl alcohol  Information required

METHOD OF ASSAY

Determination of Allyl isovalerate  Information required

ALLYL TIGLATE* (Tentative)**

SYNONYMS
Allyl tiglate, allyl 2-methylcrotonate

DEFINITION

Chemical name
Allyl (E)-2-methyl-2-butenoate

C.A.S. number
7493-71-2

Chemical formula
C<sub>8</sub>H<sub>12</sub>O<sub>2</sub>

Structural formula

\[
\begin{align*}
\text{H}_3\text{C} & \text{C} = \text{C} \text{H}_3 \\
\text{H} & \text{C} = \text{C} \text{O} \text{O} \text{C} \text{H} = \text{C} \text{H}_3
\end{align*}
\]

Molecular weight
140.18

Assay
Content not less than 98% of C<sub>8</sub>H<sub>12</sub>O<sub>2</sub>

DESCRIPTION
Colourless to pale yellow liquid with a mild fruity green odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Miscible with ethanol, ether and most fixed oils. Very slightly soluble in water.

*** B. Refractive index
\( n^\rho = 1.451 - 1.454 \)

*** C. Specific gravity
\( d^\rho = 0.942 - 0.946; \quad d^\rho_3 = 0.939 - 0.943 \)

D. Infrared spectrum
Information required**

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation. Information on infrared spectrum and the GLC method of assay are required.

PURITY TESTS

* Acid value

Not more than 1

METHOD OF ASSAY

Method A

Weigh accurately about 0.8 g of the sample and proceed as directed under the method for Ester Determination in the General Methods*, using 70.09 as the equivalence factor (e) in the calculation.

Method B

Determine by gas-liquid chromatography (Information required)

ALLYL-α-JONONE*  
(Tentative)**

SYNONYM

Allylionone

DEFINITION

Chemical name
1-(2,6,6-trimethyl-2-cyclohexenyl)-1,6-heptadien-3-one

C.A.S. number
79-78-7

Chemical formula
C_{16}H_{24}O

Structural formula

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{O} \\
\text{CH}_3 & \quad \text{CH}_2
\end{align*}
\]

Molecular weight
232.38

Assay
Content not less than 88% of C_{16}H_{24}O

DESCRIPTION

Colourless or yellowish oil liquid, with a dry woody fruity odour

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Soluble in ethanol, insoluble in water

*** B. Refractive index
n^\rho_20 : 1.503 - 1.507

*** C. Specific gravity
\rho_20^\rho : 0.929 - 0.938, \quad \rho_25^\rho : 0.928 - 0.935

*** D. Infrared spectrum
See Appendix at the end of these specifications

PURITY TESTS

*** Solubility in ethanol
1 ml dissolves in 8 ml of 70% ethanol

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The reference to identity, purity and methods of analysis were felt to require further confirmation.

METHOD OF ASSAY

**Method A**

Weigh accurately about 2.5 g of the sample and proceed as directed under the method for Aldehyde and Ketone Determination in the General Methods*, using 116.2 as the equivalence factor (e) in the calculation.

**Method B**

Determine by gas-liquid chromatography, using the following conditions.

- Column length: 1.60 m
- Column diameter: 3.0 mm
- Column material: glass
- Column packing: S.P. 1000 4%
- Column support: chromosorb 100/120 mesh
- Carrier gas: Helium
- Flow rate: 50 ml/min
- Detector type: FID
- Detector temperature: 250°
- Temperature of injection port: 250°
- Column temperature:
  - Isothermal: 120°
  - Temperature programme: 5°/min up to 200° then 10°/min
  - Final temperature: isothermal at 250°

The above conditions are to be applied together with the information provided under the General Methods*.

**Remarks:** Allow the chromatogram to develop until compounds have been eluted.

APPENDIX

**Infrared spectrum: Allyl-α-ionone**

---

**See General Methods (Guide to JECFA Specifications), FNP 5/Rev.2 (1991).**

**Infra-red spectra through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc., Philadelphia, USA.**
ALUMINIUM AMMONIUM SULFATE*

SYNONYMS
Ammonium alum; INS No.523, EEC No.523

DEFINITION

Chemical name
Aluminium ammonium sulfate
C.A.S. number
7784-25-0
Chemical formula
AlNH₄(SO₄)₂ · 12H₂O
Formula weight
453.32
Assay
Content not less than 99.5% of AlNH₄(SO₄)₂ · 12H₂O

DESCRIPTION
Large, colourless crystals, white granules, or a powder. It is odourless and has a sweetish, strongly astringent taste.

FUNCTIONAL USES
Buffer, neutralizing agent, colour fixative

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water
Insoluble in ethanol

** B. Positive test for aluminium
Passes test

** C. Positive test for ammonium
Passes test

** D. Positive test for sulfate
Passes test

PURITY TESTS

Alkalies and alkaline earths
Passes test
See description under TESTS

Fluoride
Not more than 30 mg/kg
See description under TESTS

** Selenium
Not more than 30 mg/kg
Test 0.2 g of the sample as directed in the Limit Test (Method II).

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNS 34 (1986).

**PURITY TESTS** (continued)

* **Purity Test**

  - **Arsenic**
    
    Not more than 3 mg/kg
    Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II).

  - **Lead**
    
    Not more than 10 mg/kg
    Test a solution of 1 g of the sample in 10 ml of water as directed in the Limit Test.

  - **Heavy metals**
    
    Not more than 20 mg/kg
    See description under TESTS

**TESTS**

**PURITY TESTS**

* **Alkalies and alkali earths**

  Completely precipitate the aluminium from a boiling solution of 1 g of the sample in 100 ml of water by the addition of enough ammonia TS to render the solution distinctly alkaline to methyl red TS, and filter. Evaporate the filtrate to dryness, and ignite. The weight of the residue does not exceed 5 mg.

* **Fluoride**

  **Lime suspension**

  Carefully slake about 56 g of low fluoride calcium oxide (about 2 mg/kg F) with 250 ml of water, and add 250 ml of 60% perchloric acid slowly and with stirring. Add a few glass beads and boil until copious fumes of perchloric acid are evolved. Cool, add 200 ml of water, and boil again. Repeat the dilution and boiling once more. Cool, dilute considerably, and filter through a fritted-glass filter if precipitated silicon dioxide appears. Pour the clear solution, with stirring into 1000 ml of sodium hydroxide solution (1 in 10), allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing five times in large centrifuge bottles, shaking the mass thoroughly each time. Finally shake the precipitate into a suspension and dilute to 2000 ml. Store in paraffin-lined bottles and shake well before use. (NOTE: 100 ml of this suspension should give no appreciable fluoride blank when evaporated, distilled, and titrated as directed in the Fluoride Limit Test, Method I Thorium Nitrate Colourimetric Method).

  **Procedure**

  Assemble the distilling apparatus as directed in the Limit Test (Method I), and add to the distilling flask about 1.67 g of the sample, accurately weighed, and 25 ml of dilute sulfuric acid (1 in 2). Distil until the temperature

**Fluoride (cont'd)**

reaches 160°, then maintain at 160° to 165° by adding water from the funnel, collecting 300 ml of distillate. Oxidize the distillate by the cautious addition of 2 or 3 ml of fluorine-free 30% hydrogen peroxide (to remove sulfites), allow to stand for a few min, and evaporate in a platinum dish with an excess of Lime suspension. Ignite briefly at 600°, then cool and wet the ash with about 10 ml of water. Cover the dish with a watch glass, and cautiously introduce under the cover just sufficient 60% perchloric acid to dissolve the ash. Add the contents of the dish through the dropping funnel of a freshly prepared distilling apparatus (the distilling flask should contain a few glass beads), using a total of 20 ml of the perchloric acid for dissolving the ash and transferring the solution. Add 10 ml of water and a few drops of silver perchlorate solution (1 in 2) through the dropping funnel, and continue as directed in the Fluoride Limit Test* Method I Thorium Nitrate Colorimetric Method beginning with "Distil until the temperature reaches 135°...".

Dissolve 1 g of the sample in 20 ml of water, add a few drops of dilute hydrochloric acid TS, and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 min, cool, and dilute to 25 ml with water. Test this solution as directed in the Limit Test (Method I), using 2 ml of Standard lead solution (20 µg of Pb) and 50 mg of hydroxylamine hydrochloride in the control (Solution A).

**METHOD OF ASSAY**

Weigh accurately about 1 g of the sample, dissolve in 50 ml of water, add 50 ml of 0.05 M disodium EDTA and 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), and boil gently for 5 min. Cool, and add 50 ml of ethanol and 2 ml of dithizone TS. Titrate with 0.05 M zinc sulfate to a bright rose-pink colour, and perform a blank determination. Each ml of 0.05 M disodium EDTA is equivalent to 22.67 mg of AlNH₄(SO₄)₂ · 12H₂O.

ALUMINIUM POTASSIUM SULFATE*  
(Tentative)

SYNONYMS  
Potassium alum, potash alum, INS No.522

DEFINITION  
Chemical names  
Aluminium potassium sulfate dodecahydrate  
C.A.S. number  
10043-67-1  
Chemical formula  
AlK(SO$_4$)$_2$ · 12H$_2$O  
Formula weight  
474.38  
Assay  
Aluminium potassium sulfate contains not less than 99.5% of AlK(SO$_4$)$_2$ · 12H$_2$O

DESCRIPTION  
Large, transparent crystals or crystalline fragments, or a white crystalline powder, odourless and with a sweetish, astringent taste

FUNCTIONAL USES  
Buffering agent, neutralizing agent, firming agent

CHARACTERISTICS  
IDENTIFICATION TESTS  
** A. Solubility  
Freely soluble in water. Insoluble in ethanol  
** B. pH  
3.0 - 4.0 (1 in 10 solution)  
** C. Positive test for aluminium  
PASSES test  
** D. Positive test for sulfate  
PASSES test  
** E. Colour reaction  
The sample imparts a violet colour to a non-luminous flame

PURITY TESTS  
Ammonium salts  
Heat 1 g of the sample with 10 ml of sodium hydroxide TS on a steam bath for 1 minute. The odour of ammonia is not perceptible.

Fluoride  
Not more than 30 mg/kg  
See description under TESTS

* These specifications were prepared at the 22nd session of JECFA (1978) and published in FNP 7 (1978).

PURITY TESTS

* **Selenium**

Not more than 30 mg/kg. Test 0.2 g of the sample as directed in the Limit Test (Method II).

* **Arsenic**

Not more than 3 mg/kg. Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II).

* **Lead**

Not more than 10 mg/kg. Test a solution of 1 g of the sample in 10 ml of water as directed in the Limit Test.

Heavy metals

Not more than 20 mg/kg

See description under TESTS

TESTS

PURITY TESTS

Fluoride

Thorium nitrate colorimetric method

Lime Suspension

Carefully slake about 56 g of low-fluoride calcium oxide (about 2 mg/kg F) with 250 ml of water, and add 250 ml of 60% perchloric acid slowly and with stirring. Add a few glass beads, and boil to copious fumes of perchloric acid, then cool, add 200 ml of water, and boil again. Repeat the dilution and boiling once more, cool, dilute considerably, and filter through a fritted glass filter, if precipitated silicon dioxide appears. Pour the clear solution, with stirring, into 1000 ml of a 1 in 10 sodium hydroxide solution, allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing 5 times in large centrifuge bottles, shaking the mass thoroughly each time. Finally, shake the precipitate into a suspension and dilute to 2000 ml. Store in paraffin-lined bottles and shake well before use. 100 ml of this suspension should give no appreciable fluoride blank when evaporated in a platinum dish and treated as directed below under "Procedure", beginning with "Ignite briefly at 600°, ...".

Distillation Apparatus

Connect a 125-ml distillation flask with a condenser. Equip also with a thermometer and a capillary tube, both of which must extend into the liquid. Connect a small dropping funnel or a steam generator to the capillary tube. Support the flask on an asbestos mat with a hole which exposes about one-third of the flask to the flame. To minimize the distillation blank resulting from fluoride leached from the glassware, the distillation apparatus should be treated as follows: treat the glassware with hot 10% sodium hydroxide solution, followed by flushing with tap water and rinsing with distilled water. At least once daily, treat in addition by boiling down 15-20 ml of a 1 in 2 sulfuric acid solution until the still is filled with fumes; cool, pour off the

Fluoride (cont'd)

* Heavy metals

Dissolve 1 g of the sample in 20 ml of water, add a few drops of diluted hydrochloric acid TS, and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 20 minutes, cool and dilute to 25 ml with water. Test this solution as directed in the Limit Test (Method 1), using 20 μg of lead ion and 50 mg of hydroxylamine hydrochloride in the control (Solution A).

METHOD OF ASSAY

Weigh accurately about 1 g of the sample, dissolve in 50 ml of water, add 50.0 ml of 0.05 M disodium ethylenediamine-tetraacetate, and boil gently for 5 min. Cool, and with continuous stirring add in the order given: 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml), 50 ml of ethanol, and 2 ml of dithizone TS. Titrate with 0.05 M zinc sulfate to a bright rose-pink colour, and perform a blank determination. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 23.72 mg of AlK(SO₄)₂ · 12H₂O.

ALUMINIUM POWDER*
(Tentative)**

SYNONYMS
CI Pigment Metal
INS No. 173, EEC No. E173

DEFINITION
Aluminium powder is composed of finely divided particles of aluminium. The grinding may or may not be carried out in the presence of edible vegetable oils and/or food additive quality fatty acids.** It is free from admixture with substances other than edible vegetable oils and/or food additive quality fatty acids.

Code numbers  CI (1975) No. 77000
Chemical name  Aluminium
Chemical formula  Al
Atomic weight  26.98
Assay  Not less than 99% calculated as Al on an oil-free basis.

DESCRIPTION
A silvery grey powder

FUNCTIONAL USE
Food colour, decorative pigment

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Insoluble in water
Insoluble in organic solvents
Soluble in dilute hydrochloric acid

*** B. Identification of colouring matters
A solution in dilute hydrochloric acid gives the reactions characteristic of Al^3+

PURITY TESTS

*** Loss on drying
Not more than 0.5% (105°)

**** Arsenic
Not more than 3 mg/kg

**** Lead
Not more than 20 mg/kg

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** Information required on methods of grinding.


PURITY TESTS (continued)

* Heavy metals

Not more than 40 mg/kg

Proceed as directed in the Heavy Metals Limited Test

METHOD OF ASSAY

Transfer about 0.2 g of the sample, accurately weighed, to a 500-ml flask fitted
with a rubber stopper carrying a 150-ml separating funnel, an inlet tube
connected to a cylinder of carbon dioxide and an outlet tube dipping into a water-
trap. Add 60 ml of freshly boiled and cooled water and disperse the sample;
replace the air by carbon dioxide and add, by the separating funnel, 100 ml of
a solution containing 56 g of ferric ammonium sulfate and 7.5 ml of sulfuric acid
in freshly boiled and cooled water. While maintaining an atmosphere of carbon
dioxide in the flask, heat to boiling and boil for 5 min. After the sample has
dissolved, cool rapidly to 20°, and dilute to 250 ml with freshly boiled and cooled
water. To 50 ml of this solution, add 15 ml of phosphoric acid and titrate with
0.1 N potassium permanganate.

1 ml of 0.1 N potassium permanganate = 0.8994 mg Al.

ALUMINIUM SILICATE*

SYNONYMS
Kaolin, light or heavy; INS No. 559, EEC No. 559

DEFINITION
Aluminium silicate is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. The article of commerce may be further specified as to chloride, foreign substances, particle size, loss on drying, loss on ignition and pH value.

DESCRIPTION
Aluminium silicate is a soft, whitish powder free from gritty particles, odourless and tasteless.

FUNCTIONAL USE
Anticaking agent.

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility
Insoluble in water, ethanol and mineral acids.

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

C. Positive test for silicate
Passes test
See description under TESTS

D. Positive test for aluminium
Passes test
See description under TESTS

PURITY TESTS

Water soluble substances
Not more than 0.3%
See description under TESTS

Acid soluble substances
Not more than 2%
See description under TESTS

Asbestos
Absent as determined by the method given in the specifications for Talc.

Arsenic
Not more than 3% mg/kg
See description under TESTS

Heavy metals
Not more than 10 mg/kg
See description under TESTS

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP No.4 (1978).

TESTS

IDENTIFICATION TESTS

C. Positive test for silicate

Mix about 500 mg of the sample with about 200 mg of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, add 5 ml of water, and allow to stand for 3 min. Heat the bottom of the crucible gently, detach the melt, and transfer it to a beaker with the aid of about 50 ml of water. Add gradually hydrochloric acid until no effervescence is observed, then add 10 ml more of the acid, and evaporate the mixture on a steam bath to dryness. Cool, add 20 ml of water, boil and filter the mixture through an ash-free filter paper. An insoluble residue of silica remains. (Note: Retain the filtrate for identification test D). Transfer the gelatinous residue into a platinum dish, and cautiously add 5 ml of hydrofluoric acid (warning: toxic, corrosive, must not contact skin; work under fume hood). The precipitate dissolves. (If it does not dissolve, repeat the evaporation with hydrofluoric acid.) Heat and hold in the vapours a glass stirring rod with a drop of water on the tip. The drop becomes turbid.

D. Positive test for aluminium

Add ammonia TS to the filtrate obtained in test C above. A white gelatinous precipitate is formed which is insoluble in excess ammonia but dissolves in sodium hydroxide TS.

PURITY TESTS

Water soluble substances

Weigh 5 g of the sample to the nearest mg, and boil with 50 ml of water for 30 min., adding water from time to time to maintain approximately the original volume. Filter, evaporate the filtrate to dryness, dry at 105° for 1 h, and weigh.

\[
\% \text{ Water soluble substances} = \frac{m}{10 \times W}
\]

in which

\[
m = \text{the weight of the residue (in mg)}, \quad W = \text{the weight of the sample (in g)}
\]

Acid soluble substances

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

\[
\% \text{ Acid soluble substances} = \frac{m}{5 \times W}
\]

in which

\[
m = \text{the weight of the residue (in mg)}, \quad W = \text{the weight of the sample (in g)}
\]

---

* Arsenic

A 10-ml portion of the Sample solution (see below) diluted to 35 ml with water meets the requirements of the Limit Test for Arsenic (Method II).

* Heavy metals

A 20-ml portion of the Sample solution (see below) diluted to 25 ml with water meets the requirements of the Limit Test for Heavy Metals (Method I), using 20 µg of lead ion (Pb) in the control (Solution A).

Sample solution for the determination of arsenic and heavy metals:

Weigh 10 g of the sample to the nearest mg, and transfer into a 250-ml flask, and add 50 ml of 0.5 N hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min., cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 3 filter paper, or equivalent, into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

ALUMINIUM SODIUM SULFATE*  
(Tentative)

SYNONYMS
Soda alum, sodium alum;  
INS No. 521, EEC No. 521

DEFINITION
Chemical name  
Aluminium sodium sulfate
C.A.S. number  
10102-71-3
Chemical formula  
AlNa(SO₄)₂ · xH₂O
Formula weight  
242.09 (anhydrous)
Assay  
Aluminium Sodium Sulfate contains, anhydrous form, not less than 96.5% of AlNa(SO₄)₂, after drying; dodecahydrate, not less than 99.5% of AlNa(SO₄)₂ after drying.

DESCRIPTION
Transparent crystals or crystalline fragments, or white crystalline powder, odourless and with a saline, astringent taste.

FUNCTIONAL USES
Buffering agent, neutralizing agent, firming agent

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility  
The dodecahydrate is freely soluble in water.  The anhydrous form is slowly soluble in water. Both forms are insoluble in ethanol.

** B. Positive test for Al  
Passes test

C. Positive test for sulfate  
Passes test

D. Flame test  
The sample imparts an intense yellow colour to a non-luminous flame.

PURITY TESTS
** Loss on drying  
Anhydrous form, not more than 10% (220° for 16 h).  Dodecahydrate, not more than 47.2% (at first 50-55° for 1 h, then 200° for 16 h).

* These specifications were prepared at the 22nd session of JECFA (1978) and published in FNP 7 (1978).

PURITY TESTS (continued)

Ammonium salts
Heat 1 g with 10 ml of sodium hydroxide TS on a steam bath for 1 minute. The odour of ammonia is not perceptible.

Fluoride
Not more than 30 mg/kg. Determine as directed in the specifications for Aluminium Potassium Sulfate in this compendium.

* Selenium
Not more than 30 mg/kg. Test 0.2 g of the sample as directed in the Limit Test (Method II).

* Arsenic
Not more than 3 mg/kg. Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II).

* Lead
Not more than 10 mg/kg. Test a solution of 1 g of the sample in 10 ml of water as directed in the Limit Test.

Heavy metals
Not more than 20 mg/kg.
See description under TESTS

TESTS

PURITY TESTS

* Heavy metals
Dissolve 1 g of the sample in 20 ml of water, add a few drops of diluted hydrochloric acid TS, and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 minutes, cool and dilute to 25 ml with water. Test this solution as directed in the Limit Test (Method I) using 20 µg of lead ion and 50 mg of hydroxylamine hydrochloride in the control (Solution A).

METHOD OF ASSAY

Weigh accurately about 0.5 g of the sample, previously dried, moisten with 1 ml of acetic acid, and dissolve it in 50 ml of water, warming gently on a steam bath until solution is complete. Cool, neutralize with ammonia TS, add 50.0 ml of 0.05 M disodium ethylenediaminetetraacetate, and boil gently for 5 min. Cool, and with continuous stirring add in the order given: 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml), 50 ml of ethanol, and 2 ml of dithizone TS. Titrate with 0.05 M zinc sulfate to a bright rose-pink colour, and perform a blank determination. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 12.10 mg of AlNa(SO₄)₂.

ALUMINUM SULFATE (ANHYDROUS)*
(Tentative)

SYNONYMS
INS No. 520, EEC No. E520

DEFINITION

Chemical name: Aluminum sulfate
C.A.S. number: 10043-01-3
Chemical formula: Al₂(SO₄)₃
Formula weight: 342.13
Assay: Aluminium Sulfate (anhydrous) contains not less than 99.5% of Al₂(SO₄)₃, calculated on the ignited basis

DESCRIPTION
White powder, shining plates, or crystalline fragments, odourless and with a sweetish taste, becoming mildly astringent

FUNCTIONAL USE
Firming agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water. Insoluble in ethanol.

** B. pH
2.9 or above (1 in 20 soln)

** C. Positive test for aluminium
Passes test

** D. Positive test for sulfate
Passes test

PURITY TESTS

** Loss on drying
Not more than 5% (about 500°, 3 h)

Alkalies and alkaline earths
Passes test
See description under TESTS

** Fluoride
Not more than 30 mg/kg
Determine as directed in the specifications for Aluminium Potassium Sulfate.

* These specifications were prepared at the 22nd session of JECFA (1978) and published in FNP 7 (1978).

PURITY TESTS (continued)

* Arsenic
Not more than 3 mg/kg
Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II)

* Lead
Not more than 10 mg/kg
Test a solution of 1 g of the sample in 10 ml of water as directed in the Limit Test

* Selenium
Not more than 30 mg/kg
Test 0.2 g of the sample as directed in the Limit Test (Method II)

* Heavy metals
Not more than 40 mg/kg
See description under TESTS

TESTS

PURITY TESTS

Alkalies and alkaline earths
To a boiling solution of 2 g of the sample in 150 ml of water add a few drops of methyl red TS. Then add ammonia TS until the colour of the solution just changes to a distinct yellow. Add hot water to restore the original volume, and filter while hot. Evaporate 75 ml of the filtrate to dryness, and ignite to constant weight. Not more than 4 mg of residue remains (about 0.4%).

* Heavy metals
Dissolve 0.5 g of the sample in 20 ml of water, add a few drops of dilute hydrochloric acid TS, and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 min, cool and dilute to 25 ml with water. Test this solution as directed in the Limit Test (Method I) using 20 μg of lead ion and 50 mg of hydroxylamine hydrochloride in the control (Solution A).

METHOD OF ASSAY

Weigh accurately about 4 g of the sample, transfer into a 250-ml volumetric flask. Dissolve in water, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 250-ml beaker, add 25.0 ml of 0.05 M disodium ethylenediaminetetra-acetate, and boil gently for 5 min. Cool, and with continuous stirring add in the order given: 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acid in 1000 ml), 50 ml of ethanol, and 2 ml of dithizone TS. Titrate with 0.05 M zinc sulfate until the colour changes from green-violet to rose-pink, and perform a blank determination, substituting 10 ml of water for the sample. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 8.553 mg of Al₃(SO₄)₂.

AMARANTH*

SYNONYMS
Cl Food Red 9; Naphtol Rot S.
INS No. 123, EEC No. E123

DEFINITION
Amaranth consists essentially of trisodium 3-hydroxy-4-(4-sulfonato-1-naphthylazo)-2,7-naphthalenedisulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Amaranth may be converted to the corresponding aluminium lake in which case only the General Specifications for Aluminium Lakes of Colouring Matters shall apply."

Class
Monoazo

Code numbers
CI (1975) No. 16185
CAS No. 915-67-3

Chemical name
Trisodium 3-hydroxy-4-(4-sulfonato-1-naphthylazo)-2,7-naphthalenedisulfonate

Chemical formula
C_{36}H_{37}N_{3}Na_{3}O_{16}S_{5}

Structural formula

Molecular weight
604.48

Assay
Content not less than 85% total colouring matters

DESCRIPTION
Reddish brown to dark reddish brown powder or granules

FUNCTIONAL USE
Food colour

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** See Annex 2 at the end of this Compendium.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water. Sparingly soluble in ethanol

** B. Identification of colouring matters
   Passes tests

PURITY TESTS

** Loss on drying at 135°
   Not more than 15%

** Chloride and sulfate calculated as sodium salts

** Water insoluble matter
   Not more than 0.2%

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Proceed as directed in the Heavy Metals Limit Test

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

Organic compounds other than colouring matters

- 4-Amino-1-naphthalenesulfonic acid
- 3-Hydroxy-2,7-naphthalenedisulfonic acid
- 6-Hydroxy-2-naphthalenesulfonic acid
- 7-Hydroxy-1,3-naphthalenedisulfonic acid
- 7-Hydroxy-1,3,6-naphthalenetrisulfonic acid
   Total not more than 0.5%

See description under TESTS

** Unsulfonated primary aromatic amines
   Not more than 0.01% calculated as aniline

** Ether extractable matter
   Not more than 0.2%

---


TESTS

PURITY TESTS

* Subsidiary colouring matters
  Use the following conditions:
  Developing solvent: No. 3
  Height of ascent of solvent front: 17 cm, then 1 h further development

* Organic compounds other than colouring matters
  Use HPLC under the following conditions:
  HPLC elution gradient: 2 to 100% at 4.0% per min (linear)

METHOD OF ASSAY

Determination of Total Colouring Matters by Titration with Titanous Chloride*

Use the following:
- Weight of sample: 0.7 - 0.8 g
- Buffer: 10 g sodium citrate
- Calculation: Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N TiCl₃: 15.11 mg

AMMONIUM ALGINATE*

SYNONYMS
INS No. 403, EEC No. E403

DEFINITION
Ammonium Alginate, the ammonium salt of alginic acid, is a hydrophilic, colloidal substance. The alginic acid is a linear high-polymer consisting mainly of β-(1→4) linked D-mannuronic acid in the pyranose ring form, with part of the mannuronic acid replaced by L-guluronic acid.

C.A.S. number 9005-34-9
Chemical formula \((\text{C}_4\text{H}_{11}\text{NO}_6)_n\)
Structural formula

\[
\begin{array}{c}
\text{H} \\
\text{O} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{COONH}_4 \\
\end{array}
\]

unit of the salt of mannuronic acid

Molecular weight

<table>
<thead>
<tr>
<th>Structural unit:</th>
<th>193.16 (theoretical)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(217.00 actual average)</td>
</tr>
<tr>
<td>Macromolecule:</td>
<td>32 000-250 000</td>
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</tbody>
</table>

Assay
Ammonium Alginate yields, on the dried basis, not less than 18.0% and not more than 21.0% of carbon dioxide \((\text{CO}_2)\), equivalent to not less than 88.7% and not more than 103.6% of ammonium alginate \((\text{C}_4\text{H}_{11}\text{NO}_6)_n\).

DESCRIPTION
Ammonium Alginate occurs in filamentous, grainy, granular and powdered forms. It is colourless or slightly yellow and may have a slight characteristic smell and taste.

FUNCTIONAL USES
Thickening agent and stabilizer.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Slowly soluble forming a viscous solution in water; insoluble in ethanol, ether and chloroform.

** B. Specific rotation
Clarify a 0.5% solution of the sample in sodium hydroxide TS with kieselguhr, and determine the rotation in a 20-cm tube. The specific rotation is not less than -0.8° at 20°.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

C. Precipitate formation with calcium chloride solution
   Passes test
   Proceed as directed in the same test under ALGINIC ACID

D. No precipitate formation with ammonium sulfate solution
   Passes test
   Proceed as directed in the same test under ALGINIC ACID

E. Colour reaction
   Moisten 1-5 mg of the sample with water and add 1 ml of acid ferric sulfate TS. Within 5 min a cherry-red colour develops that finally becomes deep purple.

F. Positive test for ammonium
   Heat a solution of the sample in sodium hydroxide TS. Ammonia is evolved, recognizable by its odour, and by its reaction on moist, red litmus paper.

PURITY TESTS

- **Loss on drying**
  Not more than 15% (105°, 4 h)

- **Phosphate**
  Not detectable
  Proceed as directed in the Purity Test for Phosphate under Sodium Alginate.

- **Water insolubles**
  Not more than 1% on the dried basis
  Proceed as directed in the Purity Test for Water Insolubles under Sodium Alginate.

- **Total ash**
  Not more than 4% on the dried basis
  Proceed as directed in the Total Ash Test under Alginic Acid.

- **Arsenic**
  Not more than 3 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic

- **Lead**
  Not more than 10 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Lead.

- **Heavy metals**
  Not more than 40 mg/kg
  Test 0.5 g of the sample as directed in Method II under the Limit Test for Metals using 20 μg of lead ion (Pb) in the control Solution A

METHOD OF ASSAY

Decarboxylation method
Proceed as directed under Carbon Dioxide Determination by Decarboxylation in the General Methods. Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO₂) or 27.12 mg of ammonium alginate (equivalent weight 217.00).

Gravimetric method
Dissolve 0.500 g of the sample in 10 ml of sodium hydroxide TS, add 90 ml of water, and filter if necessary. Add 15 ml of 4 N hydrochloric acid and 100 ml of 90% v/v ethanol. Allow this mixture to stand for 2 h, decant the supernatant liquid as far as possible, and centrifuge. Decant the liquid and replace it by 90% v/v ethanol. Mix well, centrifuge and decant again. This washing is repeated until the hydrochloric acid is removed. Then transfer the precipitate by means of 90% v/v ethanol to a fine glass filter, wash with dry acetone, place the filter in a vacuum desiccator, and dry to constant weight at 100°.

Calculate the percent purity of the sample by the formula:

\[
\text{% Purity} = \frac{200 \times E \times W}{\% \text{ dry substance in sample}} \times 100
\]

in which E is a conversion factor specified as 1.086 and W is the weight (in grams) of the dried precipitate.

AMMONIUM CARBONATE*

SYNONYMS
INS No. 503(i), EEC No. 503

DEFINITION
Ammonium Carbonate consists of ammonium carbamate, ammonium carbonate and ammonium hydrogen carbonate in varying proportions.

C.A.S. number
10361-29-2

Chemical formula
CH₂N₂O₅, CH₄N₂O₅ and CH₃NO₃

Structural formula
NH₄COONH₄, (NH₄)₂HCO₃, and NH₄HCO₃

Molecular weight
Ammonium carbamate 78.06; Ammonium carbonate 98.73 Ammonium hydrogen carbonate 79.06

Assay
Content not less than 30.0% and not more than 34.0% of NH₃

DESCRIPTION
White powder or hard, white or translucent masses of crystals with an odour of ammonia. On exposure to air it becomes opaque and is finally converted into white porous lumps or powder (of ammonium bicarbonate) due to loss of ammonia and carbon dioxide.

FUNCTIONAL USE
Acidity regulator, raising agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water

** B. pH
About 8.6 (1 in 20 soln)

** C. Positive test for carbonate
Passes test

** D. Positive test for ammonia
Passes test

E. Appearance on heating
When heated, it volatilizes without charring and the vapour is alkaline to moist litmus.

PURITY TESTS

Non-volatile residue
Not more than 500 mg/kg
See description under TESTS

* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982).

PURITY TESTS (continued)

**Chlorides**  
Not more than 30 mg/kg  
See description under TESTS

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

* **Arsenic**  
Not more than 3 mg/kg  
Test a solution of 1 g of the sample in 35 ml of water as directed under the Limit Test for Arsenic (Method II).

* **Heavy metals**  
Not more than 10 mg/kg  
See description under Tests

TESTS

**PURITY TESTS**

* **Non-volatile residue**  
Transfer 4 g of the sample into a tared dish, add 10 ml of water, evaporate on a steam bath, and dry for 1 h at 105°C. The weight of the residue does not exceed 2 mg.

* **Chlorides**  
Dissolve 500 mg of the sample in 10 ml of hot water, add about 5 mg of sodium carbonate, and evaporate to dryness on a steam bath. Test the residue as directed under the Limit Test. Any turbidity produced does not exceed that shown in a control containing 15 µg of chloride ion (Cl⁻).

**Sulfur compounds**  
Dissolve 4 g of the sample in 40 ml of water, add about 10 mg of sodium carbonate and 1 ml of 30% hydrogen peroxide, and evaporate the solution to dryness on a steam bath. Treat the residue as directed under the Limit Test for Sulfates* in the General Methods. Any turbidity produced does not exceed that shown in a control containing 200 µg of sulfate ion (SO₄²⁻).

* **Heavy metals**  
Dissolve the residue from the test for Non-volatile residue in 1 ml of dilute hydrochloric acid TS, and evaporate to dryness. Dissolve the residue in 50 ml water. Test a 25 ml portion of this solution as directed in the Limit Test (Method II).

**METHOD OF ASSAY**  
Place about 10 ml of water in a weighing bottle, tare the bottle and its contents, add about 2 g of the sample and weigh accurately. Transfer the contents of the bottle to a 250-ml flask and slowly add, with mixing, 50 ml of 1 N sulfuric acid. When solution has been effected, wash down the sides of the flask, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide. Each ml of 1 N sulfuric acid is equivalent to 17.03 mg of NH₃.

AMMONIUM CHLORIDE*

SYNONYMS
Sal ammoniac, ammonium muriate
INS No. 510; EEC No. 510

DEFINITION

<table>
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<tr>
<th>Chemical name</th>
<th>Ammonium chloride</th>
</tr>
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<tbody>
<tr>
<td>C.A.S. number</td>
<td>12125-02-9</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>NH₄Cl</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>53.50</td>
</tr>
<tr>
<td>Assay</td>
<td>Content not less than 99.0% on the dried basis</td>
</tr>
</tbody>
</table>

DESCRIPTION
Colourless crystals, or a white, fine or coarse, crystalline powder. It has a cool, saline taste, and is somewhat hygroscopic.

FUNCTIONAL USE
Dough conditioner, yeast food

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Freely soluble in water. Sparingly soluble in ethanol.

** B. pH 4.5 - 6.0 (1 in 20 soln)

** C. Positive test for ammonium Passes test

** D. Positive test for chloride Passes test

PURITY TESTS

** Loss on drying Not more than 2.0% (over silica gel, 4 h)

** Sulfated ash Not more than 0.5%
Test 2 g of the sample as directed in the Test for Ash (sulfated ash, Method I)

** Arsenic Not more than 3 mg/kg
Test a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II)

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* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979).

**PURITY TESTS (continued)**

* **Lead**

Not more than 10 mg/kg
Test a solution of 1 g of the sample in 10 ml of water as directed in the Limit Test

* **Heavy metals**

Not more than 20 mg/kg
Test a solution of 1 g of the sample in 25 ml of water as directed in the Limit Test

**METHOD OF ASSAY**

**Titrimetry**

Dry about 0.2 g of the sample over silica gel for 4 h, weigh accurately, and dissolve it in about 40 ml of water in a glass-stoppered flask. Add, while agitating, 3 ml of nitric acid, 5 ml of nitrobenzene, 50.0 ml of 0.1 N silver nitrate, shake vigorously, then add 2 ml of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 N ammonium thiocyanate. Each ml of 0.1 N silver nitrate is equivalent to 5.349 mg of NH₄Cl.

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AMMONIUM DIHYDROGEN PHOSPHATE*

SYNONYMS
Monobasic ammonium phosphate, monoammonium phosphate, acid ammonium phosphate, primary ammonium phosphate; INS No. 342(i)

DEFINITION
Chemical names Ammonium dihydrogen phosphate, ammonium dihydrogen tetraoxophosphate, monoammonium monophosphate, ammonium dihydrogen orthophosphate
C.A.S. number 7722-76-1
Chemical formula $\text{NH}_4\text{H}_2\text{PO}_4$
Formula weight 115.03
Assay Content not less than 96% and not more than 102% of $\text{NH}_4\text{H}_2\text{PO}_4$

DESCRIPTION
Colourless or white crystals, a white crystalline powder or granules

FUNCTIONAL USE
Buffering agent, dough conditioner, leavening agent

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility Freely soluble in water
** B. pH 4.3 - 5.0 (1 in 100 soln)
** C. Positive test for ammonium Passes test
** D. Positive test for phosphate Passes test

PURITY TESTS
** Fluoride Not more than 10 mg/kg (Method I or III)
** Arsenic Not more than 3 mg/kg
Test: a solution of 1 g of the sample in 35 ml of water as directed in the Limit Test (Method II).

** Heavy metals Not more than 10 mg/kg
Test: a solution of 2 g of the sample in 25 ml of water as directed in the Limit Test (Method I).

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

METHOD OF ASSAY

Dissolve about 500 mg of the sample, accurately weighed, in 50 ml of water, and titrate to a pH of 8.0 with 0.1 N sodium hydroxide. Each ml of 0.1 N sodium hydroxide is equivalent to 11.50 mg of NH₄H₂PO₄.
AMMONIUM HYDROGEN CARBONATE*

SYNONYMS
Ammonium bicarbonate
INS No. 503(ii), EEC No. 503

DEFINITION
Chemical name: Ammonium hydrogen carbonate
C.A.S. number: 1066-33-7
Chemical formula: \( \text{CH}_3\text{NO}_3 \)
Structural formula: \( \text{NH}_4\text{HCO}_3 \)
Molecular weight: 79.06
Assay: Content not less than 99.0% of \( \text{NH}_4\text{HCO}_3 \)

DESCRIPTION
White crystals or a crystalline powder with a slight odour of ammonia.

FUNCTIONAL USE
Raising agent

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility
Freely soluble in water. Insoluble in ethanol

** B. pH
About 8 (1 in 20 soln)

** C. Positive test for carbonate
Passes test

** D. Positive test for ammonia
Passes test

E. Appearance on heating
When heated, it volatilizes without charring and the vapour is alkaline to moist litmus.

PURITY TESTS
Non-volatile residue
Not more than 500 mg/kg
See description under TESTS

Chlorides
Not more than 30 mg/kg
See description under TESTS

Sulfur
Not more than 70 mg/kg
See description under TESTS

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNP 34 (1986).

PURITY TESTS (continued)

* Arsenic
Not more than 3 mg/kg
Test a solution of 1 g of the sample in 35 ml of water as directed under the Limit Test for Arsenic (Method II).

* Heavy metals
Not more than 10 mg/kg
See description under Tests

TESTS

PURITY TESTS

* Non-volatile residue
Transfer 4 g of the sample into a tared dish, add 10 ml of water, evaporate on a steam bath, and dry for 1 h at 105°. The weight of the residue does not exceed 2 mg.

* Chlorides
Dissolve 500 mg of the sample in 10 ml of hot water, add about 5 mg of sodium carbonate, and evaporate to dryness on a steam bath. Test the residue as directed under the Limit Test. Any turbidity produced does not exceed that shown in a control containing 15 μg of chloride ion (Cl⁻).

Sulfur compounds
Dissolve 4 g of the sample in 40 ml of water, add about 10 mg of sodium carbonate and 1 ml of 30% hydrogen peroxide, and evaporate the solution to dryness on a steam bath. Treat the residue as directed under the Limit Test for Sulfates* in the General Methods. Any turbidity produced does not exceed that shown in a control containing 280 μg of sulfate ion (SO₄²⁻).

* Heavy metals
Dissolve the residue from the test for Non-volatile residue in 1 ml of dilute hydrochloric acid TS, and evaporate to dryness. Dissolve the residue in 50 ml water. Test a 25 ml portion of this solution as directed in the Limit Test (Method I).

METHOD OF ASSAY
Place about 10 ml of water in a weighing bottle, tare the bottle and its contents, add about 2 g of the sample and weigh accurately. Transfer the contents of the bottle to a 250-ml flask and slowly add, with mixing, 50 ml of 1 N sulfuric acid. When solution has been effected, wash down the sides of the flask, add methyl orange TS, and titrate the excess acid with 1 N sodium hydroxide. Each ml of 1 N sulfuric acid is equivalent to 79.06 mg of NH₄HCO₃.

**AMMONIUM HYDROXIDE**

SYNONYMS

Aqua ammonia, strong ammonia solution; INS No.527, EEC No.527

DEFINITION

Chemical name: Ammonium hydroxide  
C.A.S. number: 7664-41-7 (ammonia); 1336-21-6 (aqua, NH₄OH)  
Chemical formula: NH₄OH  
Molecular weight: 35.05  
Assay: Ammonium Hydroxide contains not less than 27% and not more than 30% of NH₃

DESCRIPTION

Clear, colourless solution, having an exceedingly pungent, characteristic odour.

FUNCTIONAL USE

Alkali

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Positive test for ammonia  
When a glass rod, wet with hydrochloric acid, is held near the sample, dense white fumes are produced.

PURITY TESTS

** Non-volatile residue  
Not more than 0.02% by the following procedure. Evaporate 11 ml (10 g) of the sample in a tared platinum or porcelain dish to dryness, dry at 105° for 1 h, cool and weigh.

Readily oxidizable substances  
Passes test  
See description under TESTS

Arsenic  
Not more than 3 mg/kg  
See description under TESTS

Heavy metals  
Not more than 5 mg/kg  
See description under TESTS

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* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

TESTS

PURITY TESTS

Readily oxidizable substances

Dilute 4 ml of the sample with 6 ml of water, and add a slight excess of dilute sulfuric acid TS and 0.1 ml of 0.1 N potassium permanganate. The pink colour does not completely disappear within 10 min.

* Arsenic

Evaporate 10 g of the sample to about 2 ml on a steam bath, dilute to 50 ml with water. A 5-ml portion of this solution, diluted to 35 ml with water, meets the requirements of the Limit Test for Arsenic (Method II).

* Heavy metals

Transfer 20 g of the sample to a beaker, add about 5 mg of sodium chloride, evaporate to dryness on a steam bath, and dissolve the residue in 2 ml of dilute acetic acid TS and sufficient water to make 50 ml. A 10-ml portion of this solution, diluted to 25 ml with water, meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Tare accurately a 125-ml glass-stoppered conical flask containing 35 ml of 1 N sulfuric acid. Partially fill a 10-ml graduated pipet from near the bottom of the sample, previously cooled in the original sample bottle to 10° or lower (do not use vacuum for drawing up the sample). Wipe off any liquid adhering to the outside of the pipet and discard the first ml. Hold the pipet just above the surface of the acid and transfer 2 ml into the flask, leaving at least 1 ml in the pipet. Stopper the flask, mix and weigh again to obtain the weight of the sample. Add methyl red TS and titrate the excess acid with 1 N sodium hydroxide. Subtract the excess sulfuric acid from the total sulfuric acid (35 ml) to find the ml used to neutralize the sample. Each ml of 1 N sulfuric acid used to neutralize the ammonium hydroxide is equivalent to 17.03 mg of NH₃.

**AMMONIUM PERSULFATE**
(Tentative)

**SYNONYM**

INS No. 923

**DEFINITION**

- Chemical name: Ammonium persulfate
- C.A.S. number: 7727-54-0
- Chemical formula (empirical): \((\text{NH}_4)_2\text{S}_2\text{O}_8\)
- Formula weight: 228.20
- Assay: The product contains not less than 95% of \((\text{NH}_4)_2\text{S}_2\text{O}_8\)

**DESCRIPTION**

Ammonium persulfate occurs as colourless crystals, white crystalline powder. It has a sharp acrid odour.

*Caution: Powerful oxidizing substance*

**FUNCTIONAL USE**

Strengthening agent for flour

---

*These specifications were prepared at the 9th session of JECFA (1965) and published in NMRS 40ABC (1969).*
AMMONIUM POLYPHOSPHATE*  
(Tentative)**

SYNONYM
INS No. 452(v)

DEFINITION

Chemical name: Ammonium polyphosphate

Chemical formula: H$_n$P$_a$O$_{3a+t}$

Assay: Content not less than 50.5% and not more than 71.0% on an anhydrous basis calculated as P$_2$O$_5$

DESCRIPTION
Fine, white crystals, powder or glassy platelets

FUNCTIONAL USE
Information required**

CHARACTERISTICS

IDENTIFICATION TESTS

A. Solubility   Information required**
B. pH           Information required**

*** C. Positive test for ammonia     Passes test

*** D. Positive test for phosphate   Mix 0.5 g of sample with 10 ml of nitric acid and 50 ml of water, boil for 30 min, and cool. Test the resulting solution.

PURITY TESTS

*** Cyclic phosphate Not more than 8% calculated as P$_2$O$_5$ content

*** Fluoride        Not more than 10 mg/kg

*** Arsenic         Not more than 3 mg/kg

Heavy metals       Information required**

---

* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982).

** Information required on solubility, pH, level of heavy metals, and actual use of the product in commerce.

METHOD OF ASSAY

Mix about 300 mg of the sample, accurately weighed, with 15 ml of nitric acid and 30 ml of water, boil for 30 min and dilute with water to about 100 ml. Heat at 60°, add an excess of ammonium molybdate TS, and heat at 50° for 30 min. Filter, and wash the precipitate with dilute nitric acid (1 in 36), followed by potassium nitrate solution (1 in 100) until the filtrate is no longer acid to litmus. Dissolve the precipitate in 50 ml of 1 N sodium hydroxide, add phenolphthalein TS, and titrate the excess sodium hydroxide with 1 N sulfuric acid. Each ml of 1 N sodium hydroxide is equivalent to 3.086 mg of P₂O₅.
AMMONIUM SALTS OF PHOSPHATIDIC ACID*  
(Tentative)

SYNONYMS  
Ammonium phosphatides, emulsifier YN, mixed ammonium salts of phosphorylated glycerides  
INS No. 442, EEC No. E442

DEFINITION  
The product consists essentially of a mixture of the ammonium compounds of phosphatidic acids derived from the edible fat (usually partially hardened rapeseed oil). One or two or three glyceride moieties may be attached to phosphorus as indicated in the structural formula below. Moreover, two phosphorus esters may be linked together as phosphatidyl phosphatides. The product is prepared by the glycerolysis of the fat, phosphorylation by means of phosphorus pentoxide, and neutralization with ammonia.  
The article of commerce may be further specified as to water content, hexane-insoluble matter, inorganic hexane-insoluble matter, pH-value and triglyceride content.

Structural formula  
(approximate composition)

\[
\begin{align*}
    & R_1 \\
    \rightarrow & P \quad O \\
    \rightarrow & R_2 \\
    \rightarrow & R_3
\end{align*}
\]

where \( R_1, R_2 \) and \( R_3 \) each may be \(-\text{OH}, -\text{ONH}_4\) or a mono- or diglyceride moiety

Assay  
The phosphorus content is not less than 3.0 and not more than 3.4\% by weight; the ammonium content is not less than 1.2 and not more than 1.5\% (calculated as \( \text{N} \)).

DESCRIPTION  
Unctuous semisolid

FUNCTIONAL USE  
Emulsifier

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility  
Insoluble in water. Partially soluble in ethanol and in acetone. Soluble in fats

B. Positive test for phosphate  
Passes test  
See description under TESTS

C. Positive test for fatty acid  
Passes test  
See description under TESTS

** D. Positive test for glycerol  
Passes test

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Arsenic  
Not more than 3 mg/kg (Method II)

* Heavy metals  
Not more than 10 mg/kg  
Test 2.0 g of the sample as directed in Method II under the Limit Test for Heavy Metals

TESTS

IDENTIFICATION TESTS

* B. Positive test for phosphate  
Ignite 1 g of the product with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 ml of water and 5 ml of nitric acid. Add 5 ml of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained.

* C. Positive test for fatty acid  
Reflux 1 g of the product for 1 h with 25 ml of 0.5 N ethanolic potassium hydroxide. Ammonia is evolved from the end of the reflux condenser, recognizable by its odour and by its reaction on moist, red litmus paper. On cooling the residue to 0°, a precipitate of potassium soap is obtained.

METHOD OF ASSAY

**Determination of phosphate**  
Absorption spectrophotometry

**Reagents**

- Vanadate-molybdate solution  
Separately dissolve in water 20 g of ammonium molybdate and 1 g of ammonium vanadate. Mix the two solutions, add 140 ml of concentrated nitric acid and dilute to 1000 ml with water. Mix well.

- Standard phosphate solution  
Stock solution: Dissolve 3.8346 g of potassium dihydrogen phosphate, previously dried at 110°, in water and dilute to 1000 ml; 1 ml of this solution = 2.0 mg P₂O₅.

**Working solution**

- Sulfuric acid: sp gr 1.84
- Nitric acid: sp gr 1.42
- Perchloric acid: 60%, sp gr 1.54

**Procedure**

Weigh accurately 1.5 to 1.6 g of a representative sample into a small glass capsule and transfer to a 300-ml Kjeldahl flask containing 5 ml of sulfuric acid and 10 ml of nitric acid. Heat the flask, gently at first, with continual swirling, and later more strongly over a bare flame. Add further measured amounts of nitric acid from time to time, cooling the flask prior to addition, and continue the heating until the stage where the digest is clear and assumes a golden colour. Cool, add 5 ml of 60% perchloric acid and continue the oxidation until white acid fumes form in the flask. Cool again and add 5 ml of water and continue heating until white fumes are again driven off. Cool, dilute carefully with water, cool again and transfer quantitatively to a 500-ml volumetric flask. Dilute to volume with water and mix well (Test solution).

---

Determination of phosphate

**Procedure (continued)**

Carry out a blank digestion exactly as above but omit the sample and use the same volume of acid as required to wet oxidize the sample (Blank digest solution).

Into separate 100-ml volumetric flasks, add by burette:

(a) 25.0 ml of Standard phosphate working solution (= 5.0 mg P₂O₅)
(b) 30.0 ml of Standard phosphate working solution (= 6.0 mg P₂O₅) and
(c) a 25 ml aliquot of the test solution which will contain the equivalent of between 5 and 6 mg P₂O₅

Into each of the flasks containing the phosphorus standards, i.e. (a) and (b), transfer an aliquot of the blank digest solution equal in volume to (c), in order to compensate for possible traces of phosphorus derived from the acid digest reagents and which may be present in the Test Solution.

To each add 25 ml of the vanadate-molybdate reagent, mix, dilute to nearly 100 ml with water, mix well, adjust the temperature of the solution to 20°, dilute to the mark with water and re-mix.

After 10 min measure the absorbances of both the 6 mg P₂O₅ solution and the test solution against the 5 mg standard contained in the blank cell. Use optically matched 1 cm cells and measure at a wavelength of 420 nm, or with an Ilford 604 filter if using a photo-electric colorimeter.

**Calculation**

\[
\text{% Phosphorus} = \frac{5 + A_{\text{test}}}{A_{6 \text{ mg}} \ W} \times 0.873
\]

where

- \(A_{\text{test}}\) = Absorbance difference between the 5 mg standard and the test solution
- \(A_{6 \text{ mg}}\) = Absorbance difference between the 6 mg and 5 mg standards
- \(W\) = Weight of sample taken (g)

---

Determination of ammonium salt nitrogen in neutral Ammonium salts of phosphatidic acids

**Apparatus for steam distillation**

The apparatus consists of a 2-L flask fitted with a rubber bung through which pass an approximately 3" length of glass tubing, arranged so that the lower end is near the bottom of the flask, and a shorter L-shaped piece of tubing arranged such that the tube projects about 1/4" below the lower surface of the bung, to act as a steam outlet tube. The flask should be approximately 2/3 filled with distilled water made slightly acid with dilute sulfuric acid TS and contain a few pieces of sintered glass to prevent bumping when the contents of the flask are vigorously boiled to act as a steam generator. A tap funnel may be fitted to the flask if desired to facilitate replenishing the water in the flask between determinations.

The steam outlet tube is connected via a condensation trap to the inlet of a steam distillation head, fitted to a short necked 1-L round bottomed B34 necked flask. The distillation head should be such that the steam inlet tube reaches almost to the bottom of the 1-L flask and the outlet should be fitted with two splash traps, one near the top of the 1-L flask and the other near the top of B19 jointed vertical, single-surface condenser to which the distillation head connects. The vertical condenser should be fitted with an extended outlet tube, able to reach to the bottom of a 500-ml conical flask.
Determination of ammonium salt nitrogen in neutral ammonium salts of phosphatidic acids

Reagents
- Boric acid solution (2% w/v in water)
- Sodium hydroxide solution (40% w/v in water)
- 0.02N Hydrochloric acid
- Mixed indicator: Mix 5.0 of 0.1% w/v alcoholic solution of bromocresol green and 2.0 of a 0.1% w/v alcoholic solution of methyl red and dilute the mixture to 30 ml with 95% alcohol.
- Silicone fluid 200/50 MS

Procedure
Assemble and thoroughly steam out the apparatus. Accurately weigh about 0.2 g of a representative sample of neutral Ammonium salts of phosphatidic acids into a small glass phial (approx. 3/4" diameter, 1/2" deep). Transfer the phial and weighed contents to the distillation flask and add approximately 250 ml distilled water. Connect the distillation head and splash traps to the distillation flask and vertical condenser, and arrange the condenser such that the outlet dips below the surface of 10 ml of 2% boric acid and 1 ml mixed indicator contained in a 500-ml conical flask. Add to the distillation flask, via a funnel attached by means of a short piece of rubber tubing to the steam inlet tube, 75 ml 40% aqueous sodium hydroxide, and wash in with distilled water*. Detach the funnel and connect the steam inlet to the steam supply. Vigorously steam distil the contents of the distillation flask and collect 200 ml distillate in the boric acid. During the distillation gently agitate the distillation flask if necessary, to avoid the sample being deposited around the upper surfaces of the flask. When the required amount of distillate has been collected, lower the receiving flask, stop the steam supply, and wash down the inside of the condenser, and the outside of the lower end, with a small quantity of distilled water, collecting the washings in the receiving flask. Titrate the contents of the receiving flask with 0.02N hydrochloric acid.

Carry out at least one blank determination in exactly the same way but omitting the sample.

During the distillation difficulty may be experienced with frothing of the contents of the distillation flask. If so, 2 drops of silicone fluid should be added to the distillation flask at the time of adding the sample; and a similar amount included in the blank determination.

Calculation

\[
1 \text{ ml } 0.02 \text{ N HCl } = 0.2802 \text{ mg of nitrogen}
\]

\[
\% \text{ Nitrogen } = \frac{(\text{sample titre} - \text{blank titre}) \times 28.02}{(\text{sample wt. in mg})}
\]

* The sodium hydroxide may be added to the flask through a tap funnel, fitted to the distillation flask if preferred and washed in with distilled water. If so a liquid seal should be maintained in the funnel during the addition and distillation.
SYNONYMS
Isoamyl acetate, acetic acid esters of amyl alcohols

DEFINITION
A mixture of acetic acid esters of pentanols

- **Chemical name**: 3-Methylbutyl acetate
- **Chemical formula**: $\text{C}\text{\textsubscript{11}}\text{H}_{16}\text{O}_{2}$
- **Structural formula**:

```
  H
 / \  /
CH\text{\textsubscript{3}} COOCH\text{\textsubscript{2}}CH\text{\textsubscript{2}}CH
    \  /
     \CH\text{\textsubscript{3}}
```

- **Molecular weight**: 130.19
- **Assay**: Content not less than 98% of $\text{C}\text{\textsubscript{11}}\text{H}_{16}\text{O}_{2}$

DESCRIPTION
Colourless, clear liquid, having a characteristic fruitlike odour

FUNCTIONAL USE
Flavouring agent, carrier solvent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility**
Slightly soluble in water. Insoluble in glycerin.
Practically insoluble in propan-1,2-diol.
Miscible with ethanol, ether, ethyl acetate, most fixed oils and mineral oils.

** B. Refractive index**
$n_d^0$: 1.398 - 1.404

** C. Specific gravity**
$d_d^0$: 0.868 - 0.878

PURITY TESTS

** Non volatile residue**
Not more than 7 mg/100 ml

** Distillation range**
Not less than 99% v/v distils between 135° and 143°

---

* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979).

PURITY TESTS (continued)

* **Arsenic**
  Not more than 3 mg/kg (Method II)

* **Heavy metals**
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in the Limit Test (Method II)

* **Acid value**
  Not more than 1

**METHOD OF ASSAY**

Weigh accurately about 0.8 g of the sample and proceed as directed under the method for Esters Determination in the General Methods*, using 65.10 as the equivalence factor (e) in the calculation.

---

α-AMYL CINNAMIC ALDEHYDE*  
(Tentative)**

SYNONYMS
α-Amylecinnamaldehyde, α-pentylecinnamaldehyde

DEFINITION

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>2-Benzylideneheptanal, 2-pentyl-3-phenyl-2-propenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>122-40-7</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₁₅H₁₁O</td>
</tr>
<tr>
<td>Structural formula</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
</tbody>
</table>

Molecular weight 202.28

Assay Content not less than 97% of C₁₅H₁₁O

DESCRIPTION
Yellow, oily liquid, with a powerful floral odour, reminiscent of jasmine

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Very soluble in ethanol, soluble in fixed oils, practically insoluble in glycerol, propane-1,2-diol and water.

*** B. Refractive index
n₀²⁰ : 1.554 - 1.559

*** C. Specific gravity
d₂₀²⁰ : 0.9632 - 0.971;  d₁₅²⁰ : 0.963 - 0.969

D. Infrared spectrum Information required**

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation. Information on infrared spectrum is required.

PURITY TESTS

* Solubility in ethanol
1 ml dissolves in 5 ml of 80% ethanol

* Acid value
Not more than 3

METHOD OF ASSAY

Method A
Weigh accurately about 2 g of the sample and proceed as directed under the method for Aldehyde Determination in the General Methods*, using 101.1 as the equivalence factor (e) in the calculation.

Method B
Determine by gas-liquid chromatography, as directed in the Method of Assay for Allyl-α-ionone.

α-AMYL CINNAMIC ALDEHYDE DIMETHYL ACETAL*
(Tentative)**

SYNONYMS
α-Amyl cinnamaldehyde dimethyl acetal

DEFINITION

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>1,1-Dimethoxy-2-benzylideneheptane, 1,1-dimethoxy-2-pentyl-3-phenylpropene</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>91-87-2</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C_{16}H_{20}O_{2}</td>
</tr>
<tr>
<td>Structural formula</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
</tbody>
</table>

Molecular weight 248.37
Assay Content not less than 95% of C_{16}H_{20}O_{2}

DESCRIPTION
Almost colourless to yellowish oily liquid with a mild green floral odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Very soluble in ethanol and propane-1,2-diol. Insoluble in water.

*** B. Refractive index
n^20_0 : 1.503 - 1.509

*** C. Specific gravity
d^20_0 : 0.948 - 0.956

D. Infrared spectrum
See Appendix at the end of these specifications

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The reference to identity, purity and methods of analysis were felt to require further consideration. Information on the method for free aldehyde and the GLC method of assay are required.

PURITY TESTS

* Acid value

Free aldehyde

METHOD OF ASSAY

Method A

Weigh accurately about 2 g of the sample and proceed as directed under the method for Acetal Determination in the General Methods*, using 124.2 as the equivalence factor (e) in the calculation.

Method B

Determine by gas-liquid chromatography (Information required)**

APPENDIX

*** Infrared spectrum: α-Amyl Cinnamic Aldehyde Dimethyl Acetal

---


** The reference to identity, purity and methods of analysis were felt to require further consideration. Information on the method for free aldehyde and the GLC method of assay are required.

*** Intra-red spectra through the courtesy of the International Creganization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc., Philadelphia, USA.
α-AMYL CINNAMYL ALCOHOL*
(Tentative)**

SYNONYM
2-Pentyl-3-phenyl-2-propenol

DEFINITION
Chemical names
2-Benzylideneheptanol, 2-pentyl-3-phenyl-2-propenal
C.A.S. number
101-85-9
Chemical formula
C_{16}H_{20}O
Structural formula
\[
\begin{array}{c}
\text{(CH}_2\text{)}_4\text{CH}_3 \\
\text{C} = \text{C} - \text{C} \text{H}_2 \text{O} \text{H}
\end{array}
\]

Molecular weight
204.31
Assay
Content not less than 95% of C_{16}H_{20}O

DESCRIPTION
Colourless to yellowish liquid with a mild waxy and floral odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Soluble in ethanol, insoluble in water

*** B. Refractive index
n_\text{D}^\circ : 1.553 - 1.540

*** C. Specific gravity
d_\text{D}^\circ : 0.960 - 0.966

D. Infrared spectrum
See Appendix at the end of these specifications

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).
** The reference to identity, purity and methods of analysis were felt to require further confirmation. Information on the GLC method of assay is required.
PURITY TEST

* Solubility in ethanol

1 ml dissolves in 3 ml of 70% ethanol

METHOD OF ASSAY

Method A

Proceed as directed under the method for Total Alcohol Determination in the General Methods*. Weigh accurately about 1.6 g of the acetylated alcohol and use 102.2 as equivalence factor (e) in the calculation.

Method B

Determine by gas-liquid chromatography (Information required)**

APPENDIX

*** Infrared spectrum

α-Amyl Cinnamyl Alcohol

---


** The reference to identity, purity and methods of analysis were felt to require further consideration. Information on the method for free aldehyde and the GLC method of assay are required.

*** Infra-red spectra through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc., Philadelphia, USA.
ALPHA-AMYLASE AND GLUCOAMYLASE FROM *ASPERGILLUS ORYZAE, VAR.*
(Tentative)**

**SYNONYMS**

1. Diastase, ptyalin, glycogenase; INS No. 1100
2. Amyloglucosidase, acid maltase, lysosomal α-glucosidase, exo-1,4-α-glucosidase.

**SOURCES**

Commercial enzyme preparations are produced by the controlled fermentation of *Aspergillus oryzae, var.*

**ACTIVE PRINCIPLES**

1. α-Amylase
2. Glucan 1,4-α-glucosidase

**SYSTEMATIC NAMES AND NUMBERS**

1. 1,4-α-D-Glucan glucohydrolase - EC 3.2.1.1
2. 1,4-α-D-Glucan glucanohydrolase - EC 3.2.1.3

**REACTIONS CATALYZED**

1. α-Amylase hydrolyzes 1,4-α-glucosidic linkages in polysaccharides, yielding dextrins and oligo- and monosaccharides.
2. Glucoamylase hydrolyzes 1,4-α and 1,6-α-glucosidic linkages in polysaccharides, yielding glucose.

**SECONDARY ENZYME ACTIVITIES**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase</td>
<td>EC 3.1.1.3</td>
</tr>
<tr>
<td>Tannase</td>
<td>EC 3.1.1.20</td>
</tr>
<tr>
<td>Cellulase</td>
<td>EC 3.2.1.4</td>
</tr>
<tr>
<td>Endo-1,3-β-glucanase</td>
<td>EC 3.2.1.6</td>
</tr>
<tr>
<td>Pectinase</td>
<td>EC 3.2.1.15</td>
</tr>
<tr>
<td>Maltrase</td>
<td>EC 3.2.1.20</td>
</tr>
<tr>
<td>Lactase</td>
<td>EC 3.2.1.23</td>
</tr>
<tr>
<td>Endo-1,4-β-mannanase (Hemicellulase)</td>
<td>EC 3.2.1.78</td>
</tr>
<tr>
<td>Proteinase</td>
<td></td>
</tr>
</tbody>
</table>

**DESCRIPTION**

The products are typically tan amorphous powders or tan to dark-brown liquids that may be dispersed in food-grade diluents and may contain stabilizers and preservatives. They are soluble in water and practically insoluble in ethanol, chloroform and ether.

**FUNCTIONAL USES**

Preparation of and/or use in cereals and starch, fruits and vegetables, beverages, sugar and honey, confectionary, bakery and dietary food.

*These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

**Information required on non-enzymic components; characterization of microbial strains used; a measure of specific or relative activity to more explicitly define product quality; presence of β-nitropropionic acid in this product.
GENERAL SPECIFICATIONS

Must conform to the "General Specifications for Enzyme Preparations used in Food Processing".*

CHARACTERISTICS

IDENTIFICATION TESTS

1. α-Amylase activity
   The sample shows fungal α-amylase activity**

2. Glucoamylase activity
   The sample shows glucoamylase activity**

SPECIAL PURITY CRITERIA

β-Nitropropionic acid
Information required***

* See Annex 1 at the end of this compendium.


*** Information required regarding the presence of β-nitropropionic acid in this product.
**SYNONYMS**

Diastase, ptyalin, glycogenase; INS No. 1100

**SOURCES**

Commercial enzyme preparations are produced by the controlled fermentation of *Aspergillus oryzae, var.* and isolated from the growth medium.

**ACTIVE PRINCIPLE**

α-Amylase

**SYSTEMATIC NAME AND NUMBER**

1,4-α-D-Glucan glucanohydrolase - EC 3.2.1.1

**REACTIONS CATALYZED**

The enzyme preparations hydrolyze 1,4-α-glucosidic linkages in polysaccharides, yielding dextrins and oligo- and monosaccharides.

**SECONDARY ENZYME ACTIVITIES**

- Glucoamylase - EC 3.2.1.1
- Proteinases

**DESCRIPTION**

The products are typically tan amorphous powders or tan to dark-brown liquids that may be dispersed in food-grade diluents and may contain stabilizers and preservatives. They are soluble in water and practically insoluble in ethanol, chloroform and ether.

**FUNCTIONAL USES**

Starch hydrolysis, syrup production, baking and brewing

**GENERAL SPECIFICATIONS**

Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"***

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

- α-Amylase activity
  - The sample shows fungal α-amylase activity****

**SPECIAL PURITY CRITERIA**

- Beta-nitropropionic acid
  - Information required**

---

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

** Information required on major and minor enzymic activities; non-enzymic components; characterization of microbial strains used; a measure of specific or relative activity to more explicitly define product quality; presence of β-nitropropionic acid in this product.

*** See Annex 1 at the end of this compendium.

SYNONYMS
Glycogenase

SOURCE
Produced extracellularly by the controlled fermentation of *Bacillus subtilis* B1-109 (ATCC 39,701) containing the gene for α-amylase from *Bacillus megaterium* (NCIB 11568) inserted by plasmid pCPC800. When fermentation is complete, the broth is clarified by centrifugation or filtration. The clarified broth containing the soluble enzyme is ultrafiltered to produce the desired activity.

ACTIVE PRINCIPLE
Alpha-amylase

SYSTEMATIC NAME AND NUMBER
1,4-α-D-Glucan glucanohydrolase EC 3.2.1.1

REACTIONS CATALYZED
Endohydrolysis of 1,4-α-D-glucosidic linkages in polysaccharides, containing three or more 1,4-α-linked D-glucose units

SECONDARY ENZYME
Glycosyl transferase, protease

DESCRIPTION
Commercial preparations are typically tan to dark brown liquids containing the active enzyme.

FUNCTIONAL USES
Starch hydrolysis

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing***

CHARACTERISTICS

IDENTIFICATION TEST
α-Amylase activity
The sample shows bacterial α-amylase activity***

---

* New specifications prepared at the 37th session of JECFA (1990).

** The "General Specifications for Enzyme Preparations Used in Food Processing" have been tentatively revised to cover enzyme preparations produced by genetic manipulation (See Annex A at the end of this compendium). Information required: CAS number.

ALPHA-AMYLASE FROM BACILLUS STEAROTHERMOPHILUS*

SYNONYMS
Glycogenase; INS No.1100

SOURCES
Produced extracellularly by the controlled fermentation of Bacillus stearothermophilus (ATCC 39,709)

ACTIVE PRINCIPLE
α-Amylase

SYSTEMATIC NAME AND NUMBER
1,4-α-D-Glucan glucanohydrolase - EC 3.2.1.1

REACTIONS CATALYZED
Endohydrolysis of 1,4-α-D-glucosidic linkages in polysaccharides, containing three or more 1,4-α-linked D-glucose units

DESCRIPTION
The products are typically tan to dark brown liquids containing the active enzyme.

FUNCTIONAL USES
Starch hydrolysis

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"***

CHARACTERISTICS

IDENTIFICATION TEST

α-Amylase activity
The sample shows bacterial α-amylase activity ***

* New specifications prepared at the 37th session of JECFA (1990).

** See Annex 1 at the end of this compendium.

SYNONYMS
Glycogenase

SOURCES
Produced extracellularly by the controlled fermentation of Bacillus subtilis (ATCC 39,705) containing the gene for α-amylase from Bacillus stearothermophilus (ATCC 39,709) introduced by plasmid pCPC720. When fermentation is complete, the broth is clarified with calcium chloride, which flocculates the cells. The broth is separated from the cells by filtration with diatomaceous earth, and the filtered, clarified broth containing the soluble enzyme is then ultrafiltered to remove residual cells and particulate matter and to concentrate the product to the desired activity.

ACTIVE PRINCIPLE
α-Amylase

SYSTEMATIC NAME AND NUMBER
1,4-α-D-Glucan glucoamylase - EC 3.2.1.1

REACTIONS CATALYZED
Endohydrolysis of 1,4-α- and 1,6-α-glucosidic linkages in polysaccharides, containing three or more 1,4-α-linked D-glucose units

DESCRIPTION
Commercial preparations are typically tan to dark brown liquids containing the active enzyme.

FUNCTIONAL USES
Starch hydrolysis

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing***

CHARACTERISTICS

IDENTIFICATION TEST
α-Amylase activity
The sample shows bacterial α-amylase activity***

---

* New specifications prepared at the 37th session of JECFA (1990).

** The "General Specifications for Enzyme Preparations Used in Food Processing" have been tentatively revised to cover enzyme preparations produced by genetic manipulation (See Annex 1 at the end of this compendium). Information required: CAS number.

SYNONYMS
Glycogenase; INS No.1100

SOURCES
Produced extracellularly by the controlled fermentation of Bacillus subtilis strain F (ATCC 23,350, DSM 7)

ACTIVE PRINCIPLE
α-Amylase

SYSTEMATIC NAME AND NUMBER
1,4-α-D-Glucan glucanohydrolase - EC 3.2.1.1

REACTIONS CATALYZED
Endohydrolysis of 1,4-α-D-glucosidic linkages in polysaccharides, containing three or more 1,4-α-linked D-glucose units

DESCRIPTION
Commercial products are typically brown liquids, granules or powders containing the active enzyme.

FUNCTIONAL USES
Starch hydrolysis

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"**

CHARACTERISTICS

IDENTIFICATION TEST
α-Amylase activity
The sample shows bacterial α-amylase activity***

---

* New specifications prepared at the 37th session of JECFA (1990).

** See Annex 1 at the end of this compendium.

AMYLOGLUCOSIDASE FROM *ASPERGILLUS NIGER, VAR.*
(Tentative)**

SYNONYMS
Glucoamylase, acid maltase, lysosomal α-glucosidase, exo-1,4-α-glucosidase

SOURCES
Commercial enzyme preparations are produced by the controlled fermentation of *Aspergillus niger, var.*

ACTIVE PRINCIPLE
Glucan 1,4-α-glucosidase

SYSTEMATIC NAME AND NUMBER
1,4-α-β-Glucan glucohydrase - EC 3.2.1.3

REACTION CATALYZED
Glucoamylase hydrolyzes 1,4-α and 1,6-α-glucosidic linkages in polysaccharides (starch, glycogen, etc.) yielding glucose (dextrose).

SECONDARY ENZYME ACTIVITY
α-Amylase - EC 3.2.1.1

DESCRIPTION
The products are typically off-white to tan amorphous powders or tan to dark-brown liquids that may be dispersed in food-grade diluents or carriers and may contain stabilizers and preservatives. They are soluble in water and practically insoluble in ethanol, chloroform and ether.

FUNCTIONAL USES
Preparation of and/or use in starch syrups, fruit juices; manufacture of cheese.

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing***

CHARACTERISTICS

IDENTIFICATION TEST

Glucoamylase activity
The sample shows glucoamylase activity****

---

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

** Information required on major and minor enzymic activities, non-enzymic components; characterization of microbial strains used; a measure of specific or relative activity to more explicitly define product quality.

*** See Annex 1 at the end of this compendium.

**trans-ANETHOLE**

SYNONYM

trans-p-Propenylanisole

DEFINITION

Chemical names

(E)-1-Methoxy-4-(1-propenyl) benzene, 1-methoxy-trans-4-propenylbenzene

Chemical formula

\( C_{10}H_{12}O \)

Structural formula

\[
\begin{align*}
\text{C} = & \text{C} \\
\text{H} & \\
\text{H} & \\
\text{C} & \text{H}_3 \\
\end{align*}
\]

Molecular weight

148.20

Assay

Content not less than 99% of \( C_{10}H_{12}O \)

DESCRIPTION

Colourless or faintly yellow liquid at/or above 23\(^\circ\), with a sweet taste and a characteristic aniseed-like odour.

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Slightly soluble in water. Miscible with choloform and ether.

** B. Refractive index

\( n^\text{D} \) : 1.559 - 1.562 (in supercooled liquid form)

** C. Specific gravity

\( d^\text{20} \) : 0.983 - 0.988

** D. Solidification point

Not below 20\(^\circ\).

PURITY TESTS

** Solubility in ethanol

1 ml dissolves in 2 ml of 96% ethanol

** cis-Isomer

Not more than 1% w/w

See Method of Assay

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988) superseding the earlier specifications published in FNP 28 (1983).

PURITY TESTS (continued)

Aldehydes and ketones Passes test. See description under TESTS.

Phenols Passes test. See description under TESTS.

TESTS

PURITY TESTS

Aldehydes and ketones
Shake 10 ml of the sample with 50 ml of a saturated solution of sodium bisulfite in a glass-stoppered, graduated cylinder, and allow the mixture to stand for 6 h. The volume of the sample does not diminish appreciably, and no crystalline deposit separates.

Phenols
Shake 1 ml with 20 ml of water, and allow the liquid to separate. Filter the water layer through a filter paper previously moistened with water, and to 10 ml of the filtrate add 3 drops of ferric chloride TS. No purplish colour is produced.

METHOD OF ASSAY
Determine by gas-liquid chromatography as directed in the Method of Assay for d-Carvone. Cis-isomer elutes first. Then, calculate the content (and percentage of cis-isomer) by the method of area percentages (area normalization).
**ANISYL ACETONE**
(Tentative)**

**SYNONYM**
p-Methoxybenzyl acetone

**DEFINITION**

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>4-(4-Methoxyphenyl)-2-butanone</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>104-20-1</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C₁₃H₁₄O₂</td>
</tr>
<tr>
<td>Structural formula</td>
<td>![Chemical Structure]</td>
</tr>
</tbody>
</table>

**Molecular weight** 178.23

**Assay**
Content not less than 95% of C₁₃H₁₄O₂

**DESCRIPTION**
Colourless oily liquid with a floral and fruity note

**FUNCTIONAL USE**
Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

*** A. Solubility  
Soluble in ethanol and in fixed oils, slightly soluble in propane-1,2-diol, very slightly soluble in water

*** B. Refractive index  
\(n_\text{D}^25°\) : 1.520 - 1.525

*** C. Specific gravity  
\(d_\text{D}^25°\) : 1.044 - 1.050

D. Infrared spectrum  
See Appendix at the end of these specifications.

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation. Information on the GLC method of assay is required.

PURITY TEST

* Solidification point Not less than 9°

METHOD OF ASSAY

Method A
Weigh accurately about 2.5 g of the sample and proceed as directed under the method for Aldehyde and Ketone Determination in the General Methods*, using 89.12 as the equivalence factor (e) in the calculation.

Method B
Determine by gas-liquid chromatography (Information required)**

APPENDIX

** Infrared spectrum: Anisyl Acetone

---


** Infra-red spectra through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc., Philadelphia, USA.
ANNATTO EXTRACTS*

SYNONYMS
Rocou, Orlean, Terre orellana, L. Orange;
INS No. 160 b, EEC No. E160 b

DEFINITION
Annatto Extracts in oil or water-soluble annatto are obtained by extraction of the outer coating of the seeds of the annatto tree (Bixa orellana L.) as follows:

1. Annatto extract in oil, as solution or suspension, is prepared by extraction of the outer coating of the seeds with food-grade vegetable oil. It is also prepared by dilution, with food-grade vegetable oil, of the extract of the outer the coating with the following organic solvents (acetone, dichloromethane, ethanol, hexane, methanol, propan-2-ol, trichloroethylene) after removal of the solvent.

2. Water-soluble annatto is prepared either by extraction with aqueous alkali (sodium or potassium hydroxide) of the outer coating of the seeds or by hydrolysis in aqueous alkali (sodium or potassium hydroxide) of the extract of the outer coating with the following organic solvents (acetone, dichloromethane, ethanol, hexane, methanol, propan-2-ol, trichloroethylene) after removal of the solvent. Powdered forms are also prepared.

Class
Carotenoid

Code numbers
C.I. (1975) No. 75120
Schultz (1931) No. 1387
C.A.S. No. 1393-63-1

Chemical names
Annatto extract in oil contains several coloured components, the major single one being bixin, which may be present in both cis- and trans-forms. Thermal degradation products of bixin may also be present.

Water-soluble annatto contains norbixin, the hydrolysis product of bixin, in the form of the sodium or potassium salts as the major colouring principle. Both cis- and trans-forms may be present.

1. 6'-Methylhydrogen-9'-cis-6,6'-diapocarotene-6,6'-dioate
   6'-Methylhydrogen-9'-trans-6,6'-diapocarotene-6,6'-dioate
2. 9'-Cis-6,6'-diapocarotene-6,6'-dioic acid
   9'-Trans-6,6'-diapocarotene-6,6'-dioic acid

Chemical formula
Bixin: C_{25}H_{30}O_{5},
Norbixin: C_{24}H_{21}O_{4}

* These specifications were prepared at the 26th meeting of JECFA (1982) and published in FNP 25 (1982).
Cis and trans-bixins have the structures:

\[
\text{H}_3\text{CO}_2\text{C} - \text{CO}_2\text{H}
\]

\[
\text{H}_3\text{CO}_2\text{C} - \text{CO}_2\text{H}
\]

Cis- and trans-norbixins (sodium or potassium salts) have the structures:

\[
\text{RO}_2\text{C} - \text{CO}_2\text{R}
\]

\[
\text{RO}_2\text{C} - \text{CO}_2\text{R}
\]

where \( R = \text{Na} \) or \( \text{K} \)

Molecular weight

- Bixin: 394.51
- Norbixin: 380.48

Assay

Annatto extract in oil contains not less than 0.2% of total carotenoids expressed as bixin. Water-soluble annatto contains not less than 0.2% of total carotenoids expressed as norbixin.

DESCRIPTION

Annatto extract in oil: Red to reddish-brown solution or suspension.

Water-soluble annatto: Reddish-brown liquid, lumps, powder or paste.

FUNCTIONAL USE

Food colour
**CHARACTERISTICS**

**IDENTIFICATION TESTS**

* A. Solubility

<table>
<thead>
<tr>
<th>Substance</th>
<th>Test Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annatto extract in oil</td>
<td>Insoluble in water</td>
</tr>
<tr>
<td></td>
<td>Slightly soluble in ethanol</td>
</tr>
<tr>
<td>Water-soluble annatto</td>
<td>Soluble in water</td>
</tr>
<tr>
<td></td>
<td>Slightly soluble in ethanol</td>
</tr>
</tbody>
</table>

** B. Spectrophotometry

1. Annatto extract in oil, diluted with chloroform, has absorbance maxima at 439, 470 and 501 nm.
2. Water-soluble annatto, diluted with water, has absorbance maxima at 453 and 482 nm.

C. Column chromatography and Carr-Price reaction

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Passes test</td>
</tr>
<tr>
<td></td>
<td>See description</td>
</tr>
</tbody>
</table>

D. Thin-layer chromatography

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Passes test</td>
</tr>
<tr>
<td></td>
<td>See description under TESTS</td>
</tr>
</tbody>
</table>

E. Colour reaction

Concentrated sulfuric acid with annatto extract gives a cornflower-blue colour due to bixin, or a blue-green colour with norbixin.

**PURITY TESTS**

*** Arsenic

Not more than 3 mg/kg

*** Lead

Not more than 10 mg/kg

* Heavy metals

Not more than 40 mg/kg

Test 0.5 g of the sample as directed in the Limit Test (Method II).

* Residual solvents

<table>
<thead>
<tr>
<th>Substance</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane and</td>
<td>Not more than 30 mg/kg, singly or in combination</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>Not more than 30 mg/kg</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>Not more than 50 mg/kg</td>
</tr>
<tr>
<td>Methanol</td>
<td>Not more than 50 mg/kg</td>
</tr>
<tr>
<td>Hexane</td>
<td>Not more than 25 mg/kg</td>
</tr>
</tbody>
</table>


TESTS

IDENTIFICATION TESTS

C. Column chromatography and Carr-Price reaction

**Anatto extract in oil**

Dissolve enough of the sample in benzene to obtain a liquid having about the same colour as a 0.1% potassium dichromate solution. Add 3 ml of the solution at the top of the alumina column (see below) and elute slowly with benzene. Bixin is very strongly absorbed on the alumina surface and forms a brilliant orange-red zone (difference from crocetin). A very pale yellow-coloured zone migrates in general very quickly through the column and is eliminated by washing with benzene. Bixin cannot be eluted with benzene. Displace the benzene in the column with chloroform, previously dried over potassium carbonate, by washing the column three times with chloroform. When the last chloroform washing has been eluted, add 5 ml of the Carr-Price TS on the top of the column. The bixin-zone becomes immediately blue-green (differentiates bixin from crocetin).

**Water-soluble anatto**

Put 2 ml or 2 g of the sample in a 50-ml separating funnel. Add enough 2 N sulfuric acid to give strongly acid reaction. Norbixin separates as a red precipitate. Add 50 ml of benzene and shake vigorously. After separation discharge the water layer and wash the benzene phase with water until the elimination of the acid reaction. Centrifuge the generally emulsified solution of norbixin in benzene for 10 min at 2500 rpm. Decant the clear norbixin solution and dry over anhydrous sodium sulfate. Add 3-5 ml of this solution on the top of the alumina column (see below). Norbixin forms an orange-red zone at the surface of the column and gives the sample Carr-Price reaction as bixin.

**Preparation of alumina column**

**Apparatus**

A glass tube of 200 mm x 7 mm inside diameter.

**Procedure**

Close one end of the tube with a tight glass-wool plug, 1 cm thick. Fill the tube with a suspension of absorption alumina in benzene. Use standardized aluminium oxide absorbant, 80-200 mesh. The aluminium oxide after sedimentation should occupy two-thirds of the tube. Attach the tube to a suction flask. Fill the column with benzene and adjust suction to about 30 drops per min., continuing until the benzene layer is 2-3 mm above the alumina surface.

**NOTE:** It is advisable to place a glass-wool plug at the top the alumina to prevent churning when solvent is added, and to apply a reservoir of solvent at the top of the column, which will exert a mild pressure on the chromatographic column. The suction must be carefully controlled to prevent channeling in the alumina. Avoid eluting the benzene or other solvent completely. It is necessary that the surface of the alumina is kept covered with a liquid layer.
**METHOD OF ASSAY**

**Annatto extract in oil:**
Use silica gel with 12% calcium sulfate binding agent, and a mixture of acetic acid, chloroform and acetone (1:50:50 by volume) as developing solvent. The sample in oil produces at least 3 or 4 red or yellow spots. Two red spots are clearly more intense than the other spots. Dissolve the two spots in benzene and identify the one of the spots that is bixin by the method given in C.

**Water-soluble annatto:**
Prepare a chromatogram as indicated for Annatto extract in oil. Three or four spots are obtained, of which 2 spots have an orange colour and the others an orange-yellow colour. Dissolve the orange spots in benzene and identify the one of the spots that is norbixin by the method given in C.

**Annatto extract in oil**
Transfer 0.1 g to 1 g of the sample, accurately weighed, into a 100-ml volumetric flask, dissolve in chloroform, dilute to volume with chloroform, and mix. Transfer a 1 ml portion of the solution into another 100-ml volumetric flask, and dilute to 100 ml. Measure the absorbance A of this solution at the wavelength of 470 nm.

\[
\% \text{ Total carotenoid (expressed as bixin)} = \frac{A}{2.826} \times \frac{100000}{\text{sample weight (mg)}} \times 100
\]

**Water-soluble annatto**
Weigh accurately 0.1 g of the sample, add 0.01 N sodium hydroxide TS to 100 ml and shake thoroughly. Transfer a 1 ml portion of the solution into a separating funnel, add 10 ml of sodium chloride solution (1 in 10) and water to 50 ml, and add 2 ml of dilute sulfuric acid TS. Continue to extract the solution with each 10 ml of benzene until the benzene extract is not coloured. Combine benzene extract, wash three times with each 5 ml of water, and allow to stand to separate the water layer. Transfer the benzene extract into another separating funnel. Wash the water layer three times with 2 ml of benzene, and combine the washings to the benzene extract. To the benzene extract, add an equal volume of petroleum benzene, mix, and continue to extract with each 5 ml of 0.01 N sodium hydroxide TS until sodium hydroxide solution is not coloured. Combine extracts, and add 0.01 N sodium hydroxide to 100 ml. Measure the absorbance A at the wavelength of 453 nm.

\[
\% \text{ Total carotenoid (expressed as norbixin)} = \frac{A}{3.473} \times \frac{100000}{\text{sample weight (mg)}} \times 100
\]

**ANOXOMER***

*(Tentative)***

**DEFINITION**

Anoxomer is a divinylbenzene-hydroquinone-phenolic condensation polymer

**Chemical names**

Tert-butylhydroquinone polymer with divinylbenzene, p-tert-butylphenol, p-methoxyphenol, 4,4'-isopropylidene-diphenol and p-cresol; 1,4-benzenediol, 2-(1,1-dimethylethyl)-, polymer with divinylbenzene, 4-(1,1-dimethylethyl)-phenol, 4-methoxyphenol, 4,4'-(1-methylethyridene)bis phenol and 4-methylphenol

**Structural formula**

![Structural formula of Anoxomer](image)

where: $m = 13.3\%$; and $(40/86.7 \times 100)\%$ of X is

- $\text{OCH}_3$
- $\text{CH}_3$

$n = 86.7\%$  $(25/86.7 \times 100)\%$ of X is

- $\text{C-CH}_3$
- $\text{CH}_3$

$(11.7/86.7 \times 100)\%$ of X is

- $\text{CH}_3$

$(10/86.7 \times 100)\%$ of X is

- $\text{C-}$
- $\text{CH}_3$

**Molecular weight**

Approximately 3,800 (average)

**Assay**

Content not less than 98.0%

**DESCRIPTION**

A free-flowing, off-white powder

**FUNCTIONAL USE**

Antioxidant

---

* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982).

** Additional information required on molecular weight distribution, and confirmation that the referred analytical methods actually determine the phenols in the bound state within the polymer.

*** Information required on molecular weight distribution.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Insoluble in water, glycerin, and propylene glycol.
   Freely soluble in ethanol and in vegetable oils; very soluble in diethyl ether,
   chloroform, benzene, and acetone

* B. Bulk density
   Approximately 0.5 g/ml

C. Ultraviolet absorption
   A 1 in 10,000 solution in 1,4-dioxane exhibits absorption maxima at
   264 and 287 nm

D. Infrared spectrum
   See Appendix

PURITY TESTS

* Water content
   Not more than 1% (Karl Fischer Method)

* Arsenic
   Not more than 3 mg/kg (Method II)

* Heavy metals
   Not more than 10 mg/kg
   Test 2 g of the sample as directed in the Limit Test (Method II)

Low-molecular-weight antioxidant (monomer, dimer, trimer below 500 m.w.)

Not more than 1.0%, singly or in combination
   See description under TESTS

Naphthalenes
   Not more than 25 mg/kg
   See description under TESTS

High-molecular-weight antioxidant (above 50,000)

Not more than 5.0%
   See description under TESTS

Molecular weight peak
   Between 2,900 and 4,000
   See description under TESTS

** Phenol content as hydroxyanisole
   Between 40% and 43%
   See description under TESTS

** Phenol content as tert-butyl hydroquinone
   Between 11.2 and 12.9%
   See description under TESTS

** Phenol content as p-cresol
   Between 8% and 14%
   See description under TESTS

** Phenol content as tert-butylphenol
   Between 19% and 23%
   See description under TESTS

** Phenol content as bisphenol-A
   Between 7% and 11%
   See description under TESTS

** The Committee requires confirmation that the referenced analytical methods actually determine the phenols in the bound state within the polymer.
PURITY TESTS (continued)

Phenol content.  
**total**  
Between 3.2 and 3.8 meq/g titratable phenolic protons  
See description under TESTS

Residual divinyl benzene  
Not more than 50 mg/kg

Residual ethylvinyl benzene  
Not more than 100 mg/kg  
Determine by the method given for "Monomer and Naphthalene Contents"

Xylenes  
Not more than 50 mg/kg  
See description under TESTS

TESTS

PURITY TESTS

Monomer and naphthalenes  
Determine by the following method

**Principle**
When solutions of polymer are injected into a gas chromatograph, the non-volatile polymer may be trapped in a precolumn, allowing the volatile monomers to be chromatographed and identified by their retention times.

**Reagents**
- Carbon Disulfide (CS₂): Chromatography quality grade from Matheson, Coleman and Bell
- Acetone: distilled or reagent grade
- Standards: p-Cresol (pC), hydroxyanisole (IIA), t-butylphenol (tBP) and naphthalene (Naph) standards prepared from samples of ≥ 98% purity. Divinylbenzene/ethylvinyl-benzene (DVB/EVB) standard prepared from Dow or Foster Grant DVB-55, for which % DVB and % EVB have been determined previously.

**Apparatus**
- Hewlett-Packard 5711A Gas Chromatograph with flame ionization detector, equipped with injection port liners with glass inserts containing silanized glass wool plugs (to prevent polymer from reaching column).
- Glass Column, Hewlett-Packard Configuration No. 1B, ID = 2 mm, OD = 6 mm or 1/4", length = 1.83 m; packed with 5% Dexsil 300 for Gas Chromatography (Analabs, Inc.)* on Chromosorb WAW-DMCS-High Performance 100/120 mesh or equivalent
- 10 μl Hamilton syringes, or equivalent
- Varian CDS electronic integrator** or equivalent

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* Available from Analabs, Inc., Subsid. of New England Nuclear Corp. 80 Republic Dr., North Haven CT 06473 (USA).

Monomer and naphtalenes (continued)

**Procedure (continued)**

Set up the gas chromatograph as follows:

- **Oven temperature**: 155° for TBHQ analysis only and 138° for all the other analyses. (Depending on characteristics of the column and/or Gas Chromatograph used, it may be necessary to vary the oven temperatures slightly.)

- **Injection port temperature**: 250°
- **Detector temperature**: 250°
- **N₂ carrier gas flow rate**: 30 ml/min
- **Air flow rate**: 240 ml/min
- **H₂ flow rate**: 30 ml/min
- **Recorder chart speed**: 1.3 cm/min
- **Range x sensitivity**: 10 x 1

Set up the electronic integrator as follows:

**Section 1**

- **ID #**: 0 #
- **S/N**: 2N
- **IPW**: 12S
- **TAN%**: 3.20%
- **AREJ**: 50A
- **STOP**: 8.00M

Set recorder attenuator at 16 for p-C, HA, tBP, and Naph standard chromatograms, and at 4 for TBHQ, and EVB/DVB standards and for all sample analyses.

Accurately prepare a 1 mg/ml solution of TBHQ in acetone and dilute this 1:5 with CS₂ to make a TBHQ dilute standard solution of approximately 0.2 mg/ml concentration.

Accurately prepare a 1 mg/ml solution of the following standards in CS₂: p-cresol, HA, TEP, and naphthalene. Then take 1.00 ml of the p-cresol, HA, and TEP solutions and 0.50 ml of the naphthalene solution and add to 6.50 ml CS₂ to make a "combination standard". Approximate concentrations are 0.1 mg/ml p-cresol, HA, and TBP, and 0.05 mg/ml naphthalene.

To 5.00 ml CS₂ add 5.0 μl of DVB-55. Dilute this 1:20 with CS₂ to make EVB/DVB dilute standard solution.

Into a 5 ml volumetric flask accurately weigh 1 g of sample. Add 3-4 ml of CS₂ and dissolve sample. Then fill to volume with CS₂.

Inject 1.0 μl CS₂ as a solvent blank at 155°. Also inject 1.0 μl of 1:5 acetone:CS₂ as a solvent blank for the TBHQ standard.

Inject 1.0 μl of dilute TBHQ standard three reproducible times (155°, 10 x 1 x 4). The TBHQ peak should elute at approximately 4.0 min.

Make three reproducible 1.0 μl injections of polymer sample solution at same parameters as TBHQ standard.

**Note**: Any accumulation of polymer in the glass insert causes poor resolution, therefore the insert must be changed after every 2-3 injections of polymer. It is important to open the oven and cool the column before replacing the insert (air is destructive to the column at high temperatures). Also, standards and blank should always be run first using a glass...
insert which has had no polymer injected into it. Therefore change insert after analysis for tBHQ.

Make three reproducible 1.0 \( \mu l \) injection of the "combination" standard solution (138°, 10 x 1 x 16). The approximate elution time in min for each component is:

- p-C: 0.8 - 1.0 min
- HA: 1.5 - 1.8 min
- Naph: 1.9 - 2.0 min
- tBP: 2.1 - 2.3 min

Make three reproducible 1.0 \( \mu l \) injections of the dilute EVB/DBV standard solution (138°, 10 x 1 x 4). The approximate elution time in min for each component is:

- diethylbenzene = 0.8 - 1.0 min
- EVB = 1.0 - 1.1 min
- DVB = 1.2 - 1.3 min

Inject 1.0 \( \mu l \) of sample solution three reproducible times (138°, 10 x 1 x 4).

Calculations

Calculate the DBV and EVB standard concentrations as follows:

\[
\text{mg/ml EVB} = 0.05 \times 0.91 \times \% \text{ EVB in DVB-55 used} \\
\text{mg/ml DVB} = 0.05 \times 0.91 \times \% \text{ DVB in DVB-55 used}
\]

Where 0.91 is the density of DVB-55.

Calculate the content of each monomer or impurity as follows:

\[
\text{mg/kg component} = \frac{A_{\text{sample}} \times C_{\text{std}}}{C_{\text{sample}} \times A_{\text{std}}} \times 100 \times 10^4
\]

Where:

- \( A_{\text{sample}} \) = area of sample peak
- \( A_{\text{std}} \) = area of standard peak
- \( C_{\text{std}} \) = standard concentration in mg/ml
- \( C_{\text{sample}} \) = sample concentration in mg/ml

Determine by the following method

Principle

When solutions of polymer are injected into a gas chromatograph, the nonvolatile polymer may be trapped in a precolumn, allowing the volatile dimers and trimers to be chromatographed and their quantity measured. All dimers, and p-Cresol (pC), hydroxyanisole (HA), and t-butylphenol (tBP), DVB/EVB terminated trimers are eluted under the conditions given.

Reagents

- Carbon Disulfide (CX): chromatography reagent grade
- Standard: bi-diterciarybutylphenol (bi-ditBP)
Dimer and trimer (continued)

**Apparatus**
- Hewlett-Packard 5711A Gas Chromatograph with flame ionization detector, equipped with injection port liners with glass inserts containing silanized glass wool plugs (to prevent polymer reaching the column).

- Glass Column, Hewlett-Packard configuration No. 1B, ID = 2 mm, OD = 6 mm or 1/4", length = 1.83 m; column should be silanized before packing (treated with 10% dichlorodimethyl-silane in chloroform, dried, rinsed well with methanol and redried), and packed with 5% Dexsil 300 Gas Chromatography (Analabs, Inc.) on Chromosorb WAW-DMCS-High Performance 100/120 mesh, or equivalent.

- 10 µl Hamilton syringes, or equivalent.

- Varian CDS 111 electronic integrator, or equivalent.

**Procedure**
Set up the Gas chromatograph as follows:

- Oven temperature: 283°
- Injection port temperature: 250°
- Detector temperature: 300°
- N₂ carrier gas flow rate: 30 ml/min
- Air flow rate: 240 ml/min
- H₂ flow rate: 30 ml/min
- Recorder chart speed: 1.3 cm/min
- Range x sensitivity: 10 x 1

**Note:** Depending on characteristics of the column and/or Gas Chromatography used, it may be necessary to vary the oven temperature slightly.

Set up the electronic integrator as follows:

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<thead>
<tr>
<th>Section 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID #</td>
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</tr>
<tr>
<td>IPW</td>
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<tr>
<td>TAN%</td>
</tr>
<tr>
<td>AREJ</td>
</tr>
<tr>
<td>STOP</td>
</tr>
</tbody>
</table>

Set recorder attenuator at 4.

Accurately weigh about 30 mg of bi-ditertiarybutyl phenol into a four dram vial and dissolve in 8 ml CS₂. Add 2 ml of this solution to a 50 ml volumetric flask and dilute to volume with CS₂ to obtain a dilute standard solution.

Into a 5 ml volumetric flask accurately weigh 1 g of sample. Add 3-4 ml of CS₂ and dissolve sample, then dilute to volume with CS₂.

Make a 1.0 µl injection of CS₂ as a solvent blank.

Inject 1.0 µl of dilute standard solution three reproducible times. The standard elutes as three peaks at approximately 2.3, 2.5 and 3.8 min, and these individual peak areas are added together and reported as one number. (Multiple peaks are due to a small amount of oxidized standard present.)
Dimer and trimer
(continued)

Inject 1.0 μl of sample solution three reproducible times. The dimers elute between approximately 0.8 and 3.9 min. The trimers elute from about 4.0 to approximately 10 min.

Note:
Any accumulation of polymer in the glass insert causes poor resolution, therefore this must be changed after each injection of polymer. It is important to open the oven and cool the column before replacing the glass insert (air is destructive to the dexsil column at high temperatures). Also, a reproducible standard should always be run before each polymer injection as a guard against the development of leaks while cooling or reheating the column.

Calculations
Calculate the % dimer and % trimer as follows:

\[
\% \text{ dimer} = \frac{C_{\text{ad}} \times A_{\text{d}}}{A_{\text{ad}} \times C_{\text{sample}}} \times 100
\]

\[
\% \text{ trimer} = \frac{C_{\text{at}} \times A_{\text{t}}}{A_{\text{at}} \times C_{\text{sample}}} \times 100
\]

Where:
- \( C_{\text{ad}} \) = standard concentration in mg/ml
- \( A_{\text{d}} \) = area dimer in the sample
- \( A_{\text{t}} \) = area trimer in the sample
- \( C_{\text{at}} \) = area of bi-di tBP standard peaks
- \( C_{\text{sample}} \) = sample concentration in mg/ml

Molecular weight peak
Determine by the following method:

Principle
Gel permeation chromatography separates polymer molecules on the basis of size. The cross-linked polystyrene gel within the chromatography column contains pores with a range of sizes with the result that large sample molecules are excluded from some (or all) pores of the stationary phase and elute from the column first. Smaller molecules able to enter a greater percentage of pores are retarded and elute later. Ideally, there are no concurrent separation effects from partition or adsorption.

Reagents
- Tetrahydrofuran: technical grade, distilled over calcium hydride to remove water (THF)
- Standards: Narrow molecular weight distribution polystyrenes available from Waters' Associates* and Pressure Chemical. A minimum of five standards should be used to span the range from 300,000 to 1,000 Daltons. The range below 1,000 is calibrated with the polystyrene oligomers at 682, 578, 474, 370, and 266 with toluene at 92 as the final point.

Apparatus
- Columns: Toyo-Soda GMH6 + 10 Å Styragel + Toyo Soda G2000 H4
- Detector: Waters' Associates differential refractometer
- Pump: Milton Roy minipump with 16-160 ml/h flow rate capacity
- Injection valve: Rheodyne #70-10

* Available from Waters' Associates, Inc., 165 Maples Street, Milford MA 01757 (USA).
Molecular weight peak
(continued)

**Procedure**

Standard operating conditions as follows:

- **Flow rate:** 60 ml/h

- **Filtration:** 4 µm followed by 2 µm fritted stainless line filters on suction side of pump. 0.5 µm teflon Millipore sample injection filter

- **Injection loop size:** 50 µl (50 µl to 150 µl is satisfactory). RI attenuation: 4X for usual polymer sample concentration of 15 mg/ml

- **Recorder chart speed:** 0.5 cm/min

All samples dissolved in THF only.

Prepare polystyrene standards at about 2 mg/ml, run from highest to lowest molecular weight (RI setting of 8X), and draw calibration curve from the elution volumes using 4-cycle semilog paper.

Take flow rates during calibration and polymer runs using the following method which gives an average rate during the run. After sample injection, weigh a 50 ml Erlenmeyer with aluminum foil cap. Insert the solvent outflow line into a hole in the foil cap and simultaneously trip a timer. Allow the THF to collect for about 20 min periods during the calibration and about 40 min for a polymer sample (the entire run). Find the weight of THF and determine the flow rate from the equation given in the Calculations.

For routine sample injection, prepare polymer solution at approximately 15 mg/ml in THF. Anoxomer is readily soluble. Set detector attenuation at 4X. Filter through 0.5 µl Millipore into injection loop and inject into column flow. At higher concentrations, raise the attenuation to bring the peaks on scale.

**Calculations**

(a) flow rate,

\[
\text{ml/h} = \frac{(\text{weight of THF collected}) \times 3600}{0.885 \text{ g/ml} \times \text{coll. time, sec}}
\]

(b) elution volume,

\[
\text{ml} = \frac{(\text{ion to point of interest, cm}) \times (\text{ml/h})}{0.5 \text{ cm/min} \times 60 \text{ min/h}}
\]

Determine by the following method:

**Principal**

Hydroquinones and di-substituted hydroxyanisoles are oxidized quantitatively by potassium dichromate in a propionic acid medium. In DVB base polymeric antioxidants containing combinations of these two species they are titrated in two discrete steps, thereby providing a determination of both phenols in one analysis. Lithium chloride is added to the titration solvent in order to increase ionic strength and electrode response. The values obtained, taken in conjunction with proton titration results, allow phenol % iBHQ or HQ and/or phenol % HA to be calculated.

Phenol content as hydroxy-anisole and tert-butyl hydroquinone

134
Phenol content as hydroxy-anisole and tert-butyl hydroquinone
(continued)

Reagents
- **Potassium Dichromate titrant:** Approximately 0.04 M of $K_2Cr_2O_7$ in acetic acid. Water (88:12) is made by dissolving 2.90 g of reagent grade $K_2Cr_2O_7$ in 30 ml of distilled water, and while stirring making the volume to 250 ml with glacial acetic acid. The solution is then deaerated by purging with argon for 15 min. This solution should be made up fresh at least every 6 months.

- **Sample Solvent:** 0.50 M Lithium chloride in reagent grade propionic acid (21.2 g LiCl in 1 liter of propionic acid), deaerated by purging with argon for 15 min.

- **Standard:** Either Quantitative Grade $t$-butylhydroquinone ($t$-BHQ) available from Polysciences Corporation* or 2,5-di-$t$-butylhydroquinone (di-$t$BHQ) which has been recrystallized from glacial acetic acid and then sublimed may be used as standards.

Apparatus
- **Titrator** used is a Metrohm E435 or E576 recording titrator with a 1.0 ml buret assembly. A glass reference electrode (Metrohm EA 107 or EA 109) is used in combination with a platinum indicator electrode (Metrohm EA 201 or EA 202).

Note: The polymeric film which accumulates on the platinum electrode during phenol titrations causes a decrease in sensitivity and very poor titration curves. This film can be electrochemically removed as follows: to the terminals of a 6 volt lantern battery attach about 48 cm insulated wiring. Affix alligator clips to the unattached ends. Clip the negative wire to a 7.6 cm nail and the positive wire to the electrode’s plug. (CAUTION: Do not allow nail and electrode to touch, or the battery will short and become drained.) Immerse both the nail and the platinum tip in the cleaning solution (see below) for approximately one min, then rinse with water and un-clip. This procedure should be followed whenever a poor ("noisy", unstable potential) titration curve is obtained. To prepare cleaning solution, weight 0.135 g ferric chloride and 0.211 g sodium perchlorate, both of reagent grade, and transfer to a 500 ml volumetric flask, adding about 100 ml distilled $H_2O$. Pipet in 4.3 ml concentrated perchloric acid and dilute to volume.

Procedure
- **Standard and Sample Size**
  (a) 10 mg of standard are weighed out and added to the titration vessel.
  (b) When titrating poly DVB/phenol where 30-50% of phenol is hydroxyanisole (HA) and 10-20% of phenol is hydroquinone (HQ) or $t$BHQ, two different sample sizes must be used. To determine hydroquinone approximately 100-120 mg of polymer are weighed accurately and added to the titration vessel. To determine the sum of hydroxyanisole and hydroquinone approximately 30-40 mg are weighed.
  (c) When titrating a poly DVB/phenol where 80-90% of phenol is HQ or $t$BHQ and where no HA is present, 20 mg of polymer are weighed accurately and added to the titration vessel.

---

* Available from Polysciences Corporation, 6366 Gross Point Rd., Niles IL 60648 (USA).
15 ml of 0.50 M LiCl in propionic acid are added to the titration vessel and the sample is dissolved.

The instrument is set up as follows:

<table>
<thead>
<tr>
<th>mV Range</th>
<th>Compensating Potential</th>
<th>Titrating Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000 mV</td>
<td>+ 200 mV</td>
<td>2</td>
</tr>
<tr>
<td>1,000 mV</td>
<td>+ 200 mV</td>
<td>30 min/100% volume</td>
</tr>
</tbody>
</table>

The buret and electrodes are inserted, the titrant is delivered and the titration curve is recorded.

**Note:** When analyzing crude anoxomer reaction mixture (about 20% xylenes) a more accurate weighing is obtained in the following manner: accurately weigh approximately 400 mg of crude anoxomer into a 50 ml volumetric flask and dissolve in LiCl/propionic acid. (Dissolution of sample may be very slow, and is best effected by letting sample sit without stirring for 1-2 h in solvent.) Fill flask to volume, mix well, and titrate a 15 ml aliquot to determine tBHQ. Titrte a 5 ml aliquot plus 10 ml of solvent to determine tBHQ + HA.

**Interpretation**

A. For samples containing both HQ and HA. The 30-40 mg sample undergoes 2 endpoints, the first visible with difficulty. The second endpoint is located at the inflection point and the volume represents the sum of HA + HQ. The 100 mg sample is titrated only through the HQ endpoint. Some uncertainty exists regarding the placement of this endpoint. It should be placed at the intersection point, and proper placement can be insured by comparing each titration to previous titration curves which were properly placed.

B. For samples containing HQ in combination with phenols other than HA. A characteristic change in slope (bump) is seen during the endpoint. This is believed to be the point at which the hydroquinone endpoint is reached and the other phenol begins to titrate; therefore the endpoint is placed in that spot.

C. In this titration the platinum electrode is the indicator electrode and the glass electrode is the reference electrode. However, in order to prevent noise, the glass electrode (which requires good shielding) is plugged into the indicator electrode socket, and the platinum electrode into the reference electrode socket. This causes the highest point of the potential scale to be at the right side of the titration chart (it is normally on the left side).

**Calculations**

1. Standardization: $M = \frac{w}{166.22 \times 3 \times V}$

Where $M = \text{molarity of K}_2\text{Cr}_2\text{O}_7$ solution

$w = \text{weight of Quantitative Analysis Grade tBHQ in mg}$

$V = \text{volume of K}_2\text{Cr}_2\text{O}_7$ solution used, in ml

2. 30-40 mg sample containing HA + HQ:

$m\text{Moles } HQ + HA/g = \frac{V \times M \times 3 \times 1000}{w}$
Phenol content as hydroxyanisole and tert-butyl hydroquinone (continued)

100 mg sample containing HA + HQ: or sample containing HQ and no HA:

\[ \text{mMoles HQ/g} = \frac{V \times M \times 3 \times 1.000}{w} \]

Where \( V \) = volume of \( \text{K}_2\text{Cr}_2\text{O}_7 \) solution used, in ml
\( M \) = molarity of \( \text{K}_2\text{Cr}_2\text{O}_7 \) solution
\( w \) = weight of sample in mg

3. mMoles HA/g = (mMoles HA + HQ/g) - (mMoles HQ/g)

4. \% HQ (or tBHQ) =

\[ \frac{\text{mMole HQ/g}}{\text{mEq H+ /g}} \times (100 + \% \text{BPA in phenol reactants}^*) \]

5. \% HA =

\[ \frac{\text{mMole HA/g}}{\text{mEq H+ /g}} \times (100 + \% \text{BPA in phenol reactants}^*) \]

6. For anoxomer reaction mixture only:

Calculate sample weights \( w \) (for tBHQ) =

\[ \frac{\text{mg sample weighed into flask (~ 400)}}{50 \text{ ml}} \times 15 \text{ ml} \]

Calculate sample weights \( w \) (for HA + tBHQ) =

\[ \frac{\text{mg sample weighed into flask (~ 400)}}{5 \text{ ml}} \times 5 \text{ ml} \]

Calculate HQ and HA content as in 2., 3. and 4.

Calculate \% tBHQ and \% HA as in 4. and 5., using mEq titratable H+/g uncorrected for xylenes.

**Phenol content as p-cresol, tert-butylphenol and bisphenol-A**

Determine by the following method:

**Principle**

Each carbon in a chemical structure exhibits a \(^{13}\)C NMR signal which is characteristic of its chemical bonding. Each structural unit in anoxomer can be

\(^*\) This is an approximation based on the fact that both hydroxyl groups of Bisphenol A are titrated in the proton titration.

\(^{**}\) The Committee requires confirmation that the referred analytical methods actually determine the phenols in the bound state within the polymer.
identified and estimated by the presence of a set of peaks characteristic of that unit. Many peaks, of course, fall at the same very similar mg/kg values to each other and are not resolved. There are, however, 23 principal peaks that are clearly resolved in anoxomer. Each structural unit of interest has at least one peak clearly visible in the spectrum which allows verification of the presence of that unit and estimation of its monomer %.

Reagents: Deuterated chloroform (CDCl₃). NMR grade, e.g. Merck Sharp and Dohme*** 99.8% Atom % Deuterium, or equivalent.

Phenol content as p-cresol, tert-butylphenol and bisphenol-A (continued)

Apparatus
- Stir plate and 6 mm stir bar
- 4 dram (16 ml) glass vial with foil lined cap for each sample
- Varian XL-100 NMR Spectrometer

Procedure

2.0 ml of CDCl₃ is pipetted into a 4 dram glass vial. A 6 mm stir bar is added and the vial is placed on a stir plate.

1.0 g of anoxomer is weighed out on a top loading balance. The 1.0 g of antioxidant is then added to the CDCl₃ in the vial via a powder funnel.

The vial is then capped tightly and allowed to stir until the anoxomer sample is completely dissolved (10-30 min).

The stir bar is then removed and the vial is recapped. The sample is then ready to be sent for the ¹³C NMR spectrum.

An NMR Form which is available from Stanford NMR Lab is then filled out. The relevant conditions are as follows:

¹³C NMR spectrum - same parameter settings as Stanford spectrum No. 14353
0-200 mg/kg
Fully decoupled
Usual temperature (about 35°)

The sample and form are then sent to the Stanford NMR Laboratory or equivalent. The spectrum can be expected in 3-10 days.

Interpretation of the ¹³C NMR Spectrum

The first step in the analysis of the spectrum of anoxomer is the assignment of peaks. This can be done by using a combination of the spectra of model compounds and published substituent effects. Expected shifts for the five different principal phenol units obtained in this way for anoxomer are given in Table I. Expected shifts for DVB and EVB units are given in Table II.

*** Available from Merck Sharp and Dohme, Canada, Ltd., P.O. Box 899, Pointe Claire, Dorval H9Q4P7, Montreal, Quebec (Canada).
Phenol content as p-cresol, tert-butylphenol and bisphenol-A (continued)

As a first step in verification of primary structure the spectrum of the sample compound should be compared to that of a reference material. This is best done by making a transparency of the aromatic and aliphatic regions of the spectrum and overlaying them. There should be a 1:1 correspondence for all peaks both in shift value and in relative size. Note that the absolute mg/kg scale may shift a few mg/kg from spectrum to spectrum. The aromatic region scale should be set by matching the pDVB 1 (128.0 mg/kg) peaks of the two spectra. For the aliphatic region the m-pEVB 8 (15.7 mg/kg) peaks should be matched. Then all other peaks should coincide ± 1 mg/kg. Special attention should be given to the TBHQ t-butyl peak at 29.5 mg/kg since under high temperature conditions of polymerization t-butyl groups may be lost. As a second step phenol % for TBP, p-cresol, BPA and TBHQ may be estimated from sizes of the characteristic peaks. Each of the five phenol units has at least one clearly resolved peak and

Interpretation of the 13C NMR spectrum (cont’d)
hydroxyanisole and t-butylphenol units each have five clearly resolved peaks in the spectrum. This calculation is complicated by the fact that peak size by the fact that peak size is different for differently bonded carbons. Therefore, any carbon used as an internal standard must have chemical bonding similar to the one to be measured. The method of calculation used below assumes that the phenol % HA is known from dichromate titration and proton titration. The various carbons of the HA unit then may be used as internal standards for similarly bonded carbons in other phenols.

Estimation of phenol % TBP, p-Cresol, and BPA

1. Peaks used in the calculation
Equivalent response has been assumed for the following carbons:

\[
\begin{align*}
HA_3 &= TBP_3 \\
HA_2 &= p-C_2 \\
HA_2 &= BPA_5
\end{align*}
\]

These peaks should be identified in the sample spectrum. A baseline should then be constructed and the peak heights (in mm) measured and recorded.

2. Estimation of phenol % from peak heights:

\[
\begin{align*}
\text{Phenol} \% \, TBP &= \frac{\text{Peak height} \, TBP_3}{\text{Peak height} \, HA_3} \times \text{Phenol} \% \, HA \\
\text{Phenol} \% \, p-Cresol &= \frac{\text{Peak height} \, p-C_2}{\text{Peak height} \, HA_2} \times \text{Phenol} \% \, HA \\
\text{Phenol} \% \, BPA &= \frac{\text{Peak height} \, BPA_5}{\text{Peak height} \, HA_2} \times \text{Phenol} \% \, HA
\end{align*}
\]

where Phenol % HA is known.

Table I
Shift values for the various carbons of the five different phenol units in polymer (anoxomer). The spectrum or spectra used to obtain expected values is given for each unit. References are to the Dynapol collection of 13C NMR spectra* and are given in mg/kg downfield from TMS. Peaks marked with an asterisk are clearly resolved, unique to the given structure and are referred to as characteristic peaks for the given unit.

* Available with Dynapol, 1454 Page Mill Road, Palo Alto, C.A. 94304 (USA).
Phenol content as p-cresol, tert-butylphenol and bisphenol-A.

Table I (continued)

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<tbody>
<tr>
<td>HA 1</td>
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</tr>
<tr>
<td>HA 2</td>
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<tr>
<td>HA 3</td>
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<td>HA 4</td>
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<td>TBP 2</td>
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<td>TBP 3</td>
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</table>

* Available with Dynapol, 1454 Page Mill Road, Palo Alto, CA 94304 (USA).
Phenol content as p-cresol, tert-butylphenol, and bisphenol-A (continued)

Table II
Predicted values for the various carbons of the 4 different DVB, EVB units in polymer anoxomer. Predictions are based on the spectra of benzene and cumene. Shifts are downfield from TMS. Peaks marked with an asterisk are clearly resolved, unique to the given structure and are referred to as characteristic peaks for the given unit.

<table>
<thead>
<tr>
<th>Unit</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>pEVB</td>
<td>15.7</td>
<td></td>
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</tbody>
</table>

**Phenol content**

**Principle**
The weakly acidic phenolic proton of the phenolic hydroxyl is titrated under conditions for accentuating titration of weak acids. Under these conditions only one phenolic proton of a hydroquinone is titrated. Therefore this method may be used to obtain the sum of all phenols and hydroquinones in a polymer containing mixture of these.

**Reagents**
- Dimethyl sulfoxide (DMSO): distilled under 1 μ vacuum, discarding first 20% and collecting fraction boiling between 27-30° at 1 μ vacuum (approximately 20% boils above 30° and is discarded).
- Tetrabutylammonium hydroxide: 1.0 M in MeOH (25% solution) from Matheson, Coleman and Bell, or equivalent.
- Standard: primary standard grade benzoic acid.

* Available with Dynapol, 1454 Page Mill Road, Palo Alto, CA 94304 (USA).
Phenol content

total (continued)

**Apparatus**
- E 436 or E 576 Titrator
- EA 147 Combination glass, Ag/AgCl electrode
- 1 ml buret assembly

**Procedure**

Soak EA 147 electrode in 1 M HCl one to two h, before titrating to improve response. Then soak for about five min in 1 M HCl between titrations. Always rinse very well and blot dry before inserting in titration vessel.

Set up instrument as follows:

<table>
<thead>
<tr>
<th>mV range</th>
<th>Compensating potential</th>
<th>Titrating speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 436</td>
<td>1,000 mV</td>
<td>+ 200 mV</td>
</tr>
<tr>
<td></td>
<td>1,000 mV</td>
<td>+ 200 mV</td>
</tr>
<tr>
<td>E 576</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

Add 15 ml DMSO to a titration vessel and titrate DMSO blank. If electrode response is poor, either a longer soaking period in HCl is necessary, or electrode is no longer good.

Weight out approximately 120 mg of polymer or 80 mg of standard to the nearest tenth of a mg and transfer it quantitatively to the titration vessel of the titrator.

Add 15 ml of DMSO dissolve polymer or standard and titrate.

**Note:** When analyzing crude anoxomer reaction mixture (about 20% xylenes) a more accurate weighing is obtained in the following manner: accurately weigh approximately 375 mg of reaction mixture into a 25 ml volumetric flask and dissolve with DMSO. (Dissolution in DMSO may be very slow; it is sometimes helpful to let samples sit in DMSO overnight.) Fill to volume, mix well, and remove a 10 ml aliquot for titration. Place aliquot in titration vessel and add 5 ml of DMSO, mix well, and titrate.

**Interpretation**

Phenolic polymers titrated thus far titrate with a sigmoid endpoint that is shallow but distinct.

Phenolic monomers titrate with sigmoid titration curves typical of inorganic acids.

Thus, the shallowness of the inflection in the titration curve is an indication of degrees of neighbour-neighbour interaction of phenolic groups.

**Calculation**

\[
M = \frac{W_{\text{add}}}{122.12 \times (V_{\text{add}} - V_{\text{blank}})}
\]
Phenol content total (continued)

Where:

- \( M \) = molarity of titrant
- \( W_{sd} \) = weight of standard in mg
- 122.12 = molecular weight of benzoic acid
- \( V_{sd} \) = volume of titrant used by standard in ml
- \( V_{blank} \) = volume of titrant used by DMSO blank in ml at potential of standard endpoint

\[
\text{mEq titratable H}^+/g = \frac{M \times (V_{samp} - V_{blank})}{W} \times 1000
\]

Where:

- \( M \) = molarity of titrant
- \( V_{samp} \) = volume of titrant used by sample in ml
- \( V_{blank} \) = volume of titrant used by DMSO blank in ml at potential of sample endpoint
- \( W \) = weight of sample in mg

For crude anoxomer reaction mixture only:

Calculate weight of sample in mg:

\[
W = \frac{\text{mg weighed into flask (375) \times 10 ml}}{25 \text{ ml}}
\]

Calculate mEq titratable H\(^+\)/g as above. Correct for xylene content of reaction mixture as follows:

\[
\text{mEq titratable H}^+/g \text{ (corrected)} = \frac{\text{mEq titratable H}^+/g}{1 - \% \text{ xylene}} \times \frac{100}{100}
\]

Xylenes

Determine by the following method

Principle

A precolumn traps the non-volatile polymer, allowing the volatile xylenes to pass into the gas chromatography column and be properly identified and quantitated on the resulting chromatogram. Residual (< 25 mg/kg) levels of xylenes may be determined in polymeric antioxidants by this method.

Reagents

- Carbon Disulfide (CS\(_2\)): Chromatography quality grade, (from Matheson, Coleman and Bell, or equivalent)
- Standard: Reagent grade xylenes

Apparatus

- Hewlett-Packard 5711A Gas Chromatograph with flame ionization detector, equipped with injection port liners containing glass inserts with silanized glass-wool plugs (to prevent polymer from reaching column).

- Glass Column, Hewlett-Packard configuration No. 1B, ID = 2 mm, OD = 6 mm or 1/4", length = 1.83 m; packed with 5% Dexsil 300 for gas
Xylenes (continued)

chromatography (Analabs, Inc.) on Chromosorb WAW-DMSC-High Performance
100/120 mesh, or equivalent.

- 10 μl Hamilton syringes, or equivalent
- Varian CDS electronic integrator, or equivalent

Procedure

Set up the gas chromatograph as follows:

Oven temperature: 90°
Injection port temperature: 250°
Detector temperature: 250°
N₂ carrier gas flow rate: 30 ml/min
Air flow rate: 240 ml/min
H₂ flow rate: 30 ml/min
Recorder chart speed: 6 mm/min
Range x sensitivity: 10 x 1

Set up the electronic integrator as follows:

<table>
<thead>
<tr>
<th>Section</th>
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</thead>
<tbody>
<tr>
<td>ID #</td>
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<tr>
<td>S/N</td>
<td>2N</td>
</tr>
<tr>
<td>IPW</td>
<td>4S</td>
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<tr>
<td>TAN%</td>
<td>3.20%</td>
</tr>
<tr>
<td>AREJ</td>
<td>50A</td>
</tr>
<tr>
<td>STOP</td>
<td>6.00M</td>
</tr>
</tbody>
</table>

Set recorder attenuator at 4.

To 10 ml CS₂ add 2.0 μl reagent grade xylenes then dilute this solution 1:20 with
CS₂ to make the xylenes dilute standard solution.

Into a 5 ml volumetric flask, accurately weigh approximately 1 g of antioxidant
sample, add 3-4 ml CS₂ and dissolve, then fill to volume.

Inject 1.0 μl CS₂ as a solvent blank.

Make three reproducible 1.0 μl injections of the xylenes dilute standard solution.

Make three reproducible 1.0 μl injections of the polymer sample solution.

Note: Any accumulation of polymer in the glass insert causes poor resolution,
therefore this must be changed after every 2-3 injections of polymer. It is
important to open the oven and cool the column before replacing the insert (air
is destructive to the column at high temperature). Also, standards and blank
should always be run first using a glass insert which has had no polymer injected
into it.

The approximate elution time for the two xylenes peaks is between 1.0 and 1.6
min. Residual monomers elute from about 2.0 to 7.0 min; time must be allowed
for these to elute completely between each polymer injection.
Xylenes (continued)

Calculations

Calculate the % xylenes as follows:

\[
\% \text{ xylenes} = \frac{C_{\text{std}} \times A_{\text{sample}}}{C_{\text{sample}} \times A_{\text{std}}} \times 100
\]

Where:
- \( C_{\text{std}} \) = standard concentration in mg/ml
- \( A_{\text{sample}} \) = area of two xylenes peaks in sample
- \( C_{\text{sample}} \) = sample concentration in mg/ml
- \( A_{\text{std}} \) = area of two xylenes peaks in standard

METHOD OF ASSAY

Determine by the following method:

Principle

The characteristic maximum wavelength of UV absorption (\( \lambda_{\max} \)) and absorbance at this wavelength are observed to provide information regarding the uniformity of structure among antioxidant lots and weight % antioxidant in a given sample.

Reagents

- Dioxane, reagent grade
- Standard anoxomer of 99+% purity (cut 5 or 7), or other anoxomer sample for which purity has been rigorously determined using the 99+% purity sample cut 5 or 7.

Apparatus

- Cary 118 UV-Visible absorption Spectrophotometer, or equivalent
- A matched pair of Quartz cells, path length = 1 cm

Procedure

Weight accurately 10-11 mg of antioxidant standard and each sample in triplicate into four dram vials. Carefully, using a volumetric pipet, transfer 10 ml of dioxane to each of the vials. Tightly close the vials with foil lined caps and shake, or use a Vortex mixer, to dissolve the antioxidant. These are the stock solutions.

Carefully pipet 2.00 ml of each stock solution into an 8 dram vial with foil lined cap and add with pipet exactly 18.00 ml of Dioxane. These are the solutions used in the analysis.

Set up Cary 118 Spectrophotometer as follows:

- UV lamp on (30 min warm-up time)
- Select switch to UV
- Baseline Adjust switch to UV
- Auto Gain Mode
- 2.0 Absorbance Range
- Wavelength speed: 1.0 nm/sec
- Chart Speed: 50 nm/2.5 cm
- Slit: 0.1 mm

With dioxane in both sample and reference cells, scan from 350 nm to 230 nm. This is the baseline.

Empty sample cell, rinse with sample solution; then fill with sample solution and scan from 350 nm to 250 nm.
METHOD OF ASSAY (continued)

Repeat steps 1 and 4 with each sample and Standard analoxomer solution.

For each sample and for the standard note λ_{max} (about 287 nm) and note absorbance (A) at λ_{max} and 264 nm.

**Calculations**

Calculate the absorptivity of standard and samples from Beer's Law:

When cell path length is 1 cm,

\[ a_{\lambda_{max}} = \frac{A_{\lambda_{max}}}{C} \]

Where:

- \( a_{\lambda_{max}} \) = absorptivity at \( \lambda_{max} \)
- \( A_{\lambda_{max}} \) = absorbance at \( \lambda_{max} \)
- \( C \) = concentration in mg/ml

Calculate the wt % antioxidant in each sample as follows:

\[ \text{wt % antioxidant in samp.} = \frac{a_{\text{mg}}}{a_{\text{std}}} \times \text{wt % purity of standard} \]

Where:

- \( a_{\text{mg}} \) = average absorptivity of standard solutions
- \( a_{\text{std}} \) = average absorptivity of standard solutions

**Note:** Partial oxidation of hydroquinone in polymeric antioxidants leads to a new \( \lambda_{max} \) at 264 nm. Therefore, even in completely unoxidized antioxidants the absorbance at 264 nm is noted, and a second absorptivity constant calculated as follows:

\[ a_{264} = \frac{A_{264}}{C} \]

Where:

- \( a_{264} \) = absorptivity at 264 nm
- \( A_{264} \) = absorbance at 264 nm
- \( C \) = concentration in mg/ml

In addition, calculate the ratio of \( a_{\lambda_{max}} \) to \( a_{264} \) for samples and standard as follows:

\[ \text{Absorptivity ratio} = \frac{a_{\lambda_{max}}}{a_{264}} \]
APPENDIX

* Infrared spectrum: Anisomorger

Condition: At a concentration of 150 mg/ml in carbon disulfide, BaF₂ cell, pathlength = 0.059 mm.

SYNONYMS
CI Food Orange 6;
INS No. 160e, EEC No. E160e

DEFINITION
These specifications apply to predominantly all trans (Z) isomer of \( \beta \)-apo-8'-carotenal together with minor amounts of other carotenoids. Diluted and stabilized forms are prepared from \( \beta \)-apo-8'-carotenal meeting these specifications and include solutions or suspensions of \( \beta \)-apo-8'-carotenal in edible fats or oils, emulsions and water dispersible powders. These preparations may have different cis/trans isomer ratios.**

Class
Carotenoid

Code numbers
CI (1975) No. 40820
CAS No 1107-26-2

Chemical names
\( \beta \)-Apo-8'-carotenal, 8'-apo-\( \beta \)-carotene-al

Chemical formula
\( \text{C}_{40}\text{H}_{56}\text{O} \)

Structural formula

![Structural formula]

Molecular weight
416.65

Assay
Not less than 96% of total colouring matters

DESCRIPTION
Deep violet crystals with metallic lustre or crystalline powder.

\( \beta \)-Apo-8'-carotenal is sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

FUNCTIONAL USE
Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Insoluble in water. Slightly soluble in ethanol.
Sparingly soluble in vegetable oils. Soluble in chloroform.

---

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** The analytical methods described for the parent colour are not necessarily suitable for the assay of or determination of impurities in the stabilized forms. (Appropriate methods should be available from the manufacturer).

IDENTIFICATION TESTS (continued)

* B. Spectrophotometry
Determine the absorbance of the sample solution (see Method of Assay) at 461 nm and 488 nm. The ratio $A_{488}/A_{461}$ is between 0.80 and 0.84.

C. Positive test for carotenoid
The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 1 N sulfuric acid.

E. Carr-Price reaction
A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price reagent TS.

PURITY TESTS

** Sulfated ash
Not more than 0.1%
Proceed as directed under the test for Ash (Sulfated ash, Method I) using 2 g of sample.

*** Arsenic
Not more than 3 mg/kg

*** Lead
Not more than 10 mg/kg

** Heavy metals
Not more than 40 mg/kg
Test 1 g of the sample as directed in the Limit Tests (method II).

Subsidiary colouring matters
Carotenoids other than β-apo-8′-carotenal:
Not more than 3% of total colouring matters.
See description under TESTS

TESTS

PURITY TESTS

* Subsidiary colouring matters
Carotenoids other than β-apo-carotenal:
Dissolve about 80 mg of sample in 100 ml chloroform. Apply 400 μl of this solution as a streak 2 cm from the bottom of a TLC-plate (Silicagel 0.25 mm).

Pretreat the thin-layer plate by soaking in a tank with 3% KOH in methanol so that it is completely wetted. Then dry the plate for 5 min in the air and activate for 1 h at 110°C in an oven. Let cool over CaCl₂ and keep in a desiccator over CaCl₂.

---


Immediately after applying the carotenoid solution to the plate, develop the chromatogram with n-hexane/chloroform/ethylacetate (70+20+10) in a saturated chamber suitably protected from light, until the solvent front has moved 10 cm above the initial streak. Remove the plate, allow the main part of the solvent to evaporate at room temperature and mark the principal band as well as the bands corresponding to other carotenoids. Remove the silicagel adsorbent that contains the principal band, transfer it to a glass-stoppered 100 ml centrifuge tube and add 40.0 ml chloroform (solution 1).

Separately remove the silica gel of the combined bands corresponding to the other carotenoids, transfer it to a glass-stoppered, 50 ml centrifuge tube and add 20.0 ml chloroform (solution 2).

Shake the centrifuge tubes by mechanical means for 10 min and centrifuge for 5 min. Dilute 10.0 ml of Solution 1 to 50.0 ml with chloroform (solution 3).

Determine, with a suitable spectrophotometer, the absorbances of Solutions 2 and 3 in 1-cm cells at the wavelength maximum in chloroform at about 474 nm, using chloroform as a blank.

Calculation

Carotenoids other than $\beta$-apo-8'-carotenal (%) =

$$\frac{A_2 \times 10}{A_3}$$

Where

$A_2$ = absorbance of Solution 2

$A_3$ = absorbance of Solution 3

METHOD OF ASSAY

Weigh accurately about 80 mg of the sample and proceed as directed in the Spectrophotometric method in the General Methods for Food Colours*.

absorptivity (a) = 2640

Approximate wavelength of maximum absorption = 461 nm.

**β-APO-8'-CAROTENOIC ACID ETHYL ESTER**

**SYNONYMS**
CI Food Orange 7;
JNS No. 160f, EEC No. E160f

**DEFINITION**
These specifications apply to predominantly all trans (Z) isomer of β-apo-8'-carotenoic acid ethyl ester together with minor amounts of other carotenoids. Diluted and stabilized forms are prepared from β-apo-8'-carotenoic acid ethyl ester meeting these specifications and include solutions or suspensions of β-apo-8'-carotenoic acid ethyl ester in edible fats or oils, emulsions and water dispersible powders. These preparations may have different cis/trans isomer ratios.**

Class
Carotenoid

Code numbers
CI (1975) No. 40825
CAS No 1109-11-1

Chemical names
β-Apo-8'-carotenoic acid ethyl ester,
ethyl 8'-apo-β-caroten-8'-oate.

Chemical formula
C₃₀H₄₈O₂

Structural formula

![Structural formula image]

Molecular weight
460.70

Assay
Not less than 96% of total colouring matters

**DESCRIPTION**
Red to violet-red crystals or crystalline powder.

β-Apo-8'-carotenoic acid ethyl ester is sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

**FUNCTIONAL USE**
Food colour

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

*** A. Solubility
Insoluble in water. Very slightly soluble in ethanol.
Slightly soluble in vegetable oils. Soluble in chloroform.

---

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** The analytical methods described for the parent colour are not necessarily suitable for the assay of or determination of impurities in the stabilized forms. Appropriate methods should be available from the manufacturer).

IDENTIFICATION TESTS (continued)

* B. Spectrophotometry
Determine the absorbance of the sample solution (See Method of Assay) at 449 nm and 475 nm. The ratio $A_{449}/A_{475}$ is between 0.82 and 0.86.

C. Positive test for carotenoid
The colour of a solution of the sample in acetone disappears after successive additions of a 5% solution of sodium nitrite and 1 N sulfuric acid.

E. Carr-Price reaction
A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price reagent TS.

PURITY TESTS

** Sulfated ash
Not more than 0.1%
Proceed as directed under the test for Ash (Sulfated ash, Method I) using 2 g of sample.

*** Arsenic:
Not more than 3 mg/kg

*** Lead
Not more than 10 mg/kg

** Heavy metals
Not more than 40 mg/kg
Test 1 g of the sample as directed in the Limit Tests (method II).

Subsidiary colouring matters
Carotenoids other than $\beta$-apo-8'-carotenoic acid ethyl ester:
Not more than 3% of total colouring matters.
Proceed as directed in the specifications for $\beta$-Apo-8'-carotenol except that the absorbance is determined at the wavelength maximum in chloroform at about 455 nm.

METHOD OF ASSAY
Weigh accurately about 80 mg of the sample and proceed as directed in the Spectrophotometric method in the General Methods for Food Colours*.

absorptivity (a) = 2550

Approximate wavelength of maximum absorption = 449 nm.

---


ASCORBIC ACID*

SYNONYMS

Vitamin C;
INS No. 300, EEC No. E300

DEFINITION

Chemical names
L-Ascorbic acid, ascorbic acid, 2,3-dihydro-L-threo-hexono-1,4-lactone,
3-keto-L-gulofuranolactone

C.A.S. number
50-81-7

Chemical formula
C₆H₈O₆

Structural formula

```
\[ \text{CH}_2\text{OH} \]
\[ \text{H} \]
\[ \text{C} \]
\[ \text{\text{-O-}} \]
\[ \text{H} \]
\[ \text{\text{-O-}} \]
\[ \text{OH} \]
\[ \text{OH} \]
```

Molecular weight
176.13

Assay
Ascorbic acid, after drying in a vacuum desiccator over sulfuric acid for 24 h, contains not less than 99% of C₆H₈O₆

DESCRIPTION
Ascorbic acid is a white to slightly yellow, odourless crystalline solid. Its melting point** is about 190° with decomposition

FUNCTIONAL USE
Antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water; sparingly soluble in ethanol; insoluble in ether

B. Reducing activity
A solution in water immediately reduces potassium permanganate TS without heating, producing a brown precipitate

C. Reducing activity
A solution of the sample in ethanol will decolourize a solution of 2,6-dichlorophenol-indophenol TS

D. Colour reaction
Passes test
See description under TESTS

---

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Specific rotation  
The specific rotation at 25° of a 10% (w/v) solution of the sample should be +20.5° to +21.5°

* Loss on drying  
Not more than 0.4% after drying over sulfuric acid in a vacuum desiccator for 24 h

* pH  
2.4 - 2.8 (1 in 50 soln)

* Sulfated ash  
Not more than 0.1%

* Arsenic  
Not more than 3 mg/kg (Method II)

* Heavy metals  
Not more than 10 mg/kg  
Test 2 g of the sample as directed in Method I under the Limit Test for Heavy Metals

TESTS

IDENTIFICATION TESTS

D. Colour reaction  
To 2 ml of a 2.0% solution in water add 2 ml of water, 0.1 g of sodium hydrogen carbonate and about 0.02 g of ferrous sulfate. Shake and allow to stand. A deep violet colour is produced which disappears on addition of 5 ml of dilute sulfate acid TS.

METHOD OF ASSAY

Titrimetric method  
Dissolve about 0.400 g of the sample, previously dried in a vacuum desiccator over sulfuric acid for 24 hours, in a mixture of 100 ml of carbon dioxide-free water and 25 ml of dilute sulfuric acid TS. Titrate the solution at once with 0.1 N iodine, adding a few drops of starch TS as indicator as the end point is approached.

Each ml of 0.1 N iodine is equivalent to 0.008806 g of CH₃COOH.

ASCORBYL PALMITATE*

SYNONYMS
Vitamin C palmitate;
INS No. 304, EEC No. E304

DEFINITION
Chemical names
Ascorbyl palmitate; L-ascorbyl palmitate; 2,3-didehydro-L-threo-hexono-1,4-lactone-6-palmitate; 6-palmitoyl-3-keto-L-gulofuranolactone

Chemical formula
C_{25}H_{34}O_7

Structural formula

Molecular weight
414.55

Assay
Ascorbyl palmitate contains not less than 95% of C_{25}H_{34}O_7, calculated on the dried basis

DESCRIPTION
Ascorbyl palmitate is a white or yellowish-white solid, with a citrus-like odour

FUNCTIONAL USES
Antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Very slightly soluble in water; freely soluble in ethanol

** B. Melting range
107° - 117°

C. Reducing activity
A solution of the sample in ethanol will decolourize a solution of 2,6-dichlorophenol-indophenol TS

PURITY TESTS

** Specific rotation
The specific rotation at 25° of a 10% (w/v) solution of the sample in methanol should be +21° to +24°

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (continued)

* Loss on drying
  Not more than 2% after drying in a vacuum over at 56° to 60° for 1 h

* Sulfated ash
  Not more than 0.1%

* Arsenic
  Not more than 3 mg/kg (Method II)

* Heavy metals
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in Method I under the Limit Test for Heavy Metals

METHOD OF ASSAY

Titrimetric method

Add 0.800 g of the sample to a mixture of 50 ml of carbon dioxide-free water, 50 ml of chloroform and 25 ml of dilute sulfuric acid TS. Titrate the mixture at once with 0.1 N iodine making sure that the mixture is well shaken. Add a few drops of starch TS as indicator as the end point is approached.

Each ml 0.1 N iodine is equivalent to 20.73 mg of C_{22}H_{31}O_{7}.

**ASCORBYL STEARATE**

**SYNONYMS**  
Vitamin C stearate;  
INS No. 305

**DEFINITION**

**Chemical names**  
Ascorbyl stearate, L-ascorbyl stearate, 2,3-didehydro-L-threo-hexono-1,4-lactone-6-stearate; 6-stearoyl-3-keto-L-gulofuranolactone

**C.A.S. number**  
25395-66-8

**Chemical formula**  
$	ext{C}_{42}	ext{H}_{50}	ext{O}_{7}$

**Structural formula**

![Structural formula](image)

**Molecular weight**  
442.6

**Assay**  
Ascorbyl stearate contains not less than 95% of $	ext{C}_{42}	ext{H}_{50}	ext{O}_{7}$

**DESCRIPTION**  
Ascorbyl Stearate is a white or yellowish white solid with a citrus-like odour

**FUNCTIONAL USES**  
Antioxidant

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**  
Insoluble in water; soluble in ethanol

**B. Melting point**  
About 116°

**C. Reducing activity**  
A solution of the sample in ethanol will decolourize a solution of 2,6-dichlorophenol-indophenol TS

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* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS

* Loss on drying  
Not more than 2% after drying in a vacuum oven at 56 to 60° for 1 h

* Sulfated ash  
Not more than 0.1%

* Arsenic  
Not more than 3 mg/kg (Method II)

* Heavy metals  
Not more than 10 mg/kg  
Test 2 g of the sample as directed in Method I under the Limit Test for Heavy Metals

METHOD OF ASSAY

Titrimetric method

Add 0.800 g of the sample to a mixture of 50 ml of carbon dioxide-free water, 50 ml of chloroform and 25 ml of dilute sulfuric acid TS. Titrate the mixture at once with 0.1 N iodine, making sure that the mixture is well shaken. Add a few drops of starch TS as indicator as the end point is approached.

Each ml of 0.1 N iodine is equivalent to 22.13 mg of ascorbyl stearate.

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ASPARTAME*

SYNONYMS
Aspartyl phenylalanine methyl ester, APM
INS No. 951

DEFINITION
Chemical names 3-Amino-N-(α-carbomethoxy-phenethyl)-succinamic acid, N-L-α-aspartyl-L-phenylalanine-1-methyl ester
C.A.S. number 22389-47-0
Chemical formula C_{10}H_{14}N_{2}O_{3}
Structural formula

\[
\begin{align*}
\text{HOOCCH}_2\text{C} & \quad \text{CONH} \quad \text{C} \quad \text{COOCH}_3 \\
\text{NH}_2 & \quad \text{H}
\end{align*}
\]
Molecular weight 294.31
Assay Content not less than 98% and not more than 102% of C_{10}H_{14}N_{2}O_{3} on the dried basis

DESCRIPTION
White, odourless, crystalline powder, having a strong sweet taste

FUNCTIONAL USE
Sweetening agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Slightly soluble in water and in ethanol

B. Positive test for amino group
Passes test
See description under TESTS

C. Positive test for ester group
Passes test
See description under TESTS

PURITY TESTS

** Loss on drying
Not more than 4.5% (105°, 4 h)

** pH
4.5 - 6.0 (1 in 125 soln)

* These specifications were prepared at the 25th session of JECFA (1981) and published in FNP 19 (1981).


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CXAS/1983

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PURITY TESTS (continued)

* Specific rotation

\[ \alpha^D_0 = +14.5^\circ \text{ to } +16.5^\circ \text{ calculated on the dried basis.} \]
Determine in a 4 in 100/15 N formic acid soln within 30 min after preparation of the sample solution.

* Transmittance

Passes test  
See description under TESTS

* Sulfated ash

Not more than 0.2%  
Proceed as directed under the test for Ash (Sulfated ash, Method I) using 1 g of the sample.

* Arsenic

Not more than 3 mg/kg (Method II)

* Heavy metals

Not more than 10 mg/kg  
Test 2 g of the sample as directed in the Limit Test for Heavy metals (Method II).

5-Benzyl-3,6-dioxo-
2-piperazineacetic acid

Not more than 1.5%  
See description under TESTS

Other optical isomers

Passes test  
See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Positive test for amino group

Dissolve 2 g of ninhydrin in 75 ml of dimethylsulfoxide, add 62 mg of hydrindantin, dilute to 100 ml with 4 M lithium acetate buffer solution (pH 9), and filter. Transfer about 10 mg of the sample to a test tube, add 2 ml of the reagent solution, and heat. A dark purple colour is formed.

C. Positive test for ester group

Dissolve about 20 mg in 1 ml of methanol, add 0.5 ml of methanol saturated with hydroxylamine hydrochloride, mix, and then add 0.3 ml of 5 N potassium hydroxide in methanol. Heat the mixture to boiling, then cool, adjust the pH to between 1 and 1.5 with hydrochloric acid TS, and add 0.1 ml of ferric chloride TS. A burgundy colour is produced.

PURITY TESTS

Transmittance

The transmittance of a 1 in 100, 2 N hydrochloric acid solution, determined in a 1-cm cell at 430 nm with a suitable spectrophotometer, using 2 N hydrochloric acid as a reference, is not less than 0.95, equivalent to an absorbance of not more than approximately 0.022.

**5-Benzyl-3,6-dioxo-2-piperazineacetic acid**

**Apparatus**

Use a suitable gas chromatograph equipped with a hydrogen flame ionization detector and designed for handling glass columns with on-column injection (Micro-Tex 220 or equivalent), containing a 1.83-meter (6 feet) x 4-mm (i.d.) glass column packed with 3% OV-1 on 80/100-mesh Supelcoport (Supelco, Inc. or equivalent). Condition the column overnight at 250°C before readjustment and equilibration to the operating conditions. To preclude build-up of silicon oxide, clean the detector with acetone frequently.

**Operating conditions**

The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: Column temperature, 200°C; Inlet temperature, 200°C; Detector temperature, 275°C; Carrier gas, helium, flowing at a rate of 75 ml per min; Hydrogen and air flow to burner, optimized to give maximum sensitivity; Recorder, 1 mV full scale.

**Sililation reagent**

Just before use, dilute 3 parts, by volume, of N,O-bis-(trimethylsilyl) acetamide with 2 parts of dimethylformamide.

**Standard preparation**

Transfer about 25 mg of 5-Benzyl-3, 6-dioxo-2-piperazineacetic acid Reference Standard*, accurately weighed, into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Pipet 10 ml of this solution into a second 100-ml volumetric flask, dilute to volume with methanol, and mix. Pipet 3 ml of the second solution into a 2-dram vial, with Teflon-lined cap, and evaporate to dryness on a steam bath. Add 1 ml of the Sililation reagent to the residue, cap the vial tightly, shake and heat in an oven at 80°C for 30 min. Remove the vial from the oven, shake for 15 sec, and cool to room temperature.

**Sample preparation**

Transfer about 10 mg of the aspartame sample, accurately weighed, into a 2-dram vial, with Teflon-lined cap, add 1 ml of the Sililation reagent, cap tightly, shake, and heat in an oven at 80°C for 30 min. Remove the vial from the oven, shake for 15 sec, and cool to room temperature.

**Procedure**

Inject a 3-μl portion of the Standard preparation into the gas chromatograph, obtain the chromatogram, measure the height of the peak produced by the 5-benzyl-3,6-dioxo-2-piperazineacetic acid, and record it as P. Under the stated conditions, the elution time is about 7-9 min. Similarly, inject a 3-μl portion of the Sample preparation, obtain the chromatogram, measure the height of the peak produced by any 5-benzyl-3,6-dioxo-2-piperazineacetic acid contained in the sample, and record it as p.

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* Available from Food Chemicals Codex, NAS/NRC, 2101 Constitution Avenue, N.W., Washington, D.C. 20418, USA.
5-Benzyl-3,6-dioxo-2-piperazineacetic acid (continued)

Calculation

Calculate the percent of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the sample by the formula:

\[
\frac{3 \times W \times p}{500 \times w \times P} \times 100
\]

where \( W \) = the exact weight in mg of the reference standard taken

\( w \) = the exact weight in mg of the aspartame taken

Other optical isomers

Apparatus

Use a suitable amino acid analyzer (such as Hitachi KLA-5, or equivalent) which is equipped with a 550-mm x 9-mm (i.d.) column packed with approximately 50 g of strong cation exchange resin (Hitachi Custom Ion-Exchange Resin No. 2613, or equivalent), 29-m x 0.5 mm (i.d.) reaction coil, a ninhydrin supply, and a photometer with an interference filter for 570 nm and selenium photocell detector (see Figure).

Figure: Flow Diagram of Liquid Chromatograph
Other optical isomers
(continued)

Operating conditions
The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

- Column temperature: 55°
- Reaction coil temperature: 100°
- Eluant: pH 5.28 citrate buffer solution
- Pressure of eluant: 8-10 kg/cm²
- Flow rate of eluant: 60 ml/h
- Pressure of ninhydrin solution: 2-5 kg/cm²
- Flow rate of ninhydrin solution: 30 ml/h
- Photometric detector: measuring wavelength: 570 nm
- Recorder full scale: absorbance: 0-0.1

Reagents and solutions
- pH 5.28 citrate buffer solution: Dissolve 34.3 g of sodium citrate \((C_6H_5O_7 \cdot 2H_2O)\) in about 400 ml of water, add 7.5 ml of hydrochloric acid TS (35%) and 5 ml of benzyl alcohol, and add sufficient water to make 1,000 ml.
- pH 2.2 citrate buffer solution: Dissolve 1.4 g of sodium citrate \((C_6H_5O_7 \cdot 2H_2O)\), 13.0 g of citric acid \((C_6H_8O_7H_2O)\) and 10.9 g of sodium chloride in about 400 ml of water and add sufficient water to make 1,000 ml.
- Ninhydrin solution: Pour 140 ml of water into a 500-ml beaker, add 82.0 g of sodium acetate \((C_2H_3NaO_2)\), stir to dissolve completely, add 25 ml of glacial acetic acid TS and dilute to 250 ml with water. Adjust the pH of the solution to 5.51 ± 0.03 with glacial acetic acid TS or sodium acetate TS (Acetate Buffer Solution). Pour 750 ml of methylcellosolve into a 1,000-ml brown glass bottle and add 250 ml of Acetate Buffer Solution. Supply nitrogen gas through the solution, while mixing, dissolving 20 g of ninhydrin and then 0.38 g of stannous chloride \((SnCl_2 \cdot 2H_2O)\) in the solution. Allow the solution to stand for at least 24 h before use.

Preparation
- Standard Preparation: Transfer 2.50 mg of L-α-aspartyl-D-phenylalanine methyl ester Reference Standard* into a 100-ml volumetric flask, dissolve and dilute to volume with water (Solution A). Transfer 250 mg of L-α-aspartyl-L-phenylalanine methyl ester Reference Standard into another 100-ml volumetric flask, dissolve in pH 2.2 citrate buffer solution, add 10.0 ml of Solution A and dilute to volume with pH 2.2 citrate buffer solution. Store this preparation below 5°.

* Available from Ajinomoto Co. Inc., 1-5-8 Kyobashi, Chuo-ku, Tokyo 104, Japan.
Sample Preparation: Transfer 250 mg of the sample into a 100-ml volumetric flask, dissolve and dilute to volume with pH 2.2 citrate buffer solution.

**METHOD OF ASSAY**

Weigh accurately about 150 mg of the sample, previously dried at 105° for 4 h, dissolve in 35 ml of dimethylformamide, add 5 drops of thymol blue TS, and titrate with a microburette to a dark blue end-point with 0.1 N lithium methoxide. Perform a blank determination and make any necessary correction. Each ml of 0.1 N lithium methoxide is equivalent to 29.43 mg of C₆H₁₃N₂O₂.

**CAUTION:** Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminium foil while dissolving the sample and during the titration.
AVIAN PEPsin

SOURCES
Commercial preparations of Avian Pepsin contain proteolytic enzymes obtained from the forestomach (proventriculum) of chicken or turkey.

ACTIVE PRINCIPLE
Pepsin (aspartic proteinase)

SYSTEMATIC NAME AND NUMBER
None - EC 3.4.23.1

REACTION CATALYZED
The enzyme preparations hydrolyzes polypeptides yielding peptides of lower molecular weight. It clots milk.

ASSAY
Not less than 85% and not more than 115% of the declared activity

DESCRIPTION
The enzyme preparations occur as clear amber solution, tan suspension, or light tan powder.

FUNCTIONAL USE
Clotting of milk in cheese making

CHARACTERISTICS

IDENTIFICATION TESTS
Pepsin activity
The sample shows milk clotting activity
See description in the Method of Assay

PURITY TESTS

** Arsenic
Not more than 3 mg/kg

** Lead
Not more than 10 mg/kg
A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Lead, using 10 µg of lead ion in the control.

** Heavy metals
Not more than 50 mg/kg
Test 0.5 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 25 µg of lead ion in the control (Solution A).

* These specifications were prepared at the 20th session of JECFA (1976) and published in FNS 1B (1977).

PURITY TESTS (continued)

**Microbiological criteria**

* Aflatoxin

Not more than 5 µg/kg
Determine as directed in J.A.O.A.C. 49, 544 (1966); Pons et al., Determination of Aflatoxins in Agricultural Products: Use of Aqueous Acetone for Extraction.

* Salmonella spp.

Negative by the following procedure.

* Pseudomonas aeruginosa

Negative

* Coliforms

Not more than 30 per g.

* Antibiotic activity

Negative when examined by suitable methods.

**METHOD OF ASSAY**

**Milk Clotting Activity**

**Principle**
Reconstituted milk is coagulated with an avian pepsin solution. The time required to form visible clot is compared with that obtained with a reference standard.

**Definition of activity**
The time of clotting T is related to the concentration C of active enzyme by the relation $T = t_0 + kC$ where k is a proportionality factor which is constant for the same batch of milk, under constant conditions of pH, temperature and calcium ion concentration.

**Procedure**
To prepare the substrate solution, disperse 12 g of low-heat non-fat dry milk powder in 94 ml of 0.01 M calcium chloride solution contained in a 100-ml short-neck volumetric flask, stir magnetically 20-30 min then make up to mark with 0.01 M calcium chloride solution. Distribute 10-ml portions in separate test tubes and keep them at 30° ± 0.2° for at least 10 min, but not more than 1 h. To prepare the enzyme dilutions, introduce into 50-ml or 100-ml volumetric flasks 2 ml and 4 ml respectively of 1.25 M sodium acetate buffer pH 5.7, add distilled water to about two-thirds of the volume of the flask and pipet accurately measured aliquots of the enzyme sample (dissolve, if powdered), and the reference enzyme, dilute to volume. To obtain the desired dilutions (about

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METHOD OF ASSAY

Procedure (continued)
1:5,000 strength, arrived at by diluting original sample solution between 25 and 200 fold, distribute 1-ml aliquots of each dilute enzyme solution in test tubes at (18x250 mm) and incubate at 30° ± 0.2°.

To start the reaction pour rapidly the substrate solution contained in one test tube into that containing the dilute enzyme and start concomitantly a stop watch and mix the test solution twice by rapid inversion of the test tube and keep in a slanted position in the water bath kept at 30° ± 0.2°. Rotate the test tube slowly by hand until the first clots are observed whereupon the watch is stopped and the time is recorded to the nearest 0.1 sec.

Calculation
Plot the clotting times obtained with enzyme dilutions being assayed and that with the reference standard against the dilution factors employed. Straight lines are obtained for clotting times between 50 and 300 sec. The ratio of the slopes of the lines are inversely proportional to the ratio between the concentrations of enzyme.
AZODICARBONAMIDE*

SYNONYMS
Azobisformamide; INS No. 927a, EEC No. 927

DEFINITION

- **Chemical names**: Azodicarbonamide, azodicarboxylic acid diamide
- **C.A.S. number**: 123-77-3
- **Chemical formula**: \( \text{C}_2\text{H}_4\text{N}_4\text{O}_2 \)
- **Structural formula**: \( \begin{array}{c}
\text{H}_2\text{N} \hline
\text{C} \hline
\text{N} = \text{N} \hline
\text{C} \hline
\text{NH}_2
\end{array} \)
- **Molecular weight**: 116.08
- **Assay**: Azodicarbonamide, after drying in a vacuum oven at 50° for 2 h, contains not less than 98.6% of \( \text{C}_2\text{H}_4\text{N}_4\text{O}_2 \), not less than 47.2% and not more than 48.7% of N

DESCRIPTION
Yellow to orange-red, odourless, crystalline powder

FUNCTIONAL USE
Maturing agent for flour

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Practically insoluble in both water and ethanol. Slightly soluble in dimethyl sulfoxide.

** B. Melting range
MELTS above 180° with decomposition

C. Positive test for oxidation
Liberates iodine from potassium iodide TS solution in the presence of 10% sulfuric acid

** D. Positive test for carbon dioxide
Heat about 10 mg of the sample in a crucible. A drop of barium hydroxide solution held above the sample by means of a glass rod becomes turbid

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* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS

* Loss on drying
Not more than 0.5% (50°C, 2h in vacuo)

* pH
Not less than 5.0 (1 in 50 suspension made by adding 2 g of sample to 100 ml of water and agitating the mixture with a power stirrer for 5 min).

* Sulfated ash
Not more than 0.15%
Proceed as directed under the test for Ash (Sulfated ash, Method I) using 1.5 g of the sample.

* Arsenic
Not more than 3 mg/kg
A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* Lead
Not more than 10 mg/kg
A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Lead (Method II).

* Heavy metals
Not more than 30 mg/kg
Test 0.67 of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 µg of lead ion (Pb) in the control.

METHOD OF ASSAY

Azodicarbonamide

Titrimetry
Transfer about 225 mg of the accurately weighed sample, previously dried in a vacuum oven at 50°C for 2 h, into a 250-ml glass-stoppered iodine flask. Add about 23 ml of dimethyl sulfoxide to the flask, washing any adhered sample down with the solvent, stopper the flask, and place about 2 ml of the solvent in the cup or lip of the flask. Swirl occasionally until complete solution of the sample is effected, and then loosen the stopper to drain the remainder of solvent into the flask and to rinse down any dissolved sample into the solution. Add 5 g of potassium iodide followed by 15 ml of water, immediately pipet 10 ml of 0.5 N hydrochloric acid into the flask, and stopper quickly. Swirl until the potassium iodide dissolves, and allow to stand for 20-25 min protected from light. Titrate the liberated iodine with 0.1 N sodium thiosulfate to the disappearance of the yellow colour. Titrate with additional thiosulfate if any yellow colour appears within 15 min. Perform a blank determination on a solution consisting of 25 ml of dimethyl sulfoxide, 5 g of potassium iodide, 15 ml of water, and 5 ml of 0.5 N hydrochloric acid, and make any necessary correction. Each ml of 0.1 N sodium thiosulfate is equivalent to 5.804 mg of C₂H₄N₄O₂.

Nitrogen

Kjeldahl method (semimicro)*

Transfer about 50 mg of the accurately weighed sample, previously dried in a vacuum oven at 50° for 2 h, into a 100-ml Kjeldahl flask, add 3 ml of hydriodic acid (min. 57%) and digest the mixture for 75 min adding sufficient water, when necessary, to maintain the original volume. Increase the heat at the end of the digestion period and continue heating until the volume is reduced by about one-half. Cool to room temperature, add 1.5 g of potassium sulfate and 3 ml of water. Carefully add 4.5 ml of concentrated sulfuric acid and heat until iodine fumes are no longer evolved. Allow the mixture to cool, wash down the sides of the flask with water, heat until charring occurs, and again cool to room temperature. To the charred material add 40 mg of mercuric oxide, heat until the colour of the solution is pale yellow, then cool, wash down the sides of the flask with a few ml of water and digest the mixture for 3 h. Cool the digest, add 20 ml ammonia-free water, 16 ml of a 50% sodium hydroxide solution and 5 ml of a 44% sodium thiosulfate solution. Connect the flask to a distillation apparatus and distil, collecting the distillate in 10 ml of a 4% boric acid solution. Add a few drops of methyl red-methylene blue TS to the distillate and titrate with 0.05 N sulfuric acid. Perform a blank determination. Each ml of 0.05 N sulfuric acid is equivalent to 0.7004 mg of N.

AZORUBINE*

SYNONYMS
CI Food Red 3, Carmoisine
INS No. 122, EEC No. E122

DEFINITION
Azorubine consists essentially of disodium 4-hydroxy-3-(4-sulfonato-1-naphthylazo)-1-naphthalenesulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

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

Class
Monoazo

Code numbers
CI (1975) No. 14720
CAS No. 3567-69-9

Chemical name
Disodium 4-hydroxy-3-(4-sulfonato-1-naphthylazo)-1-naphthalenesulfonate.

Chemical formula
C_{29}H_{12}N_{4}Na_{3}O_{7}S_{2}

Structural formula

Molecular weight
502.44

Assay
Content not less than 85% total colouring matters

DESCRIPTION
Red powder or granules

FUNCTIONAL USE
Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Soluble in water. Sparingly soluble in ethanol

**** B. Identification of colouring matters
Passes test

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* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** See Annex 2 at the end of this Compendium.


PURITY TESTS

* Loss on drying at 135°C

* Chloride and sulfate calculated as sodium salts

* Water insoluble matter

** Arsenic

** Lead

*** Heavy metals

Subsidiary colouring matters

Not more than 15%

Not more than 0.2%

Not more than 3 mg/kg

Not more than 10 mg/kg

Not more than 40 mg/kg

Proceed as directed in the Limit Test for Heavy Metals

Not more than 1%

See description under TESTS

Organic compounds other than colouring matters

4-Amino-1-naphthalenesulfonic acid

4-Hydroxy-1-naphthalenesulfonic acid

Total no more than 0.5%

See description under TESTS

* Unsulfonated primary aromatic amines

Not more than 0.01% calculated as aniline

* Ether extractable matter

Not more than 0.2%

TESTS

PURITY TESTS

* Subsidiary colouring matters

Use the following conditions:

Developing solvent: No. 4

Height of ascent of solvent front: approximately 17 cm

* Organic compounds other than colouring matters

Use HPLC under the following conditions:

HPLC elution gradient: 1 to 100% at 2.0% per min (exponential)

METHOD OF ASSAY

Determination of Total Colouring Matters by Titration with Titanous Chloride*

Use the following:

Weight of sample: 0.5 - 0.6 g

Buffer: 15 g sodium hydrogen tartrate

Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N TiCl₃:

12.56 mg


SYNONYMS
Beetroot Red; INS No. 162, EEC No. E162

DEFINITION
Beet Red is obtained from the roots of red beets (Beta vulgaris L var rubra) as press juice or by aqueous extraction of shredded beet roots. The colour is composed of different pigments all belonging to the class betalaine. The main colouring principle consists of betacyanins (red) of which betanine accounts for 75-95%. Minor amounts of betaxanthine (yellow) and degradation products of betalaines (light brown) may be present.** Besides the colour pigments the juice or extract consists of sugars, salts and/or proteins naturally occurring in red beets. The solution may be concentrated and some products may be refined in order to remove most of the sugars, salts and proteins. Food grade acids (e.g., citric, lactic, L-ascorbic) may be added as pH controlling agents and stabilizers and carriers (e.g., maltodextrin) may be added as aids for manufacturing dry powders.

Class
Betalaine

Code number
Betalaine: CAS No 7659-95-2

Chemical names
[S-(R',R')-4-[2-[2-Carboxy-5-(β-D-glucopyranosyloxy)-2,3-dihydro-6-hydroxy-1H-indol-1-yl)ethenyl]-2,3-dihydro-2,6-pyridine-dicarboxylic acid; 1-[2-(2,6-dicarboxy-1,2,3,4-tetrahydro-4-pyridylidene)ethylidene]-5-β-D-glucopyranosyloxy]-6-hydroxyindolium-2-carboxylate.

Chemical formula
Betalaine: C_{36}H_{32}N_{2}O_{13}

Structural formula

![Structural formula of Betalaine](image)

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

** The betanine content in extracts of beetroot will suffer a progressive degradation which is accelerated by raising the pH, temperature and water activity. It is therefore expected that all commercial products will slowly lose their colour and alter their shade according to the conditions of storage.
Molecular weight: Betaine: 550.48

Assay: Content of red colour (expressed as betaine) is not less than 0.4%.

**DESCRIPTION**
Red or dark red liquid, paste, powder or solid.

**FUNCTIONAL USE**
Food colour

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

* A. Solubility
Soluble in or miscible with water.
Insoluble in or immiscible with absolute ethanol.

B. Colour reaction
Addition of an aqueous 10% w/v sodium hydroxide solution to an aqueous solution of Beet Red successively changes the colour from red to reddish violet to yellow.

* C. Spectrophotometry
Betaine in water at pH 5.4 has an absorbance maximum at about 530 nm and at pH 8.9 exhibits a broadened maximum at about 545 nm.

* D. Thin layer chromatography
Passes test
See description under TESTS

**PURITY TESTS**

**Nitrate**
Not more than 2 g nitrate anion/g of red colour (as calculated from assay).
See description under TESTS.

* **Arsenic**
Not more than 3 mg/kg

* **Lead**
Not more than 10 mg/kg

* **Heavy metals**
Not more than 40 mg/kg
Test 0.5 g of the sample as directed in the Heavy Metals Limit Test (Method II).

Basic colouring
Passes test
See description under TESTS

Other acidic colouring matters
Passes test
See description under TESTS

**TESTS**

**IDENTIFICATION TESTS**

* D. Thin layer chromatography
(a) On cellulose plates (0.25 mm) with Sörenson's phosphate buffer (pH 5.6) as solvent, Beet Red colour gives a number of spots in various colours (yellow, orange, red, purple, violet). Betaine appears as a purple spot with an Rf value of about 0.7.

D. Thin layer chromatography

Sørensen's phosphate buffer (pH 5.6)

- Solution A: 1/15 M potassium dihydrogen phosphate: Dissolve 9.08 g of KH₂PO₄ in water and dilute to 1000 ml.
- Solution B: 1/15 M disodium hydrogen phosphate: Dissolve 11.88 g of Na₂HPO₄·2H₂O in water and dilute to 1000 ml.

Sørensen's phosphate buffer is composed of a mixture of solutions A and B in the following proportions: 94.8 parts of solution A + 5.2 parts of solution B.

(b) On cellulose plates (0.10 mm) in the solvent (2 g sodium citrate + 78.5 ml water + 21.5 ml ammonia TS), betanine follows the front of the solvent as distinct from acidic water-soluble synthetic dyes. In this solvent betanine is yellow.

PURITY TESTS

Nitrate

Apparatus
A suitably sensitive potentiometric instrument, such as a pH/mV meter, with nitrate - selective electrode and reference electrode as prescribed by the manufacturer.

Solutions
- Standard nitrate solution (10,000 mg/l): Dissolve 16.31 g of potassium nitrate (KNO₃), previously dried at 105°, 24 h in 1000 ml of water.
- Buffer solution: Dissolve 6.66 g of aluminium sulfate octahydrate, Al₂(SO₄)₃·8H₂O, 3.12 g of silver sulfate (Ag₂SO₄), 1.24 g of boric acid (H₃BO₃) and 1.94 g of sulfamic acid (NH₂HSO₃) in 900 ml water, adjust to pH 3.0 with 1 M sulfuric acid and dilute with water to 1000 ml.
- Diluted buffer solution: Dilute the Buffer solution with an equal amount of water.
- Calibration solutions: Dilute the standard solution with the Diluted buffer solution in order to prepare the following solutions: 0, 100, 200, 300, 400 and 500 mg nitrate/l.

Procedure
Accurately weigh about 0.5 g of the sample in a conical flask, add 50 ml of Diluted buffer solution and dissolve by swirling.
Measure the potential of the calibration solutions and also of the sample solution. Plot the calibration curve from the potential figures against the corresponding nitrate concentrations using antilog paper with the nitrate concentrations along the linear axis. From the calibration curve read the nitrate concentration of the sample.

Basic dyes

To 1 g of the sample add 100 ml of 1% sodium hydroxide solution, and mix well. Extract 30 ml of this solution with 15 ml of diethyl ether. When extracted wash the ether layer twice with 5 ml of dilute acetic acid TS; the dilute acetic acid layer does not produce a colour.

Other acidic dyes

To 1 g of the sample add 1 ml of ammonia TS and 8 ml of water, and shake well. Discard an oily layer when separated. Proceed as directed under Paper Chromatography (Ascending chromatography) in General Methods* using 2 μl of the solution as the sample solution, and a mixture of pyridine and ammonia TS (2:1 by volume) as the developing solvent. Stop the development when the solvent front has advanced about 15 cm from the point of application. No spot is observed at the solvent front after drying under daylight, or, if any spot is observed, it shall be decolourized when sprayed with a solution of stannous chloride (2 parts of stannous chloride by weight in 5 parts of water).

METHOD OF ASSAY

Reagents and Solutions

Buffer TS (pH 5)*

Procedure

Dissolve a quantity of Beet Red accurately weighed in buffer TS (pH 5) and dilute to a suitable volume with the buffer solution (V ml in total); the maximum absorption* shall be within the range of 0.2 to 0.8. Centrifuge the solution if necessary, and measure the absorption, correcting for a blank composed of Buffer TS (pH 5). The colour content is calculated on the basis of the maximum absorption A (at about 530 nm), using the specific absorbance for betanine.

\[ A_{1\text{ cm}} = 1120. \]

Calculation

\[ \text{% Red colour} = \frac{A \times V}{1120 \times L \times W} \]

in which

\[ A = \text{maximum absorption} \]
\[ V = \text{volume of test solution measured in ml} \]
\[ L = \text{length of cell measured in cm} \]
\[ W = \text{weight of sample in g}. \]

**SYNONYM**

Benzoic aldehyde

**DEFINITION**

- **Chemical name**: Benzaldehyde
- **C.A.S. number**: 100-52-7
- **Chemical formula**: C₆H₅O
- **Structural formula**:

```
\[ \text{\begin{tikzpicture}
  \node (atom1) at (0,0) {\text{C}};
  \node (atom2) at (1,0) {\text{H}};
  \node (atom3) at (2,0) {\text{C}};
  \node (atom4) at (3,0) {\text{O}};
  \node (atom5) at (2,1) {\text{H}};
  \node (atom6) at (2,-1) {\text{H}};
  \end{tikzpicture}} \]
```

**Molecular weight**: 106.12

**Assay**: Not less than 97% of C₆H₅O

**DESCRIPTION**

Colourless liquid having an almond-like odour

**FUNCTIONAL USE**

Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

- **B. Refractive index**: \( n_\infty^D : 1.5440 - 1.5470 \)
- **C. Specific gravity**: \( d_\infty^D : 1.043 - 1.052; \quad d_\rho^D : 1.041 - 1.046 \)

**PURITY TEST**

- **Chlorinated compounds**: Passes test

**METHOD OF ASSAY**

Weigh accurately about 1 g of the sample and proceed as directed under the method for Aldehyde Determination in the General Methods**, using 53.06 as the equivalence factor \( (e) \) in the calculation.

---

* These specifications were prepared at the 11th session of JECFA (1967) and published in NMRS 44B (1969).

BENZOIC ACID*

SYNONYMS

INS No. 210, EEC No. E210

DEFINITION

Chemical names
Benzoic acid, benzenecarboxylic acid, phenylcarboxylic acid.

C.A.S. number
65-85-10

Chemical formula
C_6H_5COOH

Structural formula

[Structural formula image]

Molecular weight
122.12

Assay
After drying for 3 h over sulfuric acid, contains not less than 99.5% of C_6H_5COOH.

DESCRIPTION

A white crystalline solid, having not more than a faint characteristic odour.

FUNCTIONAL USE

Antimicrobial preservative.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Slightly soluble in water, freely soluble in chloroform and ethanol.

** B. Melting range
121.5-123.5°.

C. Sublimation test
Passes test
See description under TESTS

D. Positive test for benzoate
Passes test
See description under TESTS

PURITY TESTS

** Loss on drying
Not more than 0.5% after drying for 3 h over sulfuric acid.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (cont’d)

* pH

About 4.0 (solution in water).

* Sulfated ash

Not more than 0.05%

Proceed as directed under the test of Ash (Sulfated ash, Method I) using 2.0 g of the sample.

* Arsenic

Not more than 3 mg/kg.

A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* Heavy metals

Not more than 10 mg/kg.

See description under TESTS

* Readily carbonizable substances

Dissolve 0.5 g of the sample, weighed to the nearest mg, in 5 ml of sulfuric acid TS. The colour produced should not be darker than a light pink (Matching Fluid Q).

* Readily oxidizable substances

Passes test

See description under TESTS

Chlorinated organic compounds

Not more than 0.07% (as Cl).

See description under TESTS

TESTS

IDENTIFICATION TESTS

C. Sublimation Test

Place a pinch of the sample in a dry test tube. Wrap the test tube about 4 cm from the bottom with moistened filter paper. Heat the test tube over a low flame. Benzoic acid sublimes and crystals deposit in the colder part of the test tube leaving no residue at the bottom.

D. Positive test for benzoate

Warm gently 0.1 g of the sample with 0.1 g of calcium carbonate and 5 ml of water. Filter and add ferric chloride TS to the filtrate. A buff coloured precipitate is produced.

PURITY TESTS

* Heavy metals

Weigh 2 g of the sample to the nearest mg and volatilize over a low flame. To the residue add 2 ml of nitric acid and about 10 mg of sodium carbonate and evaporate to dryness on a steam bath. Dissolve the residue in a mixture of 1 ml of dilute acetic acid TS and 24 ml of water. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 µg of lead ion (Pb) in the control (Solution A).

Readily oxidizable substances

Add 1.5 ml of sulfuric acid to 100 ml of water, heat to boiling and add 0.1 N potassium permanganate in drops, until the pink colour persists for 30 sec. Dissolve 1 g of the sample, weighed to the nearest mg, in the heated solution, and titrate with 0.1 N potassium permanganate to a pink colour that persists for 15 sec. Not more than 0.5 ml should be required.

PURITY TESTS (cont’d)

* Chlorinated organic compounds

Weigh 0.25 g of the sample to the nearest mg, and dissolve in 10 ml of 0.1 N sodium hydroxide. Acidify with nitric acid and filter off the precipitate. Mix the precipitate with 0.5 g of calcium carbonate, dry the mixture and then ignite. Take up the ignition residue in 20 ml of dilute nitric acid TS and filter. Mix the filtrate with 0.5 ml of 0.1 N silver nitrate. The turbidity should be not more than that obtained in a similar volume by addition of 0.5 ml of 0.1 N silver nitrate and 0.5 ml of 0.01 N hydrochloric acid.

METHOD OF ASSAY

Neutralization titration

Weigh, to the nearest mg, 2.5 g of the sample, previously dried for 3 h over sulfuric acid. Dissolve in 15 ml of warm ethanol previously neutralized using phenol red TS as indicator. Add 20 ml of water and titrate with 0.5 N sodium hydroxide, using phenolphthalein TS as indicator. Each ml 0.5 N sodium hydroxide is equivalent to 61.06 mg of C₇H₆O₂.

BENZOIN GUM*  
(Tentative)

SYNONYMS  
Benzoin resin, Resine benzoique, Benzo-charz; INS No. 906

DEFINITION  
Natural gum-resin of *Styrax tonkinensis* (Benzoin Siam) and natural balsamic resin of *Styrax benzoin* or *Styrax paralleloneurus* (Benzoin Sumatra). The main constituents of Benzoin Siam are coniferyl benzoate (65-75%), benzoic acid (10-12%), other benzoate esters and vanillin. The main constituents of Benzoin Sumatra are coniferyl cinnamates, cinnamyl alcohol, benzo-resinol, phenyl propyl alcohol.

Siam benzoin yields not less than 90% of ethanol-soluble extractive, and Sumatra benzoin yields not less than 75%

C.A.S. number  
9000-05-9

FUNCTIONAL USE  
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

A. Solubility  
A solution in ethanol becomes milky upon the addition of water, and the mixture is acid to litmus

B. Heat test  
Passes test  
See description under TESTS

C. Positive test for cinnamyl alcohol  
Passes test  
See description under TESTS

PURITY TEST

** Acid-insoluble ash  
Not more than 1% in Sumatra benzoin; not more than 0.5% in Siam benzoin

Benzoic acid  
Passes test  
See description under TESTS

Foreign organic matter  
Not more than 1% in Siam benzoin  
See description under TESTS

---

* These specifications were prepared at the 21st session of JECFA (1977) and published in NMRS 57 (1977).

TESTS

IDENTIFICATION TESTS

B. Heat test
Heat a few fragments in a test tube: Sumatra benzoin evolves a sublimate consisting of plates and small rod-like crystals of cinnamic acid and its esters that strongly polarize light. Siam benzoin evolves a sublimate directly above the melted mass consisting of numerous long rod-shaped crystals of benzoic acid which do not strongly polarize light.

C. Positive test for cinnamyl alcohol
Heat about 0.5 g in a test tube with 10 ml of potassium permanganate TS. Only the Sumatra variety develops a faint odour of benzaldehyde.

PURITY TESTS

Benzoic acid
Treat about 1 g of powdered sample with 15 ml of warm carbon disulfide, filter through a small pledget of cotton, wash the cotton with an additional 5 ml of carbon disulfide, and allow the filtrate to evaporate spontaneously; the weight of the residue is not less than 6% (Sumatra benzoin); not less than 12% (Siam benzoin) of the weight of benzoin.

Foreign organic matter
Weigh about 25 g of the sample, and spread it out in a thin layer. Separate the foreign organic matter by hand, and from its weight calculate the percentage of foreign organic matter in the sample taken.

METHOD OF ASSAY

Place about 2 g of the sample, accurately weighed, in a tared extraction thimble, and insert the thimble in a Soxhlet or other soluble continuous-extraction apparatus. Place about 100 mg of sodium hydroxide in the receiving flask of the apparatus and extract the benzoin with ethanol for 5 h, or until completely extracted. Dry the extraction thimble containing the insoluble residue at 105° for 2 h. On a separate portion of benzoin, determine the water content by the Azeotropic (Toluene Distillation) Method. Calculate the weight of water in the quantity of the benzoin taken for assay, and subtract it from the original weight of the benzoin taken. The difference between this result and the weight of the residue in the extraction thimble represents the ethanol-soluble extractive.

BENZOYL PEROXIDE*

SYNONYMS
Benzoyl superoxide
INS No. 928

DEFINITION
Chemical names
Dibenzoyl peroxide

C.A.S. number
94-36-0

Chemical formula
C₁₇H₁₆O₄

Structural formula

Molecular weight
242.23

Assay
Benzoyl peroxide contains not less than 96% of \( \text{C}_17\text{H}_{16}\text{O}_4 \), calculated on the dried basis

DESCRIPTION
A colourless, crystalline solid having a faint odour of benzaldehyde.

Caution: Benzoyl peroxide is a highly reactive compound and may give rise to explosion.

FUNCTIONAL USE
Bleaching agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water. Slightly soluble in ethanol. Soluble in ether.

** B. Melting range
Between 103° and 108° with decomposition

C. Positive test for oxidation
Passes test
See description under TESTS

D. Decomposition to benzoic acid
Passes test
See description under TESTS

---

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976)

PURITY TESTS

* Water
  Not more than 10% (Karl Fisher Method)

Arsenic
  Not more than 3 mg/kg
  See description under TESTS

Lead
  Not more than 10 mg/kg
  See description under TESTS

Heavy metals
  Not more than 40 mg/kg
  See description under TESTS

TESTS

IDENTIFICATION TESTS

C. Positive test for oxidation
   Dissolve 0.1 g of the sample in 15 ml of acetone and add 3 ml of 50% potassium iodide solution. The brown colour of iodine appears immediately.

D. Decomposition to benzoic acid
   To 0.5 g of benzoyl peroxide add 50 ml of 0.6 N ethanolic potassium hydroxide, heat gradually to boiling and continue boiling for 15 min. Cool and dilute with 200 ml of water. Add sufficient 0.5 N hydrochloric acid to make strongly acid and extract with ether. Dry the ether solution over CaCl₂ and evaporate the ether. The residue of benzoic acid so obtained melts between 121.5° and 123.5° (May need to be recrystallized).

PURITY TESTS

* Arsenic
  Mix 1 g of the sample with 10 ml of sodium hydroxide TS, slowly evaporate to dryness on a steam bath, and cool. A sample solution, prepared as directed for organic compounds from the residue obtained, meets the requirements of the Limit Test for Arsenic (Method II).

* Lead
  Mix 1 g of the sample with 10 ml of sodium hydroxide TS, slowly evaporate to dryness on a steam bath, and cool. A sample solution, prepared as directed for organic compounds from the residue obtained, meets the requirements of the Limit Test for Lead.

* Heavy metals
  Mix 0.50 g of the sample with 5 ml of sodium hydroxide TS, slowly evaporate to dryness on a steam bath, cool, and dissolve the residue in 25 ml of water. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Redox titration
Dissolve about 250 mg of the sample, accurately weighed, in 15 ml of acetone in a 100-ml glass-stoppered bottle. Add 3 ml of 50% potassium iodide solution and swirl for 1 min. Titratre immediately with 0.1 N sodium thiosulfate is equivalent to 12.11 mg of C₈H₈O₄.

---

SYNONYMS

Acetic acid phenylmethyl ester, acetic acid benzyl ester, benzyl ethanoate

DEFINITION

Chemical name  Benzyl acetate
C.A.S. number  140-11-4
Chemical formula  $C_9H_{16}O_2$

** Structural formula **

$$
\text{O} \\
\text{CH}_2\text{OCCH}_3 \\
\text{C}_9\text{H}_{16}
$$

Molecular weight  150.18
Assay  Content not less than 98% of $C_9H_{16}O_2$

DESCRIPTION

Colourless, transparent liquid with a characteristic odour

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility **
Insoluble in water. Miscible with ethanol, acetone and ether. Soluble in most fixed oils

** B. Refractive index **
$n_\text{D}^20 : 1.501 - 1.504$

** C. Specific gravity **
$d_\text{D}^20 : 1.055 - 1.059; \quad d_\text{H}^20 : 1.052 - 1.056$

PURITY TEST

** Acid value **
Not more than 1

** Chlorinated compounds **
Passes test

METHOD OF ASSAY

Weigh accurately about 0.8 g of the sample, and proceed as directed under the method for Ester Determination in the General Methods**, using 75.1 as the equivalence factor (e) in the calculation.

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983) superseding the earlier specifications published in NMRS 44B (1969).

**SYNONYMS**

Phenylcarbinol, phenylmethylalcohol, benzenemethanol, α-hydroxytoluene

**DEFINITION**

**Chemical names** Benzyl alcohol, phenylmethanol

**C.A.S. number** 100-51-6

**Chemical formula** \( \text{C}_7\text{H}_8\text{O} \)

**Structural formula**

\[
\begin{array}{c}
\text{\includegraphics[width=4cm]{benzyl-alcohol-structure.png}}
\end{array}
\]

**Molecular weight** 108.14

**Assay** Content not less than 98\% of \( \text{C}_7\text{H}_8\text{O} \)

**DESCRIPTION**

Colourless, clear liquid, with a faint, aromatic odour and a sharp burning taste

**FUNCTIONAL USE**

Flavouring agent, carrier solvent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility** Soluble in water

Miscible with ethanol, chloroform and ether

**B. Refractive index** \( \eta_0^2 : 1.538 - 1.541 \)

**C. Specific gravity** \( d_20^2 : 1.042 - 1.047 \)

**PURITY TESTS**

**Distillation range** Not less than 95\% v/v distils between 202\° and 208\°

**Arsenic** Not more than 3 mg/kg (Method II)

**Heavy metals** Not more than 10 mg/kg

Test 2 g of the sample as directed in the Limit Test (Method II)

---

* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979).

PURITY TESTS (continued)

* Acid value
Not more than 0.5

* Aldehydes
(as benzaldehyde)
Not more than 0.2% v/v
See description under TESTS

* Peroxides
Passes test
See description under TESTS

* Chlorinated Compounds
Passes test

TESTS

PURITY TESTS

**Aldehydes, as benzaldehyde**
Transfer 2 ml of the sample into a 100-ml volumetric flask and add water to volume. Shake until dissolved. To 2 ml of the above solution add 3 ml of water and 0.5 ml of a saturated solution of dinitrophenylhydrazine in dilute hydrochloric acid. Cap test tube, shake and allow to stand for 10 min. Add 5 ml of 95% ethanol to 2 ml of a 10% potassium hydroxide solution and homogenize. Any red-brown colour that develops shall not be more intense than that of a control, simultaneously prepared under the same conditions, but substituting 2 ml of the sample with 2 ml of freshly prepared 0.2% v/v solution of benzaldehyde.

**Peroxides**
Flush out with carbon dioxide a ground glass necked 100-ml flask and fitted with a cool air condenser; then introduce 1 ml of the sample, 2 ml of chloroform, 0.1 g of potassium iodide and 20 ml of mixture of 1 volume chloroform and 2 volumes of glacial acetic acid. Fit the condenser to the flask and warm with small flame to initiate boiling within 30 sec. Maintain boiling for exactly 30 sec from the moment vapours appear in the condenser. Cool immediately in iced water, and add through the condenser 40 ml of carbon dioxide free water. Titrate the liberated iodine with a 0.005 N solution of sodium thiosulfate and record the number of ml of solution used as n. Perform the same operation without the sample and record the number of ml of solution used as n'. The difference (n - n') must be less than 1 (equivalent to 40 mg peroxide per litre, expressed as oxygen).

**METHOD OF ASSAY**

Weigh accurately about 1 g of the sample, proceed as directed under the method for Hydroxyl Value in the General Methods* (General Methods for Fats and Related Substances), and calculate the content by the formula,

\[
\text{Benzyl alcohol \% \ w/w} = \frac{\left[B + (WA/C) - S\right] \times N \times 10.814}{W}
\]

**BENZYL BENZOATE**

SYNONYMS

Benzoyl benzoic acid ester, benzoic acid phenylmethyl ester

DEFINITION

- **Chemical name**: Benzyl benzoate
- **C.A.S. number**: 120-51-4
- **Chemical formula**: \( C_{14}H_{12}O_2 \)
- **Structural formula**: 

![Structural formula image]

- **Molecular weight**: 212.25
- **Assay**: Content not less than 99% of \( C_{14}H_{12}O_2 \)

DESCRIPTION

Colourless oily liquid, with a faint sweet balsamic odour, and a sharp burning taste

FUNCTIONAL USE

Flavouring agent

CHARACTERISTICS

**IDENTIFICATION TESTS**

- **A. Solubility**: Practically insoluble in water, propan-1,2-diol and in glycerol. Miscible with ethanol, acetone and ether. Soluble in most fixed oils.
- **B. Refractive index**: \( n^\circ_0 = 1.568 - 1.570 \)
- **C. Specific gravity**: \( d^\circ_2 = 1.116 - 1.120 \)

**PURITY TEST**

- **Solidification point**: Not less than 18°
- **Acid value**: Not more than 1
- **Chlorinated compounds**: Passes test

METHOD OF ASSAY

Weigh accurately about 1 g of the sample, and proceed as directed under the method for Ester Determination in the General Methods**, using 106.1 as the equivalence factor (e) in the calculation.

---

* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979).

BENZYL BUTYL ETHER*  
(Tentative)**

SYNONYM  
Butyl benzyl ether

DEFINITION

Chemical name  
Benzyl butyl ether

C.A.S. number  
588-67-0

Chemical formula  
C_{11} H_{16} O

Structural formula

\[
\begin{array}{c}
\text{O} \\
\text{CH}_3
\end{array}
\]

Molecular weight  
164.25

Assay  
Information required**

DESCRIPTION  
Colourless liquid, with a floral note reminiscent of rose and geranium

FUNCTIONAL USE  
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility  
Very soluble in ethanol and in fixed oils, insoluble in water

*** B. Refractive index  
\(n^\circ : 1.485 - 1.489\)

*** C. Specific gravity  
\(d^\circ: 0.917 - 0.924\)

D. Infrared spectrum  
See Appendix at the end of these specifications

METHOD OF ASSAY  
Methodology required**

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation. Information on assay and method of assay are required.

APPENDIX

* Infrared Spectrum: Benzyl Butyl Ether

* Infra-red spectra through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc., Philadelphia, USA.
SYNONYMS
Isobutyl-benzyl-carbinol, alpha-isobutylphenethyl alcohol

DEFINITION
Chemical name 4-Methyl-1-phenyl-2-pentanol
C.A.S. number 7779-78-4
Chemical formula C₁₇H₃₄O
Structural formula

Molecular weight 178.28
Assay Content not less than 96% of C₁₇H₃₄O

DESCRIPTION
Colourless slightly oily liquid, with a straw-like odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Soluble in ethanol. Insoluble in water.

*** B. Refractive index
n²⁰ : 1.501 - 1.508

*** C. Specific gravity
d²₀ : 0.950 - 0.960

*** D. Infrared spectrum
See Appendix at the end of these specifications

PURITY TEST

*** Solubility in ethanol
1 ml dissolves in 2 ml of 70% ethanol

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation.

METHOD OF ASSAY

Method A

Proceed as directed under the method for Total Alcohol in the General Methods*. Weigh accurately about 1.4 g of the acetylated alcohol and use 89.14 as equivalence factor (e) in the calculation.

Method B

Determine by gas-liquid chromatography, using the following conditions.

- Column length: 3.00 m
- Column diameter: 3.0 mm
- Column material: glass
- Column packing: SOMB 4%
- Column support: chromosorb 80/120 mesh
- Carrier gas: Helium
- Flow rate: 50 ml/min
- Detector type: FID
- Detector temperature: 250°
- Temperature of injection port: 250°
- Column temperature:
  - Isothermal: 120°
  - Temperature program: 5°/min up to 170° then 10°/min
  - Final temperature: isothermal at 250°

The above conditions are to be applied together with the information provided under General Methods*.

Remark: Allow the chromatogram to develop until compounds have been eluted.

APPENDIX

Infrared Spectrum: Benzyl Isobutyl Carbinol**

---


** Infra-red spectra through the courtesy of the International Organization of the Flavour Industry (IOFI), Geneva, Switzerland, and of the SADTLER RESEARCH LABORATORIES, Inc., Philadelphia, USA.
**BENZYL ISOEUGENYL ETHER**
(Tentative)**

**SYNONYMS**
Isoeugenyl benzyl ether, benzyl isoeugenol

**DEFINITION**

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>(E)-1-Benzyloxy-2-methoxy-4(1-propenyl)benzene</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>120-11-6</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C_{17}H_{18}O_{2}</td>
</tr>
<tr>
<td>Structural formula</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
</tbody>
</table>

**DESCRIPTION**
White to ivory coloured crystalline powder with a mild balsamic spicy odour

**FUNCTIONAL USE**
Flavouring agent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

*** A. Solubility
Soluble in ethanol and in fixed oils, practically insoluble in propane-1,2-diol and glycerol, and water.

*** B. Melting point
57 - 59°

C. Infrared spectrum
Information required**

**PURITY TESTS**

*** Acid value
Not more than 1

---

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

** The references to identity, purity and methods of analysis were felt to require further confirmation. Information on infrared spectrum is required.

PURITY TESTS (continued)

**Free isoeugenol content**

Not more than 1%

See description under TESTS

**TEST**

**PURITY TEST**

**Free isoeugenol content**

Transfer about 5 g of the sample, accurately weighed, into a 150-ml flask having a standard taper neck. Pipet exactly 10 ml of a 1 in 10 solution of acetic anhydride in anhydrous pyridine into the flask, and pipet exactly 10 ml of this solution, preferably measured with the same pipet, into a second 150-ml flask for the residual blank titration. Connect the flasks to condensers, reflux for 1 h, and cool to a temperature below 100°. Add 25 ml of water to each flask through the condensers, and reflux again for 10 min. Cool the flasks. Add phenolphthalein TS and titrate with 0.5 N potassium hydroxide. Calculate the percent of free isoeugenol by the formula:

\[
\text{Percent of Free Isoeugenol} = \frac{(b - s) \times 100f}{w}
\]

in which \( b \) is the number of ml of 0.5 N potassium hydroxide consumed in the residual blank titration, \( s \) is the number of ml of 0.5 N potassium hydroxide consumed in the titration of the sample, \( f \) is the equivalence factor 82.10, and \( w \) is the weight of the sample, in mg.

**METHOD OF ASSAY**

Determine by gas-liquid chromatography, under the following conditions.

- Column length: 1.60 m (for consistency)
- Column diameter: 3.0 mm
- Column material: Glass
- Column packing: SP 1000 4%
- Column support: Chromosorb 100/120 mesh
- Carrier gas: Helium
- Flow rate: 50 ml/min
- Detector type: FID
- Detector temperature: 250°
- Temperature of injection port: 250°
- Column temperature: Isothermal: 250°

The above conditions are to be applied together with the information provided under General Methods*.

Remark: Allow the chromatogram to develop until compounds have been eluted.

**BLACKCURRANT EXTRACT**
*(Tentative)*

**SYNONYM**

INS No. 163(iii), EEC No. E163

**DEFINITION**

Blackcurrant extract is obtained from black currant pomace by water extraction. Maltodextrin may be added and the product spray dried. The main colouring principles consist of anthocyanins (glucosides of anthocyanidines). The remainder is mainly composed of sugars and organic acids naturally occurring in black currant.

**Class**

Anthocyanin.

**Chemical names**

The essential anthocyanidines are:

I. Delphinidin:3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)-1-benzopyrylium (chloride)

II. Cyanidin: 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-1-benzopyrylium (chloride)

**Chemical formula**

I. \( C_{13}H_{11}O_{5}X \)

II. \( C_{13}H_{11}O_{4}X \)

where \( X = \) acid moiety

**Structural formula**

![Structural formula of anthocyanin]

I. \( R = OH \)

II. \( R = H \)

\( X = \) acid moiety

**Assay**

Information required

**DESCRIPTION**

Purplish red liquid or powder having a slight characteristic odour.

**FUNCTIONAL USE**

Food colour

---

* These specifications were prepared at the 30th session of JECFA (1986) and published in FNP 37 (1986).

** Information required on use of antioxidants, levels of assay, the average specific absorption (\( E_{1%}^{1 \text{cm}} \) at pH 3, \( \lambda_{max} = 520 \text{ nm} \)) for the main colouring principle and degradation and chromatographic identification test.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water and ethanol.

** B. Spectrophotometry
   At pH 3 the absorbance maximum is about 520 nm.

C. Colour reaction
   Passes test
   See description under TESTS

D. Chromatography
   Information required

PURITY TESTS

Sulfur dioxide
   Not more than 50 mg/kg for each unit of colour intensity
   See description under TESTS and METHOD OF ASSAY

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Test 0.5 g sample as directed in Method II in the Limit Test

Basic colouring matters
   Passes test
   See description under TESTS

Other acidic colouring matters
   Passes test
   See description under TESTS

Degradation
   Information required

TESTS

IDENTIFICATION TESTS

C. Colour reaction
   Add 0.1 g of the sample to 50 ml of water and shake thoroughly. Filter if necessary. The solution shows red to purplish-red colour and it turns to blue or dark green on the addition of sodium hydroxide TS.

PURITY TESTS

Sulfur dioxide
   Distil 1 g of the sample with 100 ml of water and 25 ml of phosphoric acid solution (2 in 7) in a distilling flask with the Wagner tube (Fig. 1, next page). In an absorption flask, place 25 ml of lead acetate solution (1 in 50) previously prepared. Insert the lower end of condenser into lead acetate solution in the absorption flask. Distil until the liquid in the absorption flask reaches about 100 ml and rinse the end of

---

* See General Methods (Guide to JECFA Specifications), FNP 5/Rev.2 (1991)


Sulfur dioxide (continued)

the condenser with a little amount of water. To the distilled solution add 5 ml of hydrochloric acid and 1 ml of starch TS, and titrate with 0.01 N iodine. Each ml of 0.01 N iodine is equivalent to 0.3203 mg of SO₂.

Fig. 1
WAGNER TUBE

Basic colouring matters

Add 1 g of the sample to 100 ml sodium hydroxide solution (1 in 100) and shake well. Take 30 ml of this solution and extract with 15 ml of ether. Extract this ether extract twice with each 5 ml of dilute acetic acid TS. The acetic acid extract is colourless.

Other acidic colouring matters

Add 1 ml of ammonia TS and 10 ml of water to 1 g of the sample and shake well. Following the directions in the General Methods for Chromatography* place 0.002 ml of the solution on the chromatographic sheet and dry it. Use a mixture of pyridine and ammonia TS (2:1 by volume) as developing solvent and stop the development when the solvent front reaches about 15 cm height from the point where the sample solution was placed. No spot is observed at the solvent front after drying under daylight. If any spot is observed, it should be decolourized when sprayed with a solution of stannous chloride in hydrochloric acid (2 in 5).

METHOD OF ASSAY

In the absence of an assay method, a measurement of colour intensity by the following method may be used.

Prepare approximately 200 ml of pH 3.0 citric acid dibasic sodium phosphate buffer solution: Mix 159 volumes of 2.1% citric acid solution and 41 volumes of 0.16% dibasic sodium phosphate solution, and adjust the pH to 3.0, using the citric acid solution or dibasic sodium phosphate solution.

Weigh accurately an adequate amount of the sample so that the measured absorbance is between 0.2 and 0.7, and add pH 3.0 citric acid - dibasic sodium phosphate buffer solution to make up a 100-ml solution. Measure the absorbance* of this solution in a 1 cm cell at the wavelength of maximum absorption around 525 nm, using pH 3.0 citric acid - dibasic sodium phosphate buffer solution as the blank.

\[ \text{Colour intensity} = \frac{A \times 10}{\text{weight of sample (g)}} \]

BONE PHOSPHATE*

SYNONYMS
Edible bone phosphate, INS No. 542

DEFINITION
Bone Phosphate is a heterogeneous residual mixture of calcium phosphates, principally $3\text{Ca}_3(\text{PO}_4)_2\text{Ca(OH)}_2$, obtained by the grinding of bones which have been treated with hot water and steam under pressure.

Bone phosphate may contain unextracted fat and proteins.

Assay
Content not less than 30% and not more than 40% of Ca, and not less than 32% of $\text{P}_2\text{O}_5$.

DESCRIPTION
White to pale cream coloured, odourless, tasteless powder

FUNCTIONAL USES
Emulsifying agent, moisture retaining agent, sequestrant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in ethanol and water

** B. Positive test for phosphate
Dissolve 1 g of the sample by warming with 50 ml diluted hydrochloric acid. The resulting solution is used for positive tests for phosphate and calcium.

** C. Positive test for calcium
Passes test

PURITY TESTS

** Loss on drying
Not more than 2%

** Loss on ignition
Not more than 20%

Fluoride (total)
Not more than 1000 mg/kg
See description under TESTS

** Arsenic
Not more than 3 mg/kg
See description under TESTS

Copper
Not more than 25 mg/kg
See description under TESTS

Zinc
Not more than 250 mg/kg
See description under TESTS

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

PURITY TESTS (continued)

* Lead
  Not more than 10 mg/kg
  Use 10 μg of lead ion (Pb) in the control

Fat residue
  Not more than 2%
  See description under TESTS

* Protein residue
  Not more than 10% (N x 6.25). Kjeldahl Method (II).

* Microbiological criteria
  Total aerobic microbial count: Max 1000 in 1 g
  Salmonella: Absent in 50 g
  E. Coli: Absent in 10 g

TESTS

PURITY TESTS

* Arsenic
  Dissolve 1 g of the sample in 10 ml of dilute hydrochloric acid TS and add 25 ml of water. Test this solution as directed in the Limit Test (Method II).

* Fluoride (Total)
  Apparatus
    Suitable sensitive potentiometric instrument, such as a pH/mV-meter with F-selective electrode and reference electrode as prescribed by the manufacturer. Magnetic stirrer.

    Buffer solution
    Dissolve 36 g of cyclohexylene dinitrilotetraacetic acid (CDTA) in sufficient 1 N sodium hydroxide to make 200 ml by boiling, then cool, and filter through glass-fiber filter paper. Pipet 30 ml of this solution into a mixture consisting of 750 ml of water, 87 g of sodium chloride, and 85.5 ml of glacial acetic acid. Adjust the pH by the addition of 50% sodium hydroxide solution, then cool, and dilute to 3000 ml with water.

    Fluoride Standard Solution
    Use a solution containing 100 μg of fluoride ion (F) per ml (100 ppm), obtained commercially or prepared by dissolving 22.2 mg of sodium fluoride, previously dried at 200° for 4 h, in sufficient water to make 100.0 ml.

    Calibration solutions
    Pipet into separate 100 ml volumetric flasks 0.2, 0.5, 1.0, 1.5 and 2.0 ml of the Fluoride Standard Solution, add 50 ml of Buffer solution and dilute to 100 ml with water.

    Sample preparation
    Accurately weigh an amount of ash (obtained from the test for Loss on ignition) equal to 0.1 g of Bone Phosphate. Dissolve the ash in 1.8 ml of 1 M hydrochloric acid and transfer to a 100 ml volumetric flask. Add 50 ml of Buffer Solution and dilute to 100 ml with water.

Sample preparation (continued)

Measure the potential of the calibration solutions and also of the sample solution. Plot the calibration curve from the potential figures against the corresponding fluoride concentrations using antilog paper with the fluoride concentrations along the linear axis. From the calibration curve read the fluoride concentration of the sample.

Calculation

\[ [F^-] = \frac{a \times (100-I)}{W} \text{ mg/kg} \]

- \( a \) = F⁻ concentration of sample mg/l
- \( W \) = weight of ash (g)
- \( I \) = Loss on ignition (%)

**Copper and zinc**

General precautions

Because of the minute amounts of metals involved special care must be taken to reduce the reagent blanks to as low a value as possible and to avoid contamination during the test. All apparatus should be thoroughly cleaned with a mixture of hot dilute acids (1 part hydrochloric acid, 1 part concentrated nitric acid, and 3 parts water) followed by thorough washing with water immediately before use. The methods of preparation described should be followed exactly.

**Apparatus**

Atomic absorption spectrophotometer equipped with air/acetylene - flame and lamps for copper and zinc determination.

**Reagents**

Reagents shall be of an order of purity higher than accepted analytical reagent grade quality. Metal-free water (see below) shall be used throughout.

- Sulfuric acid, 98% H₂SO₄
- Nitric acid, sp.gr. 1.42
- Hydrochloric acid, sp. gr. 1.16-1.18 (conc.)
- Hydrochloric acid 5 M solution prepared by dilution of hydrochloric acid (conc.) with water
- Hydrochloric acid 0.5 M solution prepared by dilution of hydrochloric acid 5 M with water
- Water, metal free. Distilled water may be re-distilled from an all glass apparatus or may be passed down a column of cation exchange resin, e.g., Amberlite IR 120 (H).

**Standards**

**Standard copper solution**

Dissolve 3.928 g of pure copper sulfate CuSO₄. 5H₂O in distilled water, dilute to 1000 ml at 20°C with distilled water in a one-mark graduated flask. Dilute 10 ml to 100 ml with water in a one-mark graduated flask as required.

1 ml = 100 μg Cu.
Copper and zinc
(continued)

Standard zinc solution
Dissolve 1.000 g of pure zinc powder in a mixture of 10 ml distilled water and 5 ml hydrochloric acid special reagent (d) and dilute to 1000 ml at 20° with distilled water, in a one-mark graduated flask. Dilute 10 ml to 100 ml with water in a one-mark graduated flask as required.

1 ml = 100 μg Zn.

Sample preparation
Place about 2.5 g of the sample, accurately weighed, in a suitable crucible, add sufficient sulfuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° to 600° until all the carbon is burned off. Cool, add 4 ml of hydrochloric acid 5 M, cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness. Finally cool, add 10 ml 5 M hydrochloric acid and boil gently for a few minutes. Cool and transfer the solution to a 50-ml one-mark graduated flask washing out the Kjeldahl flask with small portions of water. Add the washings to the graduated flask and dilute to the mark with water. Solution A.

To a 100 ml one mark volumetric flask pipet 10 ml of solution A and dilute to the mark with hydrochloric acid 0.5 M. Solution B. Prepare a reagent blanks using the same quantities of reagents as used in the sample preparation for obtaining solution A and B. Blank A and Blank B.

Preparation of Calibration Curve Solutions
To a series of 100-ml one-mark volumetric flasks pipet 0, 1, 2, 3, 4 and 5 ml of each of the two standard solutions to (e) and dilute to about 50 ml. Add 20 ml of hydrochloric acid 5 M and dilute to the mark with metal-free water. These solutions then contain 0, 1.0, 2.0, 3.0, 4.0 and 5.0 μg per ml of copper and zinc.

Instrumental Conditions
Select the wavelength to be used for the particular element under consideration from the table below.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>324.7</td>
</tr>
<tr>
<td>Zinc</td>
<td>213.9</td>
</tr>
</tbody>
</table>

The recommended settings for the various instrumental parameters vary from model to model, and certain parameters require optimization at the time of use to obtain the best results. Instruments should therefore be adjusted as described in the manufacturer's instructions using wavelength settings specified above.
Copper and zinc
(continued)

**Procedure**

Set the atomic absorption spectrophotometer to the appropriate conditions. Aspirate the strongest standard containing the element to be determined and optimize the instrument settings to give full-scale or maximum deflection on the chart recorder. Measure the absorbances of the other standards and plot a graph showing the net absorbance against the concentration of the element in the standard solutions. Aspirate Solution A and the corresponding Blank A for determination of copper or Solution B and the corresponding Blank B for determination of zinc and determine the net absorbance. Using the graph prepared above, determine the concentration of the element in the sample solution.

**Copper content**

\[ [\text{Cu}] = \frac{\text{Concentration of element (\(\mu\)g/ml) } \times 50}{\text{Weight of sample taken (g)}} \text{ mg/kg} \]

**Zinc content**

\[ [\text{Zn}] = \frac{\text{Concentration of element (\(\mu\)g/ml) } \times 500}{\text{Weight of sample taken (g)}} \text{ mg/kg} \]

**Fat residue**

Accurately weigh 5-10 g sample. Without previous drying, extract in soxhlet or other suitable container with petroleum ether (40°-60°) for about 6 hours. Filter extract through small hardened paper into weighed vessel, washing paper into weighed vessel, washing paper finally with small portion of hot fresh solvent. Distil or evaporate solvent at temperature ca. 100° and dry vessel containing residue in air oven for 1h at 100°-105°C. Weigh the dried residue and calculate percentage of the sample.

**METHOD OF ASSAY**

**Calcium**

Weigh accurately about 0.150 g of the sample. Dissolve, with the aid of gentle heat if necessary, in a mixture of 5 ml of hydrochloric acid and 3 ml of water contained in a 250 ml beaker equipped with a magnetic stirrer, and cautiously add 125 ml of water. With constant stirring, add, in the following order, 0.5 ml of triethanolamine, 300 mg of hydroxynaphthol blue indicator, and, from a 50 ml buret, about 23 ml of 0.05 M disodium ethylenediaminetetraacetate. Add sodium hydroxide solution (45 in 100) until the initial red colour changes to clear blue, then continue to add it dropwise until the colour changes to violet, then add an additional 0.5 ml. The pH is between 12.3 and 12.5. Continue the titration dropwise with the 0.05 M disodium ethylenediaminetetraacetate until the appearance of a clear blue endpoint that persists for not less than 60 sec. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 2.004 mg of Ca.

**P₂O₅ content**

Proceed as directed in Method II of the Phosphate Determination as P₂O₅ in the General Methods*.

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**BRILLIANT BLACK BN**

**SYNONYMS**

CI Food Black 1, Black BN
INS No. 151, EEC No. E151

**DEFINITION**

Brilliant Black BN consists essentially of tetrasodium 4-acetamido-5-hydroxy-6-[7-sulfonato-4-(4-sulfonato-phenylazo)-1-naphthylazo]-1,7-naphthalene-disulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Brilliant Black BN may be converted to the corresponding aluminium lake in which case only the General Specifications for Aluminium Lakes of Colouring Matters apply.

Class

Biazo

Code numbers

CI (1975) No. 28840
CAS No. 2519-30-4

Chemical name

Tetrasodium 4-acetamido-5-hydroxy-6-[7-sulfonato-4-(4-sulfonatophenylazo)-1-naphthylazo]-1,7-naphthalene-disulfonate

Chemical formula

$C_{28}H_{12}N_{2}Na_{6}O_{14}S_{4}$

Structural formula

\[
\text{Structure Image}
\]

Molecular weight

867.69

Assay

Content not less than 80% total colouring matter

**DESCRIPTION**

Black powder or granules

**FUNCTIONAL USE**

Food colour

---

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** See Annex 2 at the end of this Compendium.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water.
   Sparingly soluble in ethanol

** B. Identification of colouring matters
   Passes test

PURITY TESTS

** Loss on drying at 135°
   Not more than 20%

** Chloride and sulfate calculated as sodium salts

** Water insoluble matter
   Not more than 0.2%

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Proceed as directed in the Limit Test for Heavy Metals

Subsidiary colouring matters
   Not more than 4%
   See description under TESTS

Organic compounds other than colouring matters

   Sum of 4-Acetamido-5-hydroxy-1,7-naphthalenedisulfonic acid
   4-Amino-5-hydroxy-1,7-naphthalenedisulfonic acid
   8-Amino-2-naphthalenesulfonic acid
   Sulfanilic acid
   4,4'-Diazoamino-di(benzenesulfuric acid)
   Not more than 0.8%
   See description under TESTS

** Unsulfonated primary aromatic amines
   Not more than 0.01% calculated as aniline

** Ether extractable matter
   Not more than 0.2%


**TESTS**

**PURITY TESTS**

* **Subsidiary colouring matters**

Use the following conditions:

Developing solvent: Chromatogram (i): No. 1.

Chromatogram (ii): No. 4.

Height of ascent of solvent front:

(i): approximately 17 cm

(ii): approximately 17 cm

* **Organic compounds other than coloured matters**

Use HPLC under the following conditions:

HPLC elution gradient: 2 to 100% at 2% per min (linear)

**METHOD OF ASSAY**

**Determination of Total Colouring Matters by Titration with Titanous Chloride**

Use the following:

Weight of sample: 0.6-0.7 g

Buffer: 15 g sodium hydrogen tartrate

Weight (D) of colouring matters equivalent
to 1.00 ml of 0.1 N TiCl₃: 10.86 mg

SYNONYMS
CI Food Blue 2, FD&C Blue No. 1
INS No. 133, EEC No. E133

DEFINITION
Brilliant Blue FCF consists essentially of Disodium 3-{N-ethyl-N-[4-{N-ethyl-N-(3-sulfonatobenzyl)-amino] phenyl} (2-sulfonatophenyl)methylene]-2,5-cyclohexadiene-1-ylidene] ammoniomethyl] benzenesulfonate and its isomers and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Brilliant Blue FCF may be converted to the corresponding aluminium lake in which case only the General Specifications for Aluminium Lakes of Colouring Matters shall apply.**

Class
Triarylmethane

Code numbers
CI (1975) No. 42900
CAS No. 3844-45-9

Chemical name
Disodium α-[4-(N-ethyl-3-sulfonatobenzylamino)phenyl]-α-[4-(N-ethyl-3-sulfonatobenzylimino)cyclohexa-2,5-dienyliden]toluene-2-sulfonate (an alternative chemical name)

Chemical formula
C_{37}H_{44}N_{2}Na_{x}O_{y}S_{z}

Structural formula

Molecular weight
792.86

Assay
Content not less than 85% total colouring matters

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** See Annex 2 at the end of this Compendium.
DESCRIPTION

Blue powder or granules

FUNCTIONAL USE

Food Colour

CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water. Slightly soluble in ethanol

** B. Identification of colouring matters
   Passes Test

PURITY TESTS

** Loss on drying at 135°
   } Not more than 15%

** Chloride and sulfate calculated as sodium salts

** Water insoluble matter
   Not more than 0.2%

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

*** Chromium
   Not more than 50 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Proceed as directed in the Limit Test for Heavy Metals

Subsidiary colouring matters
   Not more than 6%
   See description under TESTS

Organic compounds other than colouring matters

   Sum of 2-, 3- and 4-formylbenzenesulfonic acids
   Not more than 1.5%
   See description under TESTS

   3-[N-ethyl-N-(4-sulfophenyl) amino] methyl benzenesulfonic acid
   Not more than 0.3%
   See description under TESTS

   Leuco base:
   Not more than 5%
   See description under TESTS

---


Organic compounds other than colouring matters (continued)

* Unsulfonated primary aromatic amines
  Not more than 0.01% calculated as aniline

* Ether extractable matter
  Not more than 0.2%

TESTS

PURITY TESTS

* subsidiary colouring matters
  Use the following conditions:
  Developing solvent: No. 4
  Develop chromatogram for approximately 20 hours

* Organic compounds other than colouring matters
  Proceed as directed under Column Chromatography
  The following absorptivities may be used:
  - 3-formylbenzenesulfonic acid: 0.0495 mg/L/cm at 246 nm in dilute HCL
  - 3-[[N-ethyl-N-(4-sulfophenyl)amino] methyl] benzenesulfonic acid: 0.078 mg/L/cm at 277 nm in dilute ammonia.

* Leuco base
  Weigh accurately 120 ± 5 mg of sample and proceed as directed under "Determination of Leuco Base."
  Absorptivity (a) = 0.164 mg/L/cm at approximately 630 nm
  Ratio = 0.9706

METHOD OF ASSAY

Determination of Total Colouring Matters by Titration with Titanous Chloride*

Use the following:

  Weight of sample: 1.8 - 1.9 g
  Buffer: 15 g sodium hydrogen tartrate
  Weight (D) of colouring matters equivalent to 1.00 ml of 0.1 N TiCl₅: 39.65 mg

SYNONYM
INS No.1101(iii)

SOURCES
Commercial preparations of Bromelain are a purified proteolytic substances derived from Ananas comosus and Ananas bracteatus (L).

ACTIVE PRINCIPLE
Bromelain (cystein proteinase)

SYSTEMATIC NAMES AND NUMBERS
None - EC 3.4.22.4

REACTIONS CATALYZED
The enzyme hydrolyzes polypeptides, amides and esters, especially at linkages involving basic amino acids, or leucine or glycine, yielding peptides of lower molecular weight.

DESCRIPTION
The enzyme preparations occur as a white to light tan amorphous powder. They are soluble in water, the solutions being colourless to light yellow and somewhat opalescent. They are practically insoluble in alcohol, chloroform and ether.

FUNCTIONAL USES
Chillproofing of beer, tenderizing of meat, preparation of precooked cereals, and production of protein hydrolysates.

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"**

CHARACTERISTICS

IDENTIFICATION TESTS
Bromelain activity The sample shows plant proteolytic activity***

** These specifications were prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this Compendium.

BROMINATED VEGETABLE OILS*

SYNONYM
Brominated oils

DEFINITION
A bromine addition product of vegetable oil or oils

DESCRIPTION
A pale yellow to dark brown, viscous oily liquid having a bland or fruity odour and a bland taste

FUNCTIONAL USE
Cloud producing agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water and in ethanol
Soluble in chloroform, ethanol and in hexane

B. Positive test for bromide
Passes test
See description under TESTS

C. Positive test for bromide
Passes test
See description under TESTS

TESTS

IDENTIFICATION TESTS

To 0.2 g of the sample, add 1 g of anhydrous sodium carbonate, and heat gently to carbonize. Cool, add 5 ml of water, stir and filter. Acidify slightly the filtrate with dilute nitric acid, heat in a water bath for 5 min, and cool. The solution responds to the tests mentioned below:

B. Positive test for bromide
Addition of silver nitrate TS yields a light yellow precipitate which is insoluble in dilute nitric acid or ammonia TS. Separate the precipitate. Add strong ammonia TS to the precipitate and shake. The separated liquid when acidified with dilute nitric acid, yields white turbidity.

C. Positive test for bromide
Addition of chlorine TS yields a yellow to reddish brown colour. When a portion of the resultant solution is shaken with chloroform or carbon disulfide, the lower layer produces yellow to reddish brown colour. Another portion of the resultant solution yields a white precipitate with phenol.

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* These specifications were prepared at the 14th session of JECFA (1970) and published in NMRS 48B (1971).

SYNONYMS

DEFINITION

Brown FK consists essentially of a mixture of

I. Sodium 4-(2,4-diaminophenylazo) benzenesulfonate

II. Sodium 4-(4,6-diamino-m-tolylazo) benzenesulfonate

III. Disodium 4,4'-(4,6-diamino-1,3-phenylenebisazo)- di(benzenesulfonate)

IV. Disodium 4,4'-(2,4-diamino-1,3-phenylenebisazo)- di(benzenesulfonate)

V. Disodium 4,4'-(2,4-diamino-5-methyl-1,3-phenylenebisazo)di(benzenesulfonate)

VI. Trisodium 4,4',4'''-(2,4-diaminobenzene-1,3,5-trisazo)tri-(benzenesulfonate)

and subsidiary colouring matters together with water, sodium chloride and/or sodium sulfate as the principal uncoloured components.

This product as manufactured, and to which these specifications apply, is often diluted with sodium chloride to a total colouring matters content of about 50% to meet the needs of users.

Class

Azo (a mixture of mono-, bis- and trisazo colours).

Code numbers

CAS No. 8062-14-4

Chemical name

A mixture of

I. Sodium 4-(2,4-diaminophenylazo) benzenesulfonate

II. Sodium 4-(4,6-diamino-m-tolylazo) benzenesulfonate

III. Disodium 4,4'-(4,6-diamino-1,3-phenylenebisazo)- di(benzenesulfonate)

IV. Disodium 4,4'-(2,4-diamino-1,3-phenylenebisazo)- di(benzenesulfonate)

V. Disodium 4,4'-(2,4-diamino-5-methyl-1,3-phenylenebisazo)di(benzenesulfonate)

VI. Trisodium 4,4',4'''-(2,4-diaminobenzene-1,3,5-trisazo)tri-(benzene-sulfonate)

* These specifications were prepared at the 30th session of JECFA (1986) and published in FNP 37 (1986).

Chemical formula

I  $C_{12}H_{11}N_4NaO_3S$
II $C_{13}H_{13}N_4NaO_3S$
III $C_{14}H_{14}N_6Na_2O_3S_2$
IV $C_{15}H_{15}N_6Na_2O_3S_2$
V $C_{16}H_{16}N_6Na_2O_3S_2$
VI $C_{24}H_{17}N_6Na_3O_3S_3$

Structural formula

I

II

III
Structural formula (continued)

Molecular weight

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>314.30</td>
<td>328.33</td>
<td>520.46</td>
<td>520.46</td>
<td>534.47</td>
<td>726.59</td>
</tr>
</tbody>
</table>
Assay  
Content not less than 70% total colouring matters.
Of the total colouring matters present the proportions of the components shall not exceed:

I 26%  
II 17%  
III 17%  
IV 16%  
V 20%  
VI 16%

DESCRIPTION  
Red-brown powder or granules

FUNCTIONAL USE  
Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility  
Soluble in water. Sparingly soluble in ethanol

** B. Identification of colouring matters  
Passes test

PURITY TESTS

** Loss on drying at 135°  
Not more than 30%

** Chloride and sulfate calculated as sodium salts  

** Water insoluble matter  
Not more than 0.2%

*** Arsenic  
Not more than 3 mg/kg

*** Lead  
Not more than 10 mg/kg

* Heavy metals  
Not more than 40 mg/kg
Proceed as directed in the Limit Test for Heavy Metals

Subsidiary colouring  
Not more than 3.5%
See description under METHOD OF ASSAY

Organic compounds other than colouring matters

4-Aminobenzene-1-sulfonic acid  
Not more than 0.7%
See description under TESTS

m-Phenylenediamine and  
4-methyl-m-phenylenediamine  
Not more than 0.35%
See description under TESTS


Organic compounds other than colouring matters (continued)

* Unsulfonated primary aromatic amines other than m-phenylene diamine and 4-methyl-m-phenylene diamine
  Not more than 0.007%
  calculated as aniline

* Ether extractable matter
  Not more than 0.2%

TESTS

PURITY TESTS

Organic compounds other than colouring matters

Determine chromatographically using the following conditions:

Instrument
High performance liquid chromatograph fitted with a gradient elution accessory

Detector: A UV HPLC detector recording absorbances at 254 nm

Column: 250 x 4 mm Li Chromosorb RP8, 7 μm

Solvent system
- A: 0.075 M sodium acetate solution adjusted to pH 6.0 using glacial acetic acid.
- B: A: methanol (2:3)

Gradient:

<table>
<thead>
<tr>
<th>Minutes</th>
<th>% (a)</th>
<th>% (b)</th>
<th>Flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

METHOD OF ASSAY

Determination of the amount of component colouring matters and subsidiary colouring matters.

Apparatus

- Glass tank capable of holding 20 cm x 20 cm glass TLC plates.
- Micrometer syringe capable of delivering 0.10 ml with a tolerance of ± 0.002 ml
- Spectrophotometer

METHOD OF ASSAY
(continued)

Reagents: All of recognized analytical grade
- Chromatography solvent: A mixture of 4 parts phenol and 1 part water by volume
- Diethyl ether
- Kieselgel G
- Extraction solvent: Mix 100 ml 10% sodium carbonate solution with 500 ml methanol and dilute to 1 L with water

Procedure
Prepare a 20 x 20 cm TLC plate with a 0.5 mm thick coating of Kieselgel G. Using the micrometer syringe apply a solution containing 0.4 mg Brown FK as evenly as possible in an area near the bottom of the plate. Develop the chromatogram in the phenol/water mixture allowing the solvent to ascend the full height of the plate; then remove it from the tank and allow it to dry.

The diagram shows a typical Brown FK chromatogram with the bands numerically identified.

Remove each band from the plate and transfer it to a small beaker. The subsidiary colouring matters are located in the area between bands I and V. Remove this area of Kieselgel G. Transfer it to a beaker and wash it with a small quantity of ether to remove the phenol. Allow the residual ether to evaporate.

Add 10 ml of extraction solvent to each beaker and swirl to extract the colour. Filter through a small filter paper and measure the absorbance versus extraction solvent in 10 mm cells at the wavelength of maximum absorption. Calculate the concentration of each component colouring matter and of the subsidiary colouring matters using the absorptivity figures given in the table below.
METHOD OF ASSAY (continued)

<table>
<thead>
<tr>
<th>Component</th>
<th>Wavelength (nm)</th>
<th>Absorptivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>453</td>
<td>55.1</td>
</tr>
<tr>
<td>II</td>
<td>464</td>
<td>59.6</td>
</tr>
<tr>
<td>III</td>
<td>355</td>
<td>88.2</td>
</tr>
<tr>
<td>IV</td>
<td>412</td>
<td>70.5</td>
</tr>
<tr>
<td>V</td>
<td>448</td>
<td>60.3</td>
</tr>
<tr>
<td>VI</td>
<td>410</td>
<td>59.7</td>
</tr>
<tr>
<td>Subsidiary Colouring</td>
<td>425</td>
<td>74.2</td>
</tr>
</tbody>
</table>

Calculation of
(i) Total colouring matters content
(ii) Percentages of component colours
(iii) Percentage of subsidiary colours

Note: Component colours are expressed as percentages of the total colouring matters present, i.e. as percentages of the sum of component colours and subsidiary colours. Subsidiary colours are expressed for the purposes of the specification limit as a percentage of the sample.

Let the absorptivities of the component colours be $a_1$, $a_2$,...,$a_6$, and the absorbances of the extracts of the component colours be $A_1$, $A_2$,...,$A_6$.

Let the Absorptivity of the subsidiary colours be $a_7$ and the absorbance of the extract of the subsidiary colours be $A_7$.

The weight (in mg) of component colour I in the 10 ml extract is calculated from the expression:

$$\frac{A_1}{a_1} \times 10 \text{ mg} = W_1$$

In a similar manner calculate the weights ($W_2$, $W_3$,...,$W_7$) of the remaining component colours and the subsidiary colours.

(i) Total colouring matters content ($\%$) =

$$\frac{W_1 + W_2 + W_3 + W_4 + W_5 + W_6 + W_7}{0.4} \times 100\%$$

(ii) The percentage of component colour 1 =

$$\frac{W_1}{W_1 + W_2 + W_3 + W_4 + W_5 + W_6 + W_7} \times 100\%$$

In a similar manner calculate the percentages of the other component colours.

(iii) The percentage of subsidiary colours =

$$\frac{W_7}{0.4} \times 100\%$$
BROWN HT*

SYNONYMS
CI Food Brown 3, Chocolate brown HT
INS No. 155, EEC No. E155

DEFINITION
Brown HT consists essentially of disodium 4,4’-(2,4-dihydroxy-5-hydroxymethyl-1,3-phenylene-bisazo) di-1-naphthalenesulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Brown HT may be converted to the corresponding aluminium lake in which case only the General Specifications for Aluminium Lakes of Colouring Matters shall apply.**

Class
Bisazo

Code numbers
CI (1975) No. 20285
CAS No. 4553-89-3

Chemical name
Disodium 4,4’-(2,4-dihydroxy-5-hydroxymethyl-1,3-phenylene-bisazo) di-1-naphthalenesulfonate

Chemical formula
C_{27}H_{18}N_{2}Na_{2}O_{4}S_{2}

Molecular weight
652.57

Assay
Content not less than 70% total colouring matters

DESCRIPTION
Brown powder or granules

FUNCTIONAL USE
Food colour

---

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

** See Annex 2 at the end of this Compendium.
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
   Soluble in water. Insoluble in ethanol

** B. Identification of colouring matters
   Passes test

PURITY TESTS

** Loss on drying at 135°
   Not more than 30%

** Chloride and sulfate calculated as sodium salts
   Not more than 0.2%

** Water insoluble matter
   Not more than 0.2%

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Proceed as directed in the Limit Test for Heavy Metals

Subsidiary colouring matters
   Not more than 10%
   See description under TESTS

Organic compounds other than colouring matters

4-aminonaphthalene-1-sulfonic acid
   Not more than 0.7%
   See description under TESTS

** Unsulfonated primary aromatic amines
   Not more than 0.01% calculated as aniline

** Ether extractable matter
   Not more than 0.2%

---


TESTS

PURITY TESTS

* Subsidiary colouring matters

Use the following conditions:

Prepare the standard in the following manner:

Dilute 1.0 ml of the 1% dye solution to 100 ml with water and mix well. Transfer 0.10 ml of this solution to a test tube; add 5.0 ml of water: acetone (1:1 by vol.) and then 14.9 ml of 0.05 N sodium hydrogen carbonate solution and shake the tube to ensure mixing. Determine the net absorbance ($A_s$) of the standard.

Developing solvent: No.6
Develop chromatogram for approximately 14 h

* Organic compounds other than colouring matters

Use HPLC under the following conditions:

HPLC elution gradient: 1 to 100% at 2.0% per min (exponential)

METHOD OF ASSAY

Determination of Total Colouring Matters by Spectrophotometry

Use the following conditions:

Solvent: pH 7 phosphate buffer
Dilution of solution A: 10 ml to 250 ml
Absorptivity ($a$): 40.3
Approximate wavelength of maximum absorption: 460 nm

BUTAN-1-OL*

SYNONYMS
Butyl alcohol, n-butyl alcohol, 1-hydroxybutane, n-butanol, n-propyl carbinol, NBA

DEFINITION
Chemical names 1-Butanol, butan-1-ol
C.A.S. number 76-36-3
Chemical formula C₆H₁₄O
Structural formula CH₃ — CH₂ — CH₂ — CH₂ — OH
Molecular weight 74.12
Assay Not less than 99.5%

DESCRIPTION
Colourless, clear, slightly viscous, with a characteristic odour

FUNCTIONAL USES
Extraction solvent, flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Soluble in water. Miscible with ethanol and ether.
* B. Specific gravity 0.810 - 0.812

PURITY TESTS

** Distillation range 116° - 118°. Warning: Check for peroxides**
** Non-volatile residue Not more than 2 mg/100 ml
** Water-content Not more than 0.1% (Karl Fischer Method)
** Arsenic Not more than 3 mg/kg (Method II)
** Heavy metals Not more than 10 mg/kg
See description under TESTS

Acidity Not more than 0.003% w/w (as acetic acid)
See description under TESTS

Aldehydes and ketones (as butanal) Not more than 0.2% w/w
See description under TESTS

* This specification was prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984).

PURITY TESTS (continued)

*Other alcohols, ethers, and volatile impurities*  
Not more than 0.5%, with not more than 0.1% of any single impurity.  
See Method of Assay.

TESTS

**PURITY TESTS**

- **Heavy metals**  
  Evaporate 2 g of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid TS, and slowly evaporate to dryness again on a steam bath. Moisten the residue with 1 drop of hydrochloric acid TS, add 10 ml of hot water and digest for 2 min. Cool and dilute to 25 ml with water. Test the solution as directed in the Limit Test (Method II).

- **Acidity**  
  To 60 g of the sample add a few drops of phenolphthalein TS, and titrate with 0.1 N ethanolic potassium hydroxide to a pink end-point which persists for at least 15 sec. Not more than 0.3 ml is required.

- **Aldehyde and ketones**  
  (as butanal)  
  Proceed as directed under Determination of Aldehydes and Ketones in the General Methods, using 10 g of the sample and 36.06 as the equivalence factor (e) in the calculation.

**METHOD OF ASSAY**  

Using the procedures for gas chromatography described in the General Methods*, establish the following conditions:

- Column: 1.8 m length, 6 mm diameter steel column packed with 10% P.E.G. 400 on Chromosorb W (60/80 mesh), or equivalent.
- Carrier gas: Helium, at flow rate of 45 ml/min
- Detector: Flame ionization type
- Temperatures: Injection port, 150°
- Column: 90°
- Detector: 150°

Inject 1 to 5 μl of sample, obtain chromatogram, and determine the content of each constituent by the method of area normalization.

---

BUTAN-2-OL

SYNONYMS
Secondary butyl alcohol, 2-hydroxybutane

DEFINITION

Chemical names
2-Butanol, butan-2-ol

C.A.S. number
78-92-2

Chemical formula
C₆H₁₂O

Structural formula
\[
\begin{array}{c}
\text{CH}_3 \\
\text{OH}
\end{array}
\]

Molecular weight
74.12

DESCRIPTION
Colourless, clear, slightly viscous, flammable liquid, with a characteristic odour

FUNCTIONAL USES
Extraction solvent, flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water. Miscible with ethanol and ether

** B. Specific gravity
0.806 - 0.809

PURITY TESTS

** Distillation range
98.5° - 100.5°

** Non-volatile residue
Not more than 2 mg/100 ml

** Water content
Not more than 0.2% w/w (Karl Fischer)

** Arsenic
Not more than 3 mg/kg (Method II)

** Heavy metals
Not more than 10 mg/kg
See description under TESTS

Acidity
Not more than 0.003% w/w (as acetic acid)
See description under TESTS

Aldehydes and ketones
Not more than 0.3% w/w
See description under TESTS

---

* This specification was prepared at the 25th session of JECFA (1981) and published in FNP 19 (1981).

TESTS

PURITY TESTS

* Heavy metals

Evaporate 2 g of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid TS, and slowly evaporate to dryness again on a steam bath. Moisten the residue with 1 drop of hydrochloric acid TS, add 10 ml of hot water and digest for 2 min. Cool, and dilute to 25 ml with water. Test the solution as directed in the Limit Test (Method II).

Acidity

To 60 g of the sample add a few drops of phenolphthalein TS, and titrate with 0.1 N ethanolic potassium hydroxide to a pink end-point which persists for at least 15 sec. Not more than 0.3 ml is required.

* Aldehydes and ketones
  (as butanal)

Proceed as directed under Determination of Aldehydes and Ketones in the General Methods, using 10 g of the sample and 36.06 as the equivalence factor (e) in the calculation.

**BUTAN-1,3-DIOL**

**SYNONYMS**

1,3-Butylene glycol, 8-butylene glycol

**DEFINITION**

Chemical name: Butan-1,3-diol  
C.A.S. number: 107-88-0  
Chemical formula: \( \text{C}_4\text{H}_8\text{O}_2 \)  
Structural formula: \( \text{CH}_2 - \text{CH} \rightarrow \text{CH}_2\text{CH}_2-\text{OH} \rightarrow \text{OH} \)  
Molecular weight: 90.12  
Assay: Content not less than 99% w/w

**DESCRIPTION**

Clear colourless, odourless, hygroscopic, viscous liquid having a slight characteristic taste

**FUNCTIONAL USE**

Carrier solvent

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

** A. Solubility**  
Miscible with water, acetone and ether  
Soluble in fixed oils, ethanol, ether

** B. Specific gravity**  
1.004 - 1.006

**PURITY TESTS**

** Distillation range**  
200-215°

** Water content**  
Not more than 0.5% w/w (Karl Fischer Method)

** Colour**  
Not more than Colour Standard No. 10

---

* These specifications were prepared at the 23rd session of JECFA (1979) and published in FNP 12 (1979).

PURITY TESTS (continued)

* Arsenic: Not more than 3 mg/kg (Method II)

* Heavy metals: Not more than 10 mg/kg
  Test 2 g of the sample as directed in the Limit Test (Method II)

METHOD OF ASSAY

Weigh accurately about 0.2 g of the sample, proceed as directed under the method for Hydroxyl Value in General Methods*, and calculate the content by the formula:

\[
\text{Butan-1,3-diol \% w/w} = \frac{(B + (WA/C) - S) \times N \times 4.506}{w}
\]

BUTYL ACETATE*

SYNONYMS

Acetic acid butyl ester, butyl ethanoate

DEFINITION

Chemical name
Butyl acetate
C.A.S. number
123-86-4
Chemical formula
C₆H₁₂O₂
Structural formula
CH₃COOCH₂CH₂CH₂CH₃
Molecular weight
116.16
Assay
Not less than 95% of C₆H₁₂O₂

DESCRIPTION
Colourless mobile liquid having fruity odour

FUNCTIONAL USE
Flavouring agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Refractive index
n³₀ : 1.3920 - 1.400

** B. Specific gravity
d³₀ : 0.880 - 0.887;  d₃₅ : 0.876 - 0.883

PURITY TEST

** Acid value
Not more than 1

METHOD OF ASSAY

Weigh accurately about 1 g and proceed as directed under the method for Ester Determination in the General Methods**, using 58.08 as the equivalence factor (e) in the calculation.

---

* These specifications were prepared at the 11th session of JECFA (1967) and published in NMRS 44B (1969).

**BUTYL p-HYDROXYBENZOATE**

**SYNONYMS**
Butylparaben, butyl p-oxybenzoate

**DEFINITION**
Chemical names: Butyl p-hydroxybenzoate, n-butyl ester of p-hydroxybenzoic acid.

C.A.S. number: 94-26-8

Chemical formula: C₁₁H₁₄O₃

Structural formula:

\[
\text{HO-} \quad \text{COOCH₂CH₂CH₂CH₃}
\]

Molecular weight: 194.23

**Assay**
Butyl p-Hydroxybenzoate, when dried for 5 h over silica gel, contains not less than 99.0% of C₁₁H₁₄O₃.

**DESCRIPTION**
Almost odourless, small, colourless crystals or white, crystalline powder.

**FUNCTIONAL USE**
Antimicrobial preservative.

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**
Slightly soluble in water. Soluble in ethanol, ether, acetone and propylene glycol. Slightly soluble in glycerol.

**B. Melting range**
69-72°

C. Positive test for p-hydroxybenzoic acid
Passest test
See description under TESTS

D. Positive test for phenol
Passest test
See description under TESTS

---

* These specifications were prepared at the 17th Session of JECFA (1974) and published in FNP 4 (1978)

PURITY TESTS

* Loss on drying
Not more than 0.5% after drying for 5 h over silica gel.

* Sulfated ash
Not more than 0.1%

Acidity
Passes test
See description under TESTS

* Arsenic
Not more than 3 mg/kg.
Proceed as directed in the specifications for Benzoic Acid

* Heavy metals
Not more than 10 mg/kg.
Test 2 g of the sample as directed in the Limit Test (Method II)

p-Hydroxybenzoic acid and salicylic acid
Passes test
See description under TESTS

TESTS

IDENTIFICATION TESTS

C. Positive test for p-hydroxybenzoic acid
To 0.5 g of the sample add 10 ml of sodium hydroxide TS. Boil for 30 min and concentrate to about 5 ml. Cool, acidify with dilute sulfuric acid, collect the precipitate on a filter, and wash thoroughly with water. Dry at 105° for 1 h. The p-hydroxybenzoic acid so obtained melts between 213 and 217°.

D. Positive test for phenol
To 10 mg of the sample add 5 ml of water and 5 drops mercuric nitrate TS, and heat in a water bath for 2 min. Cool, and add 1 drop of dilute sulfuric acid and 1 drop of sodium nitrite TS. Heat again in water bath for 2 min. A red colour should be produced.

PURITY TESTS

Acidity
Heat 750 mg of the sample with 15 ml of water at 80° for 1 min, cool, and filter. The filtrate should be acid or neutral to litmus. To 10 ml of the filtrate add 0.2 ml of 0.1 N sodium hydroxide and 2 drops of methyl red TS. A yellow colour should be produced.

p-Hydroxybenzoic acid and salicylic acid
Dissolve 0.5 g of the sample in 30 ml of ether, add 20 ml of a 1 in 100 sodium hydrogen carbonate solution, shake, and separate the water layer. Wash the water layer with two 20 ml portions of ether, add 5 ml of dilute sulfuric acid and 30 ml of ether, and shake. Separate the ether layer, and shake with about 10 ml of water. Filter the ether layer, and wash the vessel and the filter with a small amount of ether. Combine the washings and the filtrate, evaporate ether on a water bath, and dry the residue over sulfuric acid to constant weight. The weight of the residue should not exceed 5 mg. Dissolve any residue in 25 ml of water, heat to about 70°, filter, and add a few drops of dilute ferric chloride TS. No violet to reddish violet colour should be produced.

METHOD OF ASSAY

Neutralization titration

Transfer about 2 g of the sample, accurately weighed and previously dried for 5 h over silica gel, into a flask. Add 40.0 ml of 1 N sodium hydroxide, and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h, and cool. Add 5 drops of bromothymol blue TS, and titrate the excess sodium hydroxide with 1 N sulfuric acid, matching the colour to a buffer solution (pH 6.5) containing the same proportion of indicator. Perform a blank determination with the reagents and make necessary correction. Each ml of 1 N sodium hydroxide is equivalent to 194.2 mg of C_{11}H_{11}O_{3}. 
**BUTYLATED HYDROXYANISOLE**

**SYNONYMS**

BHA; INS No.320, EEC No.320

**DEFINITION**

Chemical names  3-Tertiary-butyl-4-hydroxyanisole, a mixture of 3- and 2-tertiary-butyl-4-hydroxyanisole

C.A.S. number  25013-16-5

Chemical formula  C_{11}H_{16}O_{2}

**DESCRIPTION**

White or slightly yellow crystals or waxy solid, with a faint characteristic odour

**FUNCTIONAL USE**

Antioxidant

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

**A. Solubility**

Insoluble in water

Freely soluble in ethanol and propane-1,2-diol

**B. Colour reaction**

Passes test

See description under TESTS

**PURITY TESTS**

**Sulfated ash**

Not more than 0.05%

Proceed as directed under the test of Ash (Sulfated ash, Method I) using 5 g of the sample.

---

* These specifications were prepared at the 33rd session of JECFA (1988) and published in FNP 38 (1988).

PURITY TESTS (continued)

* Arsenic
Not more than 3 mg/kg (Method II)

* Heavy metals
Not more than 10 mg/kg
Test 2 g of the sample as directed under in the General Methods (Method II).

Phenolic impurities
Not more than 0.5%
See procedure under TESTS

TESTS

IDENTIFICATION TESTS

B. Colour reaction
To 5 ml of a 1 in 10,000 solution of the sample in 72% ethanol, add 2 ml of sodium borate TS and 1 ml of a 1 in 10,000 solution of 2,6-dichloroquinonechlorimide in absolute ethanol, and mix. A blue colour appears.

Determine by Thin-Layer Chromatography*, using silica gel G plates.

- Solution 1: Dissolve 0.25 g of the sample in 10 ml of ether.

- Solution 2: Dilute 1 ml of Solution 1 to 10 ml with ether, and then dilute 1 ml of the resulting solution to 20 ml with ether. Use the final dilution as solution 2.

Procedure

Spot 2 µl each of Solution 1 and of Solution 2 on separate TLC plates. Place each plate in a developing chamber containing chloroform as solvent, and allow the solvent front to ascend to a point 15 cm above the sample spots. Develop the chromatograms by spraying with an aqueous mixture of equal volumes of 2% ferric chloride solution and 1% potassium ferricyanide solution mixed prior to use. The blue colours produced may be intensified by spraying with 2N hydrochloric acid.

Chromatogram 1 Any blue spots appearing (other than the major spot and the spot at Rf 0.35) are not more intense than the major spot appearing on Chromatogram 2.

METHOD OF ASSAY

Internal standard solution*

Accurately weight 500 mg, dissolve in acetone and make up to 250 ml with acetone. Standard solution: Accurately weight 90 mg of 3-butylated hydroxyanisole and 10 mg of 2-butylated hydroxyanisole and dissolve in Internal standard solution to make 100 ml.

Procedure

Dissolve 10 mg of the sample, accurately weighed, in the Internal standard solution to make 50 ml. Inject aliquots of the solution into a gas chromatograph equipped with a hydrogen flame ionization detector. Either of the following GC conditions or equivalent may be used:

A: The internal standard elutes after 3-tert-butyl-4-hydroxyanisole:

Column: 2 mm i.d. x 1.5 m Glass Column
Packing: 10% XE-60 on 100-200 mesh
Column Temp.: 155°
Injector Temp.: 250°
Detector Temp.: 250°
Carrier Gas: N₂, 30 ml/min

B: The internal standard elutes before 3-tert-butyl-4-hydroxyanisole:

Column: 3 mm i.d. x 2 m Glass Column
Packing: 5% Versamide-900 on 80/100 mesh Chromosorb W-AW-DMCS
Column Temp.: 170°
Injection Temp.: 225°
Detector Temp.: 250°
Carrier Gas: N₂, 30 ml/min

A standard curve of 2- and 3-butylated hydroxyanisole peak height/internal standard peak height versus concentration is prepared by using internal standard solutions having various concentrations of butylated hydroxyanisole. The concentrations of 2- and 3-butylated hydroxyanisole are determined by reference to a standard curve. The sum of per cent 2-isomer and per cent 3-isomer gives per cent of total in the sample.

Assay preparation

Dissolve about 100 mg of the sample, accurately weighed, in Internal standard solution, and dilute with Internal standard solution to 10 ml.

* Select either diphenylamine or 4-tertiary-butylphenol.
METHOD OF ASSAY
(cont'd)

Chromatographic system

The gas chromatograph is equipped with a flame-ionization detector, and contains a 1.8-m x 2-mm stainless steel column packed with 10 percent liquid phase on the support, the column is maintained isothermally at a temperature between 175° and 185°, and helium is used as the carrier gas. Chromatograph a sufficient number of injections of the Standard preparation, and record the areas as directed under Procedure, to ensure that the relative standard deviation does not exceed 2.0% for the 3-tert-butyl-4-hydroxyanisole isomer and 6.0% for the 2-tert-butyl-4-hydroxyanisole isomer. The resolution between the isomers is not less than 1.3 and the tailing factor does not exceed 2.0.

Liquid phase
25% 2-cyanoethyl : 75% methyl-polysiloxane.

Support

Siliceous earth for chromatography has been fluxcalcined by mixing diatomite with Na₂CO₃ flux and calcining above 900°. The siliceous earth is acid-washed, then water-washed until neutral, but not base-washed. The siliceous earth is silanized by treating with an agent such as dimethyldichlorosilane to mask the surface silanol group.

Procedure

Separately inject suitable portions (about 5 μl) of the Standard preparation and the Assay preparation into the gas chromatograph, and record the chromatograms. Measure the areas under the peaks for each isomer and the internal standard in each chromatogram, and calculate the quantity, in mg, of each isomer in the sample taken by the formula 10C₅(Rₜ/Rₛ), in which C₅ is the concentration, in mg, of the isomer in the Standard solution, Rₜ is the ratio of the area of each isomer standard to that of the Internal standard in the chromatogram from the Standard preparation, and Rₛ is the ratio of the area of each isomer to that of the internal standard in the chromatogram from the Assay preparation. Calculate the weight, in mg, of C₁₁H₁₆O₂ in the sample taken by adding the quantities of the two isomers.
BUTYLATED HYDROXYTOLUENE*

SYNONYMS
BHT; INS No.321, EEC No.E321

DEFINITION

- Chemical names: 2,6-Ditertiary-butyl-p-cresol, 4-methyl-2,6-ditertiary-butyl-phenol
- C.A.S. number: 128-37-0
- Chemical formula: C_{15}H_{20}O
- Structural formula:

```
   OH
  /   \(CH_3)\_3C\_C(CH_3)\_3
 /   /
C(CH_3)\_3
 /  \
CH_3
```

- Molecular weight: 220.36
- Assay: Content not less than 99.0%

DESCRIPTION
White, crystalline or flaked solid, odourless or having a characteristic faint aromatic odour.

FUNCTIONAL USE
Antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water and propane-1,2-diol.
Freely soluble in ethanol.

** B. Melting range
Between 69° and 72°

D. Absorbance maximum
The absorption in the range 230 to 320 nm of a 2 cm layer of a 1 in 100,000 solution in dehydrated ethanol exhibits a maximum only at 278 nm.

C. Colour reaction
Passes test
See description under TESTS

* These specifications were prepared at the 37th session of JECFA (1990) superseding the earlier specifications published in FNP 37 (1986).

PURITY TESTS

- **Solidification**
  The solidification point determined as directed in General Methods is not lower than 69.2°C.

- **Sulfated ash**
  Not more than 0.005%.
  Test 20 g of the sample as directed in the Limit Test for Ash (Method I).

- **Arsenic**
  Not more than 3 mg/kg (Method II)

- **Heavy metals**
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in the Limit Test (Method II).

**Phenolic impurities**
Not more than 0.5%
See description under TESTS

TESTS

IDENTIFICATION TESTS

D. Colour reaction
To 10 ml of a 1 in 100,000 solution of the sample in methanol add 10 ml of water, 2 ml of sodium nitrite solution (3 in 1000) and 5 ml of dianisidine dihydrochloride solution (200 mg of 3,3-dimethoxy-benzidine dihydrochloride dissolved in a mixture of 40 ml of methanol and 60 ml of 1 N hydrochloric acid). An orange red colour develops within 3 min. Add 5 ml of chloroform, and shake. The chloroform layer exhibits a purple or magenta colour that fades when exposed to light.

PURITY TESTS

**Phenolic impurities**
Determine by Thin-Layer Chromatography*, using silica gel G plates.

- **Solution 1**: Dissolve 0.25 g of the sample in 10 ml of ether.
- **Solution 2**: Dilute 1 ml of Solution 1 to 10 ml with ether, and then dilute 1 ml of the resulting solution to 20 ml with ether. Use the final dilution as Solution 2.

Spot 2 µl each of Solution 1 and of Solution 2 on separate TLC plates. Place each plate in a developing chamber containing chloroform as solvent, and allow the solvent front to ascend to point 15 cm above the sample spots. Develop the chromatograms by spraying with an aqueous mixture of equal volumes of 2% ferric chloride solution and 1% potassium ferrocyanide solution mixed prior to use. The blue colours produced may be intensified by spraying with 2N hydrochloric acid. Any blue spots appearing on Chromatogram 1 (other than the major spot) are not more intense than the major spot appearing on Chromatogram 2.

METHOD OF ASSAY

Gas Chromatography Method

**Internal standard solution**
Accurately weigh 500 mg, dissolve in acetone and make up to 250 ml with acetone.

**Standard solution**
Accurately weigh 100 mg of butylated hydroxytoluene and dissolve in acetone to make 50 ml.

**Procedure**
Dissolve 10 mg of the sample, accurately weighed, in the internal standard solution to make 50 ml. Inject aliquots of the solution into a gas chromatograph equipped with a hydrogen flame ionization detector. The glass column (1.5 m x 3 mm i.d.) is prepared by packing with 10% XE-60 on 100-200 mesh Aeropak 30 (Varian Aerograph), or equivalent, and maintained isothermally at 155°. The injector and detector temperatures are 225° and 250° respectively. The nitrogen carrier-gas flow rate is 30 ml/min.

A standard curve of butylated hydroxytoluene peak height/ internal standard peak height versus concentration is prepared by using internal standard solutions having various concentration of butylated hydroxytoluene. The concentrations of butylated hydroxytoluene is determined by reference to a standard curve.

* Select either diphenylamine or 4-tertiary butylphenol.
CALCIUM ACETATE*

SYNONYMS
INS No. 263, EEC No. E263

DEFINITION

Chemical name
Calcium acetate

C.A.S. number
62-54-4

Chemical formula
Anhydrous: \( \text{C}_4\text{H}_6\text{CaO}_4 \); Monohydrate: \( \text{C}_4\text{H}_6\text{CaO}_4 \cdot \text{H}_2\text{O} \);
\( \text{C}_4\text{H}_6\text{CaO}_4 \cdot x\text{H}_2\text{O} \quad (x < 1) \)

Structural formula
\[
\begin{align*}
\text{CH}_3\text{COO} \\
\mid \\
\text{Ca} \\
\mid \\
\text{CH}_3\text{COO}
\end{align*}
\]

Molecular weight
Anhydrous: 158.17; Monohydrate: 176.18

Assay
After drying at 155°, to constant weight, contains no less than 98% of \( \text{C}_4\text{H}_6\text{CaO}_4 \).

DESCRIPTION
Anhydrous Calcium Acetate is a white, hygroscopic, bulky, crystalline solid with a slightly bitter taste. A slight odour of acetic acid may be present. The monohydrate may be needles, granules or powder.

FUNCTIONAL USES
Antimold and antirope agent, stabilizer, buffer.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water, insoluble in ethanol.

B. Positive test for acetate
Passes test
See description under TESTS

** C. Positive test for calcium
Add ammonium oxalate TS to a solution of the sample. A white precipitate forms that is soluble in hydrochloric acid, but insoluble in acetic acid.

PURITY TESTS

** Loss on drying
Not more than 11% after drying at 155° to constant weight (monohydrate)

---

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (cont’d)

* pH

Water insolubles

6 - 9 (1 in 10 soln).

Not more than 0.3%.

See description under TESTS

* Arsenic

Not more than 3 mg/kg.

A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* Lead

Not more than 10 mg/kg.

A sample solution prepared as directed for organic compounds meets the requirements of Limit Test for Lead. Use 10 μg of lead control.

Heavy metals

Not more than 30 mg/kg.

See description under TESTS

Formic acid and oxidizable impurities

Not more than traces.

See description under TESTS

Aldehydes

Not more than traces.

See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Positive test for acetate

1. Cover the open end of a hard glass tube containing the sample with a piece of filter paper, slightly larger than the mouth of the tube and moistened with freshly prepared alkaline o-nitrobenzaldehyde TS. Suspend the tube through an asbestos plate and heat slowly with a gas flame. The filter paper turns blue to blue-green, indicating the presence of acetate.

2. Add a 1 in 2 sulfuric acid solution to the sample and heat. Acetic acid is evolved recognizable by its odour.

PURITY TESTS

Water insolubles

Dissolve 10 g of the sample, weighed to the nearest mg, in 100 ml of hot water. Filter through a Gooch crucible, tared to an accuracy of ± 0.2 mg, and wash any residue with water. Dry the crucible for 2 h at 105°. Cool, weigh and calculate as percentage. (The weight of the dried residue should not exceed 30 mg).

* Heavy metals

A solution of 0.8 g of the sample, weighed to the nearest mg, in 25 ml of water meets the requirements of the Limit Test for Heavy Metals (Method I) using 15 μg of lead ion (Pb) and 0.3 g of the sample in the control (Solution A).

PURITY TESTS (continued)

Formic acid and oxidizable impurities
Dissolve 1 g of the sample in 5 ml of water. Add 2.5 ml of 0.1 N potassium dichromate and 6 ml of sulfuric acid and allow to stand for 1 min. Add 20 ml of water, cool to 15° and add 1 ml of potassium iodide TS. A faint yellow or brown colour should be produced immediately.

Aldehydes
Dissolve 2 g of the sample in 10 ml of water and distil. To the first 5 ml of the distillate, add 10 ml of mercuric chloride TS and make alkaline with N sodium hydroxide. Allow to stand for 5 min, and acidify with dilute sulfuric acid TS. The solution should show no more than a faint turbidity.

METHOD OF ASSAY

Calcium content
Chelatometry
Dissolve in a beaker 2.5 g of the sample, weighed to the nearest mg, in 5 ml of hot dilute hydrochloric acid TS. Cool, transfer to a 250-ml volumetric flask, dilute to volume with water, and mix. Transfer 50 ml of the solution to a 400-ml beaker, add 100 ml of water, 25 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation*, and 3 ml of naphthol green TS. Titrate with 0.05 M disodium ethylenediamine-tetracacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetracacetate is equivalent to 7.909 mg of C₄H₆CaO₄.

Acid content
Half fill a chromatographic column (1.5 cm in diameter, 20 cm long) with a strong cation-exchange resin (Amberlite IR 120, Amberlite IR 100, Duolit C III, Dowex 50, Lewatit KS, Ion Exchanger I Merck). Add 0.1 N hydrochloric acid through the top of the column, with the outflow orifice closed until the resin is completely covered and let stand 1-2 h. Drain the acid and rinse the column with water (about 1 liter) until 20 ml of eluate forms a red colour, when one drop each of 0.02 N sodium hydroxide and phenolphthalein TS is added. Weigh, to the nearest mg, 0.05 g of the sample, previously dried at 155° to constant weight, into a flask. Dissolve in 15 ml of water and pour slowly on to the column. Wash the flask and the column with about 200 ml of water and collect the total filtrate in a conical flask. Add two drops of phenolphthalein TS and titrate with 0.1 N sodium hydroxide using a microburette. Each ml of 0.1 N sodium hydroxide is equivalent to 7.909 mg of C₄H₆CaO₄.

* An alternative indicator is hydroxynaphthol blue, of which 0.25 g is used. In this case the naphthol green TS is omitted.
CALCIUM ALGINATE*

SYNONYMS

INS No. 404, EEC No. E404

DEFINITION

Calcium Alginate, the calcium salt of alginic acid, is a colloidal substance. The alginic acid is a linear high-polymer consisting mainly of β-(1→4) linked D-mannuronic acid in the pyranose ring form, with part of the mannuronic acid replaced by L-guluronic acid.

C.A.S. number

9005-35-0

Chemical formula

(C₆H₁₀Ca₄O₁₂)ₙ

Structural formula

![Structural formula of Calcium Alginate](image)

Unit of the salt of mannuronic acid

Molecular weight

Structural unit: 195.16 (theoretical)  
(219.00 actual average)  
Macromolecule: 32 000-250 000

Assay

Calcium Alginate yields, on the dried basis, not less than 18.0% and not more than 21.0% of carbon dioxide (CO₂), equivalent to not less than 89.6% and not more than 104.5% of calcium alginate.

DESCRIPTION

Calcium Alginate occurs in filamentous, grainy, granular and powdered forms. It is colourless or slightly yellow and may have a slight characteristic smell and taste.

FUNCTIONAL USES

Thickening agent and stabilizer.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Insoluble in water and ether; slightly soluble in ethanol; slowly soluble in solutions of sodium polyphosphate, sodium carbonate, and substances that combine with the calcium.

** B. Specific rotation

Clarify a 0.5% solution of the sample in sodium hydroxide TS with kieselguhr, and determine the rotation in a 20-cm tube. The specific rotation is not less than −0.8° at 20°.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

C. Precipitate formation with calcium chloride solution
   Passes test
   Proceed as directed in the same test under Alginic Acid

D. No precipitate formation with ammonium sulfate solution
   Passes test
   Proceed as directed in the same test under Alginic Acid

E. Colour reaction
   Moisten 1-5 mg of the sample with water and add 1 ml of acid ferric sulfate TS. Within 5 min, a cherry-red colour develops that finally becomes deep purple

F. Positive test for calcium
   Dissolve the sulfated ash of the sample in dilute acetic acid TS and filter. Treat the filtrate with ammonium oxalate TS. The white precipitate formed is soluble in hydrochloric acid

PURITY TESTS

* Loss on drying
  Not more than 15% (105°, 4 h)

Phosphate
  Not detectable
  Proceed as directed in the Purity Test for Phosphate under Sodium Alginate

* Total ash
  Not less than 13% and not more than 24% on the dried basis.
  Proceed as directed in the Total Ash test under Alginic Acid

* Arsenic
  Not more than 3 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic

* Lead
  Not more than 10 mg/kg
  A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Lead

* Heavy metals
  Not more than 40 mg/kg
  Test 0.5 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 µg of lead ion (Pb) in the control Solution A

METHOD OF ASSAY

Decarboxylation method
  Proceed as directed under Carbon Dioxide Determination by Decarboxylation in the General Methods.* Each ml of 0.25 N sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO₂) or 27.38 mg of calcium alginate (equivalent weight 219.00).

Gravimetric method
Dissolve 0.500 g of the sample in 10 ml of sodium hydroxide TS, add 90 ml of water, and filter if necessary. Add 15 ml of 4 N hydrochloric acid and 100 ml of 90% v/v ethanol. Allow this mixture to stand for 2 h, decant the supernatant liquid as far as possible, and centrifuge. Decant the liquid and replace it by 90% v/v ethanol. Mix well, centrifuge and decant again. This washing is repeated until the hydrochloric acid is removed. Then transfer the precipitate by mean of 90% v/v ethanol to a fine glass filter, wash with dry acetone, place the filter in a vacuum desiccator, and dry to constant weight at 100°.

Calculate the percent purity of the sample by the formula:

\[
\% \text{ Purity} = \frac{200 \times F \times W}{\% \text{ dry substance in sample}} \times 100
\]

in which \( F \) is a conversion factor specified as 1.108 and \( W \) is the weight (in grams) of the dried precipitate.
CALCIUM ALUMINIUM SILICATE* 

SYNONYMS 
Aluminium calcium silicate, calcium aluminosilicate, calcium silicoaluminate, sodium calcium silicoaluminate; INS No.556, EEC No.556 

DEFINITION 

Chemical name: Calcium aluminosilicate 
Assay: Calcium aluminium silicate contains, calculated on volatile matter free basis: 
- not less than 44% and not more than 50% of silicon dioxide (expressed as SiO₂); 
- not less than 3% and not more than 5% of aluminium oxide (expressed as Al₂O₃); 
- not less than 32% and not more than 38% of calcium oxide (expressed as CaO); 
- not less than 0.5% and not more than 4% of sodium oxide (expressed as Na₂O). 

DESCRIPTION: Fine, white, free-flowing powder. 
FUNCTIONAL USE: Anticaking agent. 
CHARACTERISTICS 
IDENTIFICATION TESTS 

** Solubility: Insoluble in water and ethanol. 
PURITY TESTS 

** Loss on ignition: Not less than 14% and not more than 18% (ignition at 1000° to constant weight). 
** Loss on drying: Not more than 10% (105°, 2 h). 
** Fluoride: Not more than 50 mg/kg 
See description under TESTS. 
** Arsenic: Not more than 3 mg/kg. 
See description under TESTS. 

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984). 
PURITY TESTS (continued)

**Lead**

Not more than 10 mg/kg.
See description under TESTS.

**Heavy metals**

Not more than 30 mg/kg.
See description under TESTS.

**TESTS**

**PURITY TESTS**

* **Fluoride**

Weigh 1 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method I or III).

* **Arsenic**

Dilute a 10-ml portion of the Sample solution (see below) to 35 ml with water and test as directed in the Limit Test (Method II).

* **Lead**

Test a 10-ml portion of the Sample solution (see below) as directed in the Limit Test.

* **Heavy metals**

Dilute a 6.7-ml portion of the Sample solution (see below) to 25 ml with water and test as directed in the Limit Test.

**Sample solution for the determination of arsenic, heavy metals and lead**

Weigh 10 g of the sample to the nearest mg, and transfer into a 250-ml flask, and add 50 ml of 0.5 N hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min, cool, and let the undissolved material settle. Decant the supernatant liquid through a Whatman No. 3 filter paper, or equivalent, into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

**METHOD OF ASSAY**

**Silicon dioxide**

Transfer 500 mg of the sample, previously dried at 105° for 2 h and weighed accurately, into a 250 ml beaker. Wash the walls of beaker with a few ml of water, then add 30 ml of 72% perchloric acid and 15 ml of hydrochloric acid. Heat on a hot-plate until dense white fumes appear. Let cool. Add 15 ml of hydrochloric acid and reheat until dense fumes appear. Let cool, add 70 ml of water, and filter through Whatman No. 40 filter paper or equivalent.

---

Silicon dioxide
(cont’d)
Wash the paper and the precipitate with hot water to remove perchloric acid. Then transfer the paper and the precipitate to a tared platinum crucible, and ignite at 900° to constant weight. Moisten the residue with a few drops of water, then add 15 ml of hydrochloric acid* and 8 drops of sulfuric acid, and heat on a hotplate until white fumes of sulfur trioxide appear. Let cool. Add 5 ml of water, 10 ml of hydrofluoric acid* and 3 drops of sulfuric acid, then evaporate to dryness on a hotplate. Carefully heat over a flame until fumes of sulfur trioxide no longer appear. Then ignite at 900° to constant weight. The weight loss after the addition of hydrofluoric acid represents the weight of SiO₂ in the sample taken.

Aluminium oxide
Fuse the residue obtained in the silicon dioxide determination with 2 g of potassium pyrosulfate for 5 min. Cool, dissolve the fusion in water, and dilute to 250 ml in a volumetric flask. Transfer 100 ml of the solution into a 600 ml beaker, add 100 ml of water and 5 drops of bromotymol blue TS, and heat to a low boil. Add ammonium hydroxide, dropwise, until a blue colour appears, then boil the solution for 5 min to expel the excess ammonia. Filter through Whatman No. 41, or equivalent filter paper, and wash the precipitate with six portions of a 1-in-50 hot ammonium chloride solution. Transfer the filtrate and precipitate into a tared platinum crucible, char the paper, and ignite over a Meker burner to constant weight. The weight of the residue, corrected for the ash content of the filter paper and multiplied by 2.5, represents the weight of Al₂O₃ in the original sample.

Calcium oxide
To the combined filtrate and washings retained in the silicon dioxide determination, add, while stirring, about 30 ml of 0.05 M disodium ethylenediaminetetraacetate from a 50-ml burette. Then add 15 ml of sodium hydroxide TS and 300 g of hydroxynaphthol blue indicator, and continue the titration to a blue end point. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 2.804 mg of CaO.

Sodium oxide
Transfer about 500 mg of the sample, previously dried at 105° for 2 h, and accurately weighed, into a tared platinum dish, and moisten with 8 to 10 drops of water. Add 25 ml of 70% perchloric acid and 10 ml of hydrofluoric acid* and heat on a hot plate in a hood until dense white fumes of perchloric acid appear. Add 10 ml of hydrofluoric acid*, heat again to dense white fumes, and dissolve the residue in sufficient water to make 250 ml. Set a suitable flame photometer to a wavelength of 589 nm. Adjust the instrument to zero transmittance against water, then adjust it to 100% transmittance with a standard solution containing 200 µg of sodium, in the form of the chloride, per ml. Read the percent transmittance of three other standard solutions containing 50, 100 and 150 µg each of sodium per ml, and plot the standard curve as % transmittance vs. concentration of sodium. Place a portion of the sample solution in the photometer, read the percent transmittance in the same manner, and by reference to the standard curve determine the concentration (C) of sodium, in µ per ml in the sample solution. Calculate the quantity, in mg, of Na₂O in the sample taken by the formula:

\[
250 \times C \times \frac{1.348}{1000} - F
\]

in which F, the quantity of sodium oxide equivalent to any sodium sulfate present in the sample, is found as follows:

* WARNING: Toxic, corrosive, must not contact skin; work under fume hood
Correction for sodium sulfate content

Weigh accurately 12.5 g of the sample, previously dried at 105° for 2 h and stir it with 240 ml of water for at least 5 min. with a high speed mixer. Transfer the mixture into a 250-ml graduated cylinder, and wash the mixer container with water, adding the washings to the cylinder to make 250 ml. Stopper the cylinder, invert it several times to mix the sample, and determine the conductivity of the slurry using a suitable conductance bridge assembly. By means of a standard curve, obtained from solutions containing 50, 100, 200 and 500 mg of sodium sulfate per 100 ml, determine the concentration (C), in mg per 100 ml, of sodium sulfate in the sample slurry, and calculate the correction factor (F) by the formula:

\[ F = 0.437 \times 2.5 \times C \times \frac{W}{W} \]

in which w is the weight of the sample taken for the sodium oxide determination, and W is the weight of the sample taken for the preparation of the slurry.
CALCIAL ASORBATE*

SYNONYMS

INS No. 302, EEC No. E302

DEFINITION

Chemical names Calcium ascorbate dihydrate, calcium salt of 2,3-didehydro-L-threo-hexono-1,4-lactone dihydrate

C.A.S. number 5743-27-1

Chemical formula \(\text{C}_6\text{H}_4\text{O}_7\text{Ca} \cdot 2\text{H}_2\text{O}\)

Structural formula

![Structural formula](image)

Molecular weight 426.35

Assay Content not less than 98% of \(\text{C}_6\text{H}_4\text{O}_7\text{Ca} \cdot 2\text{H}_2\text{O}\)

DESCRIPTION

White to slightly yellow odourless crystalline powder

FUNCTIONAL USE

Antioxidant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Soluble in water; slightly soluble in ethanol and insoluble in ether

** B. Calcium Passes test

Positive test for calcium

** C. Specific rotation \(\alpha_0 = 95^\circ \text{ to } +97^\circ \) (1 g in 20 ml solution)

---

* These specifications were prepared at the 25th session of JECFA (1981) and published in FNP 19 (1981).

PURITY TESTS

pH

6.0 to 7.5 (1 in 10 solution)

• Fluoride

Not more than 10 mg/kg (Method I)

• Arsenic

Not more than 3 mg/kg (Method II)

• Heavy metals

Not more than 10 mg/kg

Test 2 g of the sample as directed in the Limit Test (Method I)

METHOD OF ASSAY

Weigh accurately about 0.4 g of the sample into a 250 ml flask and add 50 ml of carbon dioxide free water. Immediately titrate with 0.1 N iodine, adding a few drops of starch TS as indicator as the end point is approached.

Each ml of 0.1 N iodine is equivalent to 10.66 mg of C\textsubscript{13}H\textsubscript{4}O\textsubscript{5}Ca \cdot 2H\textsubscript{2}O

CALCIUM BENZOATE*

SYNONYMS
Monocalcium benzoate
INS No. 213, EEC No. E213

DEFINITION
Chemical name
Calcium benzoate

C.A.S. number
2090-05-3

Chemical formula
Anhydrous: C₁₄H₁₀CaO₄; Monohydrate: C₁₄H₁₀CaO₄·H₂O
Trihydrate: C₁₄H₁₀CaO₄·3H₂O

Structural formula

\[
\begin{array}{c}
\text{Ca}^{2+} \\
\end{array}
\]

Molecular weight
Anhydrous: 282.31; Monohydrate: 300.32
Trihydrate: 336.36

Assay
Content not less than 99% of C₁₄H₁₀CaO₄ on the dried basis

DESCRIPTION
White or colourless crystals, or white powder

FUNCTIONAL USE
Antimicrobial preservative

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water

** B. Melting range for benzoic acid
121.5-123.5*°
Proceed as directed in the specifications for Sodium Benzoate

** C. Positive test for benzoate
Passes test

** D. Positive test for calcium
Passes test

PURITY TESTS

** Loss on drying
Not more than 17.5% (105*° to constant weight)

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

PURITY TESTS (cont'd)

Water insoluble matter

Not more than 0.3%

See description under TESTS

Acidity or alkalinity

Passes test.

Proceed as directed in the specifications for Sodium Benzoate

* Fluoride

Not more than 10 mg/kg.

Weigh 5 g of the sample to the nearest mg and proceed as directed in the Limit Test (Method I or III).

* Arsenic

Not more than 3 mg/kg

Proceed as directed in the specifications for Benzoic Acid

* Heavy metals

Not more than 10 mg/kg.

Test 2 g of the sample as directed in the Limit Test (Method II).

Readily oxidizable substances

Passes test.

Proceed as directed in the specifications for Benzoic Acid.

* Chlorinated organic compounds

Not more than 0.07% (as Cl)

TESTS

PURITY TESTS

Water insoluble matter

Dissolve 10 g of the sample, weighed to the nearest mg, in 100 ml of hot water.

Filter through a Gooch crucible, tared to an accuracy of + 0.2 mg, and wash any residue with hot water. Dry the crucible for 2 h. at 105°. Cool, weigh and calculate as percentage.

METHOD OF ASSAY

Chelatometry

Weigh accurately 0.6 g of the sample, previously dried at 105° for 4 h., dissolved in a mixture of 20 ml of water and 2 ml of dilute hydrochloric acid TS, and dilute about 100 ml with water. While stirring (preferably with a magnetic stirrer) add about 30 ml of 0.05 M disodium ethylenediaminetetraacetate from a 50-ml buret, then add 15 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation** and 3 ml of naphthol green TS, and continue the titration until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 14.116 mg of C₂H₁₀CaO₆.


** An alternative indicator is hydroxynaphthol blue, of which 0.25 g is used. In this case the naphthol green TS is omitted.
CALCIUM CARBONATE*

SYNONYMS

Chalk: INS No.170(i), EEC No.E170

DEFINITION

<table>
<thead>
<tr>
<th>Chemical names</th>
<th>Calcium carbonate, carbonic acid calcium salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.S. number</td>
<td>471-34-1</td>
</tr>
<tr>
<td>Chemical formula</td>
<td>CaCO₃</td>
</tr>
<tr>
<td>Formula weight</td>
<td>100.09</td>
</tr>
<tr>
<td>Assay</td>
<td>Calcium Carbonate contains not less than 98.0% CaCO₃ after drying at 200° for 4 h.</td>
</tr>
</tbody>
</table>

DESCRIPTION

Calcium Carbonate occurs as an odourless and tasteless, white micro-crystalline powder.

FUNCTIONAL USE

Anticaking agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Insoluble in water and ethanol.

** B. Positive test for carbonate

The sample dissolves with effervescence when treated with dilute acetic acid TS.

C. Positive test for calcium

Dissolve the sample in dilute acetic acid TS and add ammonium oxalate TS. The white precipitate formed is soluble in hydrochloric acid, but insoluble in acetic acid.

PURITY TESTS

** Loss on drying

Not more than 2% after drying at 200° for 4 h.

Acid insoluble substances

Not more than 0.2%. See description under TESTS.

** Arsenic

Not more than 3 mg/kg.

A solution of 1 g of the sample, weighed to the nearest mg, in 10 ml of dilute hydrochloric acid TS and 25 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

Barium

Not more than 0.03%. See description under TESTS.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (continued)

* **Fluoride**

Not more than 50 mg/kg.

Weigh 1 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method III).

**Free alkali**

Not more than 0.05%.

See description under TESTS.

**Magnesium and alkali salts**

Not more than 1%.

See description under TESTS.

* **Lead**

Not more than 10 mg/kg.

See description under TESTS.

* **Heavy metals**

Not more than 30 mg/kg.

See description under TESTS.

**TESTS**

**PURITY TESTS**

* **Acid insoluble substances**

Weigh 5 g of the sample to the nearest mg and suspend in 25 ml of water. Cautiously add with agitation 25 ml of a 1 in 2 solution of hydrochloric acid and add water to make a volume of about 200 ml. Heat the solution to boiling, cover, digest on a steam bath for 1 h, cool, and filter. Wash the precipitate with water until the last washing shows no chloride with silver nitrate TS, and then ignite it. Cool, weigh and calculate as percentage. (The weight of the residue should not exceed 10 mg).

**Barium**

Weigh 1 g of the sample to the nearest mg and mix with 10 ml of water. Add 15 ml of dilute hydrochloric acid TS and dilute to 30 ml with water and filter. To 20 ml of the filtrate add 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS and allow to stand for 15 min. The solution should show no more turbidity than a comparison solution containing 1 mg barium/ml. To prepare the comparison solution, add 20 ml of water to 0.3 ml of barium standard solution (1.799 g barium chloride in 1 L), and then 2 g of sodium acetate TS, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS.

**Free alkali**

Add 3 g of the sample to 30 ml of freshly boiled and cooled water, stir for 3 min and filter. To 20 ml of the filtrate add 2 drops of phenolphthalein TS. Though a red colour is produced, it should disappear when 0.2 ml of 0.1 N hydrochloric acid is added.

Magnesium and alkali salts

Mix 1 g of the sample with 40 ml of water, carefully add 5 ml of hydrochloric acid, mix and boil for 1 min. Rapidly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS, dropwise, until the mixture is just alkaline and cool. Transfer the mixture to a 100-ml cylinder, dilute with water to 100 ml, let stand for 4 h or overnight and then decant the clear supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. Cool, weigh and calculate as percentage. (The weight of the residue should not exceed 5 mg).

* Lead

Neutralize a 5-ml portion of the Sample solution (see below) with ammonia TS, using phenolphthalein as indicator, and dilute to 20 ml with water. This solution meets the requirements of the Limit Test for Lead, using 10 µg of lead ion (Pb) in the control.

* Heavy metals

Neutralize 3.3 ml of the Sample solution (see below) with sodium hydroxide TS, using phenolphthalein as indicator, and dilute to 25 ml with water. This solution meets the requirements of the Limit Test for Heavy Metals (Method I), using 20 µg of lead ion (Pb) in the control (Solution A).

Sample solution for the determination of lead and heavy metals:

Cautiously dissolve 5 g of the sample, weighed to the nearest mg, in 25 ml of a 1 in 2 hydrochloric acid solution and evaporate to dryness on a steam bath. Dissolve the residue in about 15 ml of water and dilute to 25 ml.

METHOD OF ASSAY

Weigh, to the nearest 0.1 mg, 200 mg of the sample previously dried at 200°C for 4 h. Transfer into a 400-ml beaker, add 10 ml of water and swirl to form a slurry. Cover the beaker with a watch glass and introduce 2 ml of dilute hydrochloric acid TS from a pipette inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the sample. Wash down the sides of the beaker, the outer surface of the pipette and the watch glass, and dilute to about 100 ml with water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 M disodium ethylenediaminetetraacetate from a 50-ml burette, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxynaphthol blue indicator, and continue the titration to a blue end point. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 5.004 mg of CaCO₃.

CALCIUM CHLORIDE*

SYNONYMS
INS No. 509, EEC No. E509

DEFINITION

Chemical name
Calcium chloride

C.A.S. number
10043-52-4

Chemical formula
Anhydrous: CaCl₂; Dihydrate: CaCl₂ • 2H₂O; Hexahydrate: CaCl₂ • 6H₂O

Formula weight
Anhydrous: 110.99; Dihydrate: 147.02; Hexahydrate: 219.08

Assay
Anhydrous Calcium Chloride contains not less than 93% of CaCl₂
Calcium Chloride dihydrate contains not less than 99.0% and not more than the equivalent of 107.0% of CaCl₂ • 2H₂O
Calcium Chloride hexahydrate contains not less than 98.0% and not more than the equivalent of 110% of CaCl₂ • 6H₂O

DESCRIPTION
Anhydrous Calcium Chloride occurs as white, deliquescent lumps or porous masses.
Calcium Chloride dihydrate occurs as white, hard, deliquescent fragments or granules.
Calcium Chloride hexahydrate occurs as colourless, very deliquescent crystals.

FUNCTIONAL USE
Firming agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Anhydrous Calcium Chloride: Freely soluble in water and ethanol
Calcium Chloride dihydrate: Freely soluble in water. Soluble in ethanol
Calcium Chloride hexahydrate: Very soluble in water and ethanol.

** B. Positive test for chloride
Passes test

** C. Positive test for calcium
Passes test

PURITY TESTS

Free alkali
Not more than 0.15% as Ca(OH)₂
See description under TESTS

Magnesium and alkali salts
Not more than 5%
See description under TESTS

** Fluoride
Not more than 40 mg/kg (Method III)

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS (continued)

* Arsenic

Not more than 3 mg/kg

A solution of 1 g of the sample in 35 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

* Lead

Not more than 10 mg/kg

A solution of 1 g of the sample in 20 ml of water meets the requirements of the Limit Test for Lead.

* Heavy metals

Not more than 40 mg/kg

Dissolve 0.5 g of the sample in 2 ml of dilute acetic acid TS, and add water to make 25 ml. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

TESTS

PURITY TESTS

Free alkali

Dissolve 1 g of the sample in 20 ml of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS. If the solution is pink, the pink colour is discharged by adding 2 ml of 0.02 N hydrochloric acid.

Magnesium and alkali salts

Dissolve 1 g of anhydrous calcium chloride, or the corresponding weight of a hydrate, in about 50 ml of water, add 500 mg of ammonium chloride, mix and boil for about 1 min. Quickly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonium TS dropwise until the mixture is just alkaline, and cool. Transfer the mixture into a 100-ml cylinder, dilute with water to 100 ml, let stand for 4 h or overnight, and then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 25 mg.

METHOD OF ASSAY

Chelatometry

Weigh accurately about 1 g of anhydrous calcium chloride, or the corresponding weight of a hydrate, transfer to a 250-ml beaker, and dissolve in a mixture of 100 ml of water and 5 ml of dilute hydrochloric acid TS. Transfer the solution to a 250-ml volumetric flask, dilute with water to volume and mix. Pipet 50 ml of the solution into a suitable container, add 100 ml of water, 15 ml of sodium hydroxide TS, 40 mg of murexide indicator (ammonium purpurate) and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediaminetetra-acetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 5.55 mg of CaCl₂; 7.35 mg of CaCl₂ • 2H₂O; or 10.95 mg of CaCl₂ • 6H₂O.

CALCIUM CITRATE*

SYNONYMS
INS No. 333, EEC No. E333

DEFINITION
Chemical names
Tricalcium citrate, tricalcium salt of 2-hydroxy-1,2,3-propanetricarboxylic acid, tricalcium salt of \( \beta \)-hydroxy-tricarballylic acid

C.A.S. number
813-94-5

Chemical formula
\( \text{C}_{12}\text{H}_{16}\text{Ca}_{3}\text{O}_{14} \cdot 4\text{H}_{2}\text{O} \)

Structural formula

\[
\begin{array}{c}
\text{CH}_3 - \text{COO} \\
\text{HO} - \text{C} - \text{COO} \\
\text{CH}_2 - \text{COO} \\
\end{array}
\]

Ca\(_3\) \( \cdot 4\text{H}_2\text{O} \)

Molecular weight
570.51

Assay
Calcium Citrate, after drying at 150° for 4 h, contains not less than 97.5% of \( \text{C}_{12}\text{H}_{16}\text{Ca}_{3}\text{O}_{14} \)

DESCRIPTION
Odourless, fine white powder

FUNCTIONAL USES
Sequestrant, buffer, firming agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Very slightly soluble in water. Insoluble in ethanol.

B. Positive test for citrate
Passes test
See description under TESTS

C. Positive test for calcium
Passes test
See description under TESTS

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* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS

* Loss on drying  
Not less than 10% and not more than 14% (150*, 4 h)

* Fluorides  
Not more than 30 mg/kg (Method I or III)

** Arsenic  
Not more than 3 mg/kg  
See description under TESTS

** Lead  
Not more than 10 mg/kg  
See description under TESTS

** Heavy metals  
Not more than 20 mg/kg  
See description under TESTS

** Free acid and alkali  
Passes test  
See description under TESTS

** Oxalate  
Passes test  
See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Positive test for citrate  
Dissolve 1 g of the sample in 20 ml of water and 5 ml of dilute nitric acid TS. Dilute 5 ml of this solution to 10 ml. Add 1 ml of mercuric sulfate TS, heat to boiling and add 0.1 N potassium permanganate. A white precipitate is formed.

C. Positive test for calcium  
Ignite 0.5 g of the sample at as low a temperature as possible, cool, and dissolve the residue in 10 ml of water and 1 ml of glacial acetic acid. Filter and add 10 ml of ammonium oxalate TS. A voluminious white precipitate appears which is soluble in hydrochloric acid.

PURITY TESTS

* Arsenic  
A solution of 1 g of the sample in 5 ml of dilute hydrochloric acid and 30 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

* Lead  
Dissolve 1 g of the sample in 10 ml of water and 1 ml of dilute hydrochloric acid, and neutralize to phenolphthalein TS. This solution meets the requirements of the Limit Test for Lead.

* Heavy metals  
Dissolve 1 g of the sample in 20 ml of water and 2 ml of dilute hydrochloric acid, add 1.5 ml of stronger ammonia TS, and dilute to 25 ml with water. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 µg of lead ion (Pb) in the control (Solution A).

PURITY TESTS (continued)

- **Free acid and alkali**
  
  To 1 g of the sample, add 5 ml of water, shake well for 1 min, and add 2 drops of phenolphthalein TS. No pink colour is produced. Add 0.5 ml of 0.1 N sodium hydroxide. A pink colour is produced.

- **Oxalate**
  
  Dissolve 1 g of the sample in 5 ml of warm dilute hydrochloric acid TS and filter the solution if necessary. Add 2 g of sodium acetate and dilute to 10 ml with water. No turbidity is produced within 1 h.

**METHOD OF ASSAY**

Weigh accurately about 350 mg of the sample, previously dried at 150° for 4 h, dissolve in a mixture of 10 ml of water and 2 ml of dilute hydrochloric acid TS, and dilute to about 100 ml with water. While stirring (preferably with a magnetic stirrer) add about 30 ml of 0.05 M disodium ethylenediaminetetra-acetate from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxynaphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 8.303 mg of C_{12}H_{10}Ca_3O_{14}.

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CALCIUM CYCLAMATE*

SYNONYM

INS No. 952

DEFINITION

Chemical names

Calculated cyclohexylsulfamate, Calcium cyclohexanesulfamate,  
C.A.S. number

139-06-0

Chemical formula

\[
\text{CaH}_{24}\text{CaN}_{2}\text{O}_{4}\text{S}_{2} \cdot 2\text{H}_{2}\text{O}
\]

Structural formula

\[
\begin{array}{c}
\text{NH} \bigarrow \text{SO}_{3} \\
\end{array}
\text{Ca} \cdot 2\text{H}_{2}\text{O}
\]

Molecular weight

432.57

Assay

Content not less than 98% and not more than 101% on the dried basis

DESCRIPTION

White colourless crystals or crystalline powder

FUNCTIONAL USE

Sweetening agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility  
Soluble in water, sparingly soluble in ethanol

B. Precipitation test  
Passes test  
See description under TESTS

** C. Positive test for calcium  
Passes test

PURITY TESTS

** Loss on drying  
Not less than 6% and not more than 9% (140°, 2 h)

** Selenium  
Not more than 30 mg/kg

** Heavy metals  
Not more than 10 mg/kg  
Proceed as directed in the specifications for ACESULFAME POTASSIUM

Cyclohexylamine  
Not more than 25 mg/kg  
See description under TESTS

* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

PURITY TESTS (continued)

**Dicyclohexylamine**

Not more than 2 mg/kg
See description under TESTS

TESTS

**IDENTIFICATION TESTS**

**B. Precipitation test**

To 10 ml of a 1 in 100 solution of the sample add 1 ml of hydrochloric acid, mix, add 1 ml of barium chloride TS. The solution remains clear, but upon the addition of 1 ml sodium nitrite TS, a white precipitate is formed.

**PURITY TESTS**

**Cyclohexylamine**

**Methyl orange-boric acid solution**

Dissolve 200 mg of methyl orange and 3.5 g of boric acid in 100 ml of water, heating on a steam bath to effect solution. Allow to stand for at least 24 h, and filter before use.

**Standard solution**

Transfer 100 mg of cyclohexylamine into a 100-ml volumetric flask, dissolve in 50 ml of water and 0.5 ml of hydrochloric acid, dilute to volume with water, and mix. Transfer 5 ml of the solution into a second 100-ml volumetric flask, dilute to volume with water, and mix. Transfer 5 ml of the solution into a third 100-ml volumetric flask, dilute to volume with water, and mix. Each ml of this solution contains 2.5 μg of cyclohexylamine.

**Test preparation**

Transfer 10 g of the sample into a 100-ml volumetric flask, dissolve in water, dilute to volume with water, and mix.

**Procedure**

Transfer 10 ml each of the Standard preparation and of the Test preparation into two separate 50-ml glass-stoppered centrifuge tubes, and transfer 10 ml of water to a third tube to serve as a blank. To each tube add 3.0 ml of disodium ethylenediaminetetra acetate solution (prepared by dissolving 10 g of disodium ethylenediaminetetraacetate and 3.4 g of sodium hydroxide in 100 ml of water) and 15 ml of a 20:1 mixture of chloroform and n-butanol, shake the tubes for 2 min, and centrifuge. Remove and discard the aqueous layer in each tube, and then transfer 10 ml of the chloroform solution from each tube into separate centrifuge tubes. To each tube add 2 ml of Methyl orange-boric acid solution, shake the tubes for 2 min, and centrifuge. Remove and discard the aqueous layer in each tube, then add to each tube 1 g of anhydrous sodium sulfate, shake well, and allow to settle. Transfer 5 ml of each clear chloroform solution into separate test tubes, add 0.5 ml of a 50:1 mixture of methanol and sulfuric acid, and mix. Concomitantly determine the absorbance of the solutions in 1-cm cells at 520 nm with a suitable spectrophotometer, using the blank to set the instrument. The absorbance of the solution from the Test preparation does not exceed that from the Standard preparation.
**METHOD OF ASSAY**

Dissolve 50 g of the sample in 300 ml of water, add 3 ml of sodium hydroxide TS, and extract with 50 ml and 30 ml of chloroform. Combine the extracts, add 2 g of anhydrous potassium carbonate and filter. Wash the container and the residue on the filter paper several times with 5 ml chloroform, combine the washings to the filtrate and concentrate to 1 ml under 30°. To this solution add 1 ml of nitrobenzene standard solution (100 mg in 500 ml chloroform) as an internal standard and examine for dicyclohexylamine by Gas chromatography* using a flame ionization detector under the conditions described below. Calculate the dicyclohexylamine content from a standard curve.

- Dicyclohexylamine (C_{12}H_{23}N) for the standard curve,

  \[ n^2_d : 1.480 - 1.488 \]

  \[ d^{25}_{20} : 0.905-0.915 \]

  Boiling point: 254-256°

1.5 m stainless steel column 3-4 mm of inside diameter packed with 60-80 mesh diatomaceous earth (gas chromatographic grade) in a solution of methanolic potassium hydroxide: the final potassium hydroxide concentration should be about 3% of the diatomaceous support. Evaporate off the methanol, add a chloroform solution of polyethylene glycol 6000, and evaporate the chloroform. The content of polyethylene glycol 6000 should be about 10% of the diatomaceous support.

- Column temperature: 130-140°

- Carrier gas: Nitrogen or helium Flow rate should be that to outflow nitrobenzene after about 7 min

**Dicyclohexylamine**

Dissolve 50 g of the sample in 300 ml of water, add 3 ml of sodium hydroxide TS, and extract with 50 ml and 30 ml of chloroform. Combine the extracts, add 2 g of anhydrous potassium carbonate and filter. Wash the container and the residue on the filter paper several times with 5 ml chloroform, combine the washings to the filtrate and concentrate to 1 ml under 30°. To this solution add 1 ml of nitrobenzene standard solution (100 mg in 500 ml chloroform) as an internal standard and examine for dicyclohexylamine by Gas chromatography* using a flame ionization detector under the conditions described below. Calculate the dicyclohexylamine content from a standard curve.

- Dicyclohexylamine (C_{12}H_{23}N) for the standard curve,

  \[ n^2_d : 1.480 - 1.488 \]

  \[ d^{25}_{20} : 0.905-0.915 \]

  Boiling point: 254-256°

1.5 m stainless steel column 3-4 mm of inside diameter packed with 60-80 mesh diatomaceous earth (gas chromatographic grade) in a solution of methanolic potassium hydroxide: the final potassium hydroxide concentration should be about 3% of the diatomaceous support. Evaporate off the methanol, add a chloroform solution of polyethylene glycol 6000, and evaporate the chloroform. The content of polyethylene glycol 6000 should be about 10% of the diatomaceous support.

- Column temperature: 130-140°

- Carrier gas: Nitrogen or helium Flow rate should be that to outflow nitrobenzene after about 7 min

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### SYNONYMS
Monocalcium phosphate, monobasic calcium phosphate

*INS No. 341 (i), EEC No. E341*

### DEFINITION
Calcium Dihydrogen Phosphate contains approximately 80% of the monobasic salt, the balance consisting mostly of the dibasic salt. It contains not less than 16.4% and not more than 17.9% Ca, and not less than .....% and not more than .....% phosphate expressed as P (data to be supplied by manufacturers on the basis of the analytical range found in the product in practice).

- **Chemical name**: Calcium dihydrogen phosphate, calcium dihydrogen tetraoxophosphate
- **C.A.S. number**: 7758-23-8
- **Chemical formula**: Anhydrous: Ca(H₂PO₄)₂; Dihydrous: Ca(H₂PO₄)₂·H₂O
- **Formula weight**: Anhydrous: 234.05 Dihydrous: 252.08

### DESCRIPTION
This product occurs as white crystals or granules or as granular powder.

### FUNCTIONAL USE
As buffer, firming agent, leavening agent, texturing agent and in fermentation processes.

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* These specifications were prepared at the 9th session of JECFA (1965) and published in NMRS 40A,B,C (1967).

CALCIUM DI-L-GLUTAMATE*

SYNONYMS

Calcium glutamate
INS No. 623, EEC No. 623

DEFINITION

Chemical name
Monocalcium di-L-glutamate

C.A.S. number
19238-49-4

Chemical formula
\( \text{C}_{n}\text{H}_{m}\text{CaN}_{2}\text{O}_{x} \cdot x\text{H}_{2}\text{O} \)

Structural formula
\[
\left[ \text{HOOC-CH-CH}_{2}-\text{CH}_{2}-\text{COO} \right] \text{Ca} \cdot x\text{H}_{2}\text{O}, \ x = 0, 1, 2 \text{ or } 4 \\
\text{NH}_{2} \\
2
\]

Molecular weight
332.32 (anhydrous)

Assay
Content not less than 98.0% and not more than 102.0% of \( \text{C}_{n}\text{H}_{m}\text{CaN}_{2}\text{O}_{x} \) on the anhydrous basis

DESCRIPTION
White, practically odourless crystals or crystalline powder, having a characteristic taste

FUNCTIONAL USE
Flavour enhancer, salt substitute

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water.

B. Positive test for glutamic acid
Passes test
Proceed as directed in the Positive test for glutamic acid under Monoammonium L-Glutamate.

** C. Positive test for calcium
Passes test

PURITY TESTS

** Specific rotation
\( [\alpha]_{D} : +27.4 \text{ to } +29.2^\circ \)
Test a solution of 10 g of sample (dried basis) in 100 ml of 2N hydrochloric acid, using a 200-mm tube.

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

PURITY TESTS (continued)

* Water content 
Not more than 19 % (Karl Fisher Method)

* Chlorides 
Not more than 0.2%. 
Test 0.07 g of the sample as directed in the Chlorides Limit Test using 0.4 ml of 0.01 N hydrochloric acid in the control.

* Arsenic 
Not more than 3 mg/kg. 
Test 1 g of the sample as directed in the Arsenic Limit Test (Method II).

* Lead 
Not more than 10 mg/kg. 
Test 1 g of the sample as directed in the Lead Limit Test using 10 μg of lead ion (Pb) in the control.

* Heavy metals 
Not more than 20 mg/kg. 
Test a solution of 1 g of the sample in 25 ml of water as directed in the Heavy Metals Limit Test (Method I).

Pyrrolidone carboxylic acid 
Passes test.

Proceed as directed in the Purity Test for Pyrrolidone carboxylic acid under Monosodium L-Glutamate.

METHOD OF ASSAY

Dissolve about 250 mg of the sample, previously dried and weighed accurately, in 6 ml of formic acid, and add 100 ml of glacial acetic acid. Titrate with 0.1 N perchloric acid* determining the end-point potentiometrically. Run a blank determination in the same manner and correct for the blank.

Each ml of 0.1 N perchloric acid is equivalent to 8.308 mg of C₉H₈CaN₂O₅. Calculate the content on the anhydrous basis.

CALCIUM DISODIUM ETHYLENEDIAMINETETRAACETATE*

SYNONYMS
Calcium disodium EDTA, calcium disodium edetate; INS No. 385, EEC No. E385

DEFINITION
Chemical names
N,N'-1,2-Ethanediylbis[N-(carboxymethyl)-glycinate](4-)
N,N',O,O',O',O'-calciate(2-)disodium;
Calcium disodium ethylenediaminetetraacetate;
Calcium disodium (ethylene-dinitrilo)-tetraacetate.

C.A.S. number
662-33-9

Chemical formula
C_{10}H_{12}CaN_{2}Na_{5}O_{7} \cdot 2H_{2}O

Structural formula

\[
\text{NaOOC}\begin{array}{c}
\text{CH}_{2} \text{CH}_{2} \\
\text{N} \\
\text{H} \\
\text{C} \\
\text{Ca} \\
\text{N} \\
\text{CH}_{2} \\
\text{OOC}
\end{array} \begin{array}{c}
\text{CH}_{2} \text{COONa} \\
\text{H}_{2} \text{C} \\
\text{COO}
\end{array} \begin{array}{c}
\cdot 2 \text{H}_{2} \text{O}
\end{array}
\]

Molecular weight
410.31

Assay
Calcium Disodium Ethylenediaminetetraacetate contains not less than 97% and not more than the equivalent of 102% \( C_{10}H_{12}CaN_{2}Na_{5}O_{7} \) calculated on the anhydrous basis.

DESCRIPTION
Calcium Disodium Ethylenediaminetetraacetate occurs as white, odourless crystalline granules or as a white to nearly white powder, slightly hygroscopic, with a faint saline taste.

FUNCTIONAL USES
Sequestrant, preservative.

CHARACTERISTICS
IDENTIFICATION TESTS

** A. Solubility
Freely soluble in water, practically insoluble in ethanol.

B. Positive test for calcium
Passes test
See description under TESTS

C. Positive test for sodium
Passes test
See description under TESTS

D. Chelating activity to metal ions
Passes test
See description under TESTS

** These specifications were prepared at the 30th session of JECFA (1986) and published in FNP 37 (1986).

PURITY TESTS

pH

6.5 - 7.5 (1 in 100 soln).

* Arsenic

Not more than 3 mg/kg (Method II)

* Lead

Not more than 10 mg/kg

* Heavy metals

Not more than 20 mg/kg (Method II)

Magnesium chelating substances

Passes test

See description under TESTS

TESTS

IDENTIFICATION TESTS

* B. Positive test for calcium

To 5 ml of 1% solution of the sample add 2 ml of dilute acetic acid TS and 1 ml of ammonium oxalate TS. The white precipitate formed is soluble in hydrochloric acid.

* C. Positive test for sodium

To 1 ml of 1% solution of the sample add 5 ml of uranyl zinc acetate TS. A yellow crystalline precipitate appears within a few minutes.

D. Chelating activity to metal ions

To 5 ml of water in a test tube add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS. A deep red solution develops. Add about 50 mg of the sample and mix. The deep red colour disappears.

Transfer 1 g of the sample, accurately weighed, to a small beaker, and dissolve it in 5 ml of buffer prepared by dissolving 67.5 g of ammonium chloride in 200 ml of water, adding 570 ml of strong ammonia TS, and diluting with water to 1000 ml. To the buffered solution add 5 drops of eriochrome black TS, and titrate with 0.1 M magnesium acetate to the appearance of a deep wine-red colour. Not more than 2.0 ml should be required.

Transfer about 1.2 g of the sample, accurately weighed, into a 250-ml beaker, and dissolve in 75 ml of water. Add 25 ml of dilute acetic acid TS and 1.0 ml of diphenylcarbazone solution (1 g in 100 ml ethanol). Titrate slowly with 0.1 M mercuric nitrate to the first appearance of a purplish colour. Each ml of 0.1 M mercuric nitrate (see below) is equivalent to 37.43 mg of Cu₂H₄Ca₃N₅Na₂O₁₀.

Mercuric nitrate solution: Dissolve about 35 g of mercuric nitrate Hg(NO₃)₂·H₂O in a mixture of 5 ml of nitric acid and 500 ml of water and dilute with water to 1000 ml. Standardize the solution as follows: Transfer an accurately measured volume of about 20 ml of the solution into an Erlenmeyer flask and add 2 ml of nitric acid and 2 ml of ferric ammonium sulfric TS. Cool to below 20° and titrate with 0.1 N ammonium thiocyanate to the first appearance of a permanent brownish colour. Calculate the molarity. (0.1 M = 32.46 g Hg(NO₃)₂ per litre).

CALCIUM GLUCONATE*

SYNONYMS
INS No. 578, EEC No. 578

DEFINITION
Chemical names
Calcium di-d-gluconate monohydrate, calcium di-gluconate

C.A.S. number
299-28-5

Chemical formula
C_{12}H_{22}CaO \cdot H_2O

Structural formula

\[
\begin{align*}
\text{HOH}_2C &- C - C - C - C - \text{COO}^- \\
\text{H} & \quad \text{H} \quad \text{OH} & \quad \text{H}
\end{align*}
\]

Ca^{2+} \cdot H_2O

\[
\begin{pmatrix}
\text{OH} & \text{OH} & \text{H} & \text{OH} \\
\text{H} & \text{H} & \text{OH} & \text{H}
\end{pmatrix}
\]

\[
\begin{pmatrix}
\text{OH} & \text{OH} & \text{H} & \text{OH} \\
\text{H} & \text{H} & \text{OH} & \text{H}
\end{pmatrix}
\]

2

Molecular weight
448.39

Assay
Calcium Gluconate, when dried at 105° for 16 h, contains not less than 98% and not more than the equivalent of 102% of C_{12}H_{22}CaO \cdot H_2O

DESCRIPTION
Odourless and tasteless, white, crystalline granules or powder, stable in air

FUNCTIONAL USE
Buffer, firming agent, sequestrant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water. Insoluble in ethanol

** B. Colour reaction
To 1 ml of a 1 in 40 solution, add 1 drop of ferric chloride TS. A deep yellow colour is produced

C. Derivation to phenylhydrazide of gluconic acid
Passes test

See description under TESTS

** D. Positive test for calcium
Passes test

* These specifications were prepared at the 18th session of JECFA (1974) and published in NMRS 54B (1975).

PURITY TESTS

- **Loss on drying**
  Not less than 3% (105°, 16 h)

- **pH**
  6 - 8 (1 in 20 soln)

- **Arsenic**
  Not more than 3 mg/kg

- **Heavy metals**
  Not more than 10 mg/kg

Sucrose and reducing sugars
Passes test
See description under TESTS

TESTS

IDENTIFICATION TESTS

C. Derivation to phenylhydrazide of gluconic acid
Warm, in a test tube, 5 ml of a 1 in 10 solution, add 0.7 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine. Heat on a water bath for 30 min, cool, and scratch the inner wall of the tube with a glass rod. Crystals are formed. Filter, and dissolve the crystals in 10 ml of hot water. Add a small portion of active carbon, shake thoroughly, and filter. Cool, and scratch the inner wall of the tube with a glass rod. Crystals are formed. Filter the crystals, and dry. The crystals decompose at about 210°.

PURITY TESTS

Sucrose and reducing sugars
Dissolve 0.5 g of the sample in 10 ml of hot water. Add 2 ml of dilute hydrochloric acid TS, boil for about 2 min, and cool. Add 5 ml of sodium carbonate TS, allow to stand for 5 min, dilute with water to 20 ml, and filter. Add 5 ml of the clear filtrate to about 2 ml of alkaline cupric tartrate TS, and boil for 1 min. No red precipitate should be formed immediately.

METHOD OF ASSAY

Chelatometry
Weigh accurately about 0.5 g of the sample previously dried at 105° for 16 h and dissolve in 5 ml of dilute hydrochloric acid. Add 50 ml of water, 25 ml of sodium hydroxide TS and about 0.1 g of 2-hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphtholic acid. Titrate with 0.05 M EDTA immediately. At the end-point, the red colour changes completely to blue. Each ml of 0.05 M EDTA is equivalent to 22.42 mg of C_{16}H_{22}CaO·H_{2}O.

SYNONYMS
Calcium guanylate
INS No. 629, EEC No. 629

DEFINITION
Chemical name
Calcium guanosine-5'-monophosphate

C.A.S. number
38966-30-2

Chemical formula
$C_{19}H_{17}CaN_{5}O_{9}P \cdot xH_2O$

Structural formula

\[
\begin{array}{c}
\text{O} \\
\text{N}
\end{array}
\]

Molecular weight
401.20 (anhydrous)

Assay
Content not less than 97.0% and not more than 102.0% of $C_{19}H_{17}CaN_{5}O_{9}P$
calculated on the dried basis

DESCRIPTION
Odourless, white or off-white crystals, or powder, having a characteristic taste.

FUNCTIONAL USE
Flavour enhancer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Sparingly soluble in water.

** B. UV absorbance
Passes test
See description under TESTS

** C. Positive test for calcium
Passes test

D. Positive test for ribose
Passes test
See description under TESTS

* These specifications were prepared at the 18th session of JECFA (1974) and published in NMRS 54B (1975).

IDENTIFICATION TESTS (continued)

E. Positive test for organic phosphate
   Passes test
   See description under TESTS

PURITY TESTS

* Loss on drying
   Not more than 23% (120º, 4 h)

* pH
   7.0 - 8.0 (1 in 2,000 soln)

* Arsenic
   Not more than 3 mg/kg
   Proceed as directed in the specifications for Glutamic Acid

* Lead
   Not more than 10 mg/kg
   Proceed as directed in the specifications for Glutamic Acid

* Heavy metals
   Not more than 20 mg/kg
   Proceed as directed in the specifications for Glutamic Acid

Water soluble matter
   Passes test
   See description under TESTS

Amino acids
   Not detectable by the following test.
   To 5 ml of a 1 in 2,000 solution add 1 ml of ninhydrin TS and heat for 3 min.
   No colour is produced.

Related foreign substances
   Chromatographically not detectable
   See description under TESTS

TESTS

IDENTIFICATION TESTS

B. UV absorbance
   A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 256 ± 2 nm. The ratio A250/A260 is between 0.95 and 1.03, and the ratio A280/260 is between 0.63 and 0.71.

D. Positive test for ribose
   To 3 ml of a 3 in 10,000 solution of the sample in water, add 0.2 ml of a 1 in 10 solution of orcinol in ethanol and subsequently 3 ml of a 1 in 1,000 hydrochloric acid solution of ferric ammonium sulfate. Heat in a water bath for 10 min. A green colour is produced.

E. Positive test for organic phosphate
   To 5 ml of a 1 in 2,000 solution add 2 ml of magnesia mixture TS. No precipitate is formed. Add 5 ml of nitric acid, boil for 10 min, neutralize with strong ammonia TS, add water to make to 100 ml, add ammonium molybdate TS, and warm. A yellow precipitate, which dissolves in sodium hydroxide TS or ammonia TS.

PURITY TESTS

**Water soluble matter**

To 1 g of the sample, add 50 ml of water, allow to stand for 10 min with occasional shaking, filter through analytical grade filter paper (Whatman No. 42 or equivalent). Evaporate a 25 ml portion of the solution to dryness on a water bath and dry the residue at 105°C for 1 h. Residue weigh less than 80 mg.

**Related foreign substances**

Proceed as directed under "Thin-layer chromatography" in the General Methods*, using 10 μl of a 1 in 2,000 solution of the sample as the sample solution, a mixture of 80 volumes of a saturated solution of ammonium sulfate, 18 volumes of a 13.6% w/v solution of sodium acetate and 2 volumes of isopropanol as the developing solvent, and microcrystalline cellulose as the absorbent. Stop the development when the solvent front has advanced about 10 cm from the point of the application, dry the plate in air, and observe under ultraviolet light (about 254 nm) in a dark place. Only a spot of 5'-guanylic acid is detected.

METHOD OF ASSAY

Weigh accurately about 500 mg of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10.0 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 260 nm using 0.01 N hydrochloric acid as the reference. Calculate the content of C_{9}H_{12}CaN_{2}O_{5}P in the sample by the formula:

\[
\text{Content (\%) = } \frac{A \times 250,000 \times 100}{294.1 \times \text{weight of sample (mg)} \times (100 - \text{loss on drying (\%)})}
\]

CALCIUM HYDROGEN SULFITE*

SYNONYMS
Calcium bisulfite, INS No. 227, EEC No E227

DEFINITION

Chemical name
Calcium hydrogensulfite

Chemical formula
Ca(HSO₃)₂

Formula weight
202.22

Assay
Contains not less than 6 and not more than 8% of sulfur dioxide w/v.

DESCRIPTION
Clear greenish-yellow aqueous solution having a distinct odour of sulfur dioxide. It exists only in aqueous solution and is prepared by saturating calcium hydroxide solution with sulfur dioxide.

FUNCTIONAL USES
Combined preservative and firming agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Positive test for calcium salt
Passes tests

** B. Positive test for bisulfite
Passes tests

PURITY TESTS

Iron
Not more than 30 mg/kg
See description under TESTS

** Selenium
Not more than 10 mg/kg
0.60 g of the sample meets the requirements of the Limit Test for Selenium (Method II).

** Arsenic
Not more than 3 mg/kg
See description under TESTS

** Heavy metals
Not more than 10 mg/kg
See description under TESTS

* These specifications were prepared at the 20th session of JECFA (1976) and published in FNS 1B (1977).

TESTS

PURITY TESTS

* Iron
To 1 g of the sample, add 2 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid, dilute to 40 ml with water, and add about 40 mg of ammonium persulfate and 10 ml of ammonium thiocyanate TS. Any red or pink colour does not exceed that produced by 20 ml of Iron Standard Solution (30 µg Fe in an equal volume of solution containing the quantities of reagents used in the test).

* Arsenic
Mix 1 g of the sample with 10 ml of water, add 12 ml of dilute hydrochloric acid TS, and heat to boiling to dissolve the sample. Cool, filter, and dilute the filtrate to 35 ml with water. This solution meets the requirements of the Limit Test for Arsenic (Method II).

* Heavy metals
Mix 2 g of the sample with 10 ml of water, add 15 ml of dilute hydrochloric acid TS, and heat to boiling to dissolve the sample. Cool, and add ammonium hydroxide to a pH 7. Filter, evaporate to a volume of about 25 ml, and refilter if necessary to obtain a clear solution. This solution meets the requirements of Limit Test for Heavy Metals (Method I) using 20 µg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Sulfur dioxide

Redox titration
Measure accurately a volume of the sample, equivalent to about 100 mg of SO₂, mix with 50 ml of 0.1 N iodine in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of concentrated hydrochloric acid, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 N iodine is equivalent to 3.203 mg of SO₂.

CALCIUM HYDROXIDE*

SYNONYMS
Slaked lime; INS No.526, EEC No.526

DEFINITION
- Chemical name: Calcium hydroxide
- C.A.S. number: 1305-62-0
- Chemical formula: Ca(OH)_2
- Molecular weight: 74.09
- Assay: Calcium Hydroxide contains not less than 92.0% of Ca(OH)_2

DESCRIPTION
- White powder

FUNCTIONAL USES
- Neutralizing agent, buffer, firming agent.

CHARACTERISTICS

IDENTIFICATION TESTS
- ** A. Solubility: Slightly soluble in water. Insoluble in ethanol. Soluble in glycerol.
- ** B. Positive test for alkali: The sample is alkaline to moistened litmus paper.
- C. Positive test for calcium: Passes test

PURITY TESTS

Barium
- Not more than 0.03%
- See description under TESTS

Magnesium and alkali salts
- Not more than 6%
- See description under TESTS

Acid insoluble ash
- Not more than 1.0%
- See description under TESTS

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS (continued)

* **Arsenic**
Not more than 3 mg/kg
A solution of 1 g of the sample in 15 ml of dilute hydrochloric acid TS and 20 ml of water meets the requirements of the Limit Test for Arsenic (Method II)

* **Fluoride**
Not more than 50 mg/kg (Method I or III)

**Heavy metals**
Not more than 40 mg/kg
See description under TESTS

* **Lead**
Not more than 10 mg/kg
Dissolve 1 g of the sample in 15 ml of dilute hydrochloric acid TS, and neutralize to phenolphthalein TS. This solution meets the requirements of the Limit Test for Lead.

TESTS

IDENTIFICATION TESTS

* D. Positive test for calcium
Mix 1 g of the sample with 20 ml of water and add sufficient acetic acid to effect solution. To the solution add ammonium oxalate TS. The white precipitate formed is soluble in hydrochloric acid, but insoluble in acetic acid.

PURITY TESTS

**Barium**
Mix 1.5 g of the sample with 10 ml of water, add 15 ml of dilute hydrochloric acid TS and dilute to 30 ml with water and filter. To 20 ml of the filtrate, add 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS, and allow to stand for 15 minutes. The turbidity of the solution is not greater than that of a control prepared by adding water to 0.3 ml of barium standard solution (1.779 g barium chloride in 1000 ml of water) to make to 20 ml, adding 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS and allowing to stand for 15 minutes.

**Magnesium and alkali salts**
Dissolve 500 mg of the sample in a mixture of 30 ml of water and 10 ml of dilute hydrochloric acid TS and boil for 1 minute. Quickly add 40 ml of oxalic acid TS and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS dropwise until the mixture is just alkaline and cool. Transfer the mixture to a 100-ml cylinder, dilute to volume with water, let stand for 4 h or overnight, then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear

Magnesium and alkali salts (cont'd)

filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a flame, and continue the heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 15 mg.

* Acid insoluble ash

Dissolve 2 g of the sample in 30 ml of dilute hydrochloric acid (1 in 3) and heat to boiling. Filter the mixture, wash the residue with hot water and ignite. The weight of the residue does not exceed 20 mg.

* Heavy metals

Dissolve 0.50 g of the sample in 10 ml of dilute hydrochloric acid TS, and evaporate to dryness on a steam bath. Dissolve the residue in 25 ml of water and filter. The filtrate meets the requirements of the Limit Test for Heavy Metals (Method) using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Weight accurately about 1.5 g of the sample, transfer to a beaker, and gradually add 30 ml of dilute hydrochloric acid TS. When solution is complete, transfer to a 500-ml volumetric flask, rinse the beaker thoroughly, adding the rinsings to the flask, dilute to volume with water, and mix. Pipet 50 ml of the solution into a suitable container and add 50 ml of water and 15 ml of sodium hydroxide TS, 40 mg of murexide indicator (ammonium purpurate) and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 3.705 mg of Ca(OH)_2.
CALCIUM 5'-INOSINATE*

SYNONYMS

Calcium inosinate
INS No. 633, EEC No. 633

DEFINITION

Chemical name
Calcium inosine-5'-monophosphate

C.A.S. number
Ca salt: 38966-29-9
Ca (1:1) salt: 3387-37-9
Ca (1:1) salt hydrate: 76079-57-7

Chemical formula
$\text{Ca}_{10}\text{H}_{26}\text{CaN}_{4}\text{O}_{9}\text{P} \cdot x\text{H}_{2}\text{O}$

Structural formula

Molecular weight
386.19 (anhydrous)

Assay
Content not less than 97.0% and not more than 102.0% of $\text{Ca}_{10}\text{H}_{26}\text{CaN}_{4}\text{O}_{9}\text{P}$ on the anhydrous basis

DESCRIPTION

Odourless, white or off-white crystals, or powder, having a characteristic taste.

FUNCTIONAL USE

Flavour enhancer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Sparingly soluble in water.

** B. UV absorbance
Passes test
See description under TESTS

** C. Positive test for calcium
Passes test

---

* These specifications were prepared at the 18th session of JECFA (1974) and published in NMRS 54B (1975).

**IDENTIFICATION TESTS** (continued)

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D.</strong> Positive test for ribose</td>
<td>Passes test&lt;br&gt;See description under TESTS</td>
</tr>
<tr>
<td><strong>E.</strong> Positive test for organic phosphate</td>
<td>Passes test&lt;br&gt;See description under TESTS</td>
</tr>
</tbody>
</table>

**PURITY TESTS**

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td>7.0 - 8.0 (1 in 2,000 soln)</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td>Not more than 23% (Karl Fischer)</td>
</tr>
<tr>
<td><strong>Arsenic</strong></td>
<td>Not more than 3 mg/kg&lt;br&gt;Proceed as directed in the specifications for Glutamic Acid</td>
</tr>
<tr>
<td><strong>Lead</strong></td>
<td>Not more than 10 mg/kg&lt;br&gt;Proceed as directed in the specifications for Glutamic Acid</td>
</tr>
<tr>
<td><strong>Heavy metals</strong></td>
<td>Not more than 20 mg/kg&lt;br&gt;Proceed as directed in the specifications for Glutamic Acid</td>
</tr>
</tbody>
</table>

| Water soluble matter | Passes test<br>See description under TESTS |
| Amino acids | Not detectable by the following test.<br>To 5 ml of a 1 in 1,000 solution add 1 ml of ninhydrin TS and heat for 3 min.<br>No colour is produced. |
| Related foreign substances | Chromatographically not detectable<br>See description under TESTS |

**TESTS**

**IDENTIFICATION TESTS**

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B.</strong> UV absorbance</td>
<td>A 1 in 50,000 solution of the sample in 0.01 N hydrochloric acid exhibits an absorbance maximum at 250 ± 2 nm. The ratio A250/A260 is between 1.55 and 1.65, and the ratio A280/260 is between 0.20 and 0.30.</td>
</tr>
<tr>
<td><strong>D.</strong> Positive test for ribose</td>
<td>To 3 ml of a 3 in 10,000 solution of the sample in water, add 0.2 ml of a 1 in 10 solution of orcinol in ethanol and subsequently 3 ml of a 1 in 1,000 hydrochloric acid solution of ferric ammonium sulfate. Heat in a water bath for 10 min. A green colour is produced.</td>
</tr>
<tr>
<td><strong>E.</strong> Positive test for organic phosphate</td>
<td>To 5 ml of a 1 in 2,000 solution add 2 ml of magnesia mixture TS. No precipitate is formed. Add 5 ml of nitric acid, boil for 10 min, neutralize with strong ammonia TS, add water to make to 100 ml, add ammonium molybdate TS, and warm. A yellow precipitate, which dissolves in sodium hydroxide TS or ammonia TS.</td>
</tr>
</tbody>
</table>

PURITY TESTS

**Water soluble matter**

To 1 g of the sample, add 50 ml of water, allow to stand for 10 min with occasional shaking, filter through analytical grade filter paper (Whatman No. 42 or equivalent). Evaporate a 25 ml portion of the solution to dryness on a water bath and dry the residue at 105°C for 1 h. Residue weigh less than 450 mg.

**Related foreign substances**

Proceed as directed under "Thin-layer chromatography" in the General Methods*, using 5 μl of a 1 in 1,000 solution of the sample as the sample solution, a mixture of 80 volumes of a saturated solution of ammonium sulfate, 18 volumes of a 13.6% w/v solution of sodium acetate and 2 volumes of isopropanol as the developing solvent, and microcrystalline cellulose as the absorbent. Stop the development when the solvent front has advanced about 10 cm from the point of the application, dry the plate in air, and observe under ultraviolet light (about 254 nm) in a dark place. Only a spot of 5'-inosinic acid is detected.

**METHOD OF ASSAY**

Weigh accurately about 500 mg of the sample, dissolve in and make to 1,000 ml with 0.01 N hydrochloric acid. Take 10.0 ml of this solution and dilute with 0.01 N hydrochloric acid to 250 ml. Determine the absorbance A of the solution in a 1-cm cell at the wave length of 250 nm using 0.01 N hydrochloric acid as the reference. Calculate the content of \( \text{C}_{9}\text{H}_{14}\text{CaN}_{4}\text{O}_{8} \) in the sample by the formula:

\[
\text{Content (\%) } = \frac{A \times 250.000 \times 100}{314.9 \times \text{weight of sample (mg)} \times 100 - \text{Water (\%)}}
\]

**SYNONYM**  
INS No. 916

**DEFINITION**

- **Chemical name**: Calcium iodate monohydrate
- **C.A.S. number**: 7789-80-2
- **Chemical formula (empirical)**: Ca(IO₃)₂ · H₂O
- **Formula weight**: 407.90
- **Assay**: The product contains not less than 99% and not more than the equivalent of 101% of Ca(IO₃)₂ · H₂O

**DESCRIPTION**
Calcium iodate monohydrate occurs as a white powder and may have a slight odour.

**FUNCTIONAL USES**
Rapid oxidizing agent used for strengthening flour.

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*These specifications were prepared at the 9th session of JECFA (1965) and published in NMRS 40ABC (1969).*
CALCIUM LACTATE*

SYNONYMS
INS No.327, EEC No.327

DEFINITION
Chemical names
Calcium dilactate, calcium dilactate hydrate, 2-Hydroxypropanoic acid calcium salt

C.A.S. number
814-80-2

Chemical formula
Ca\(_{10}\)H\(_{14}\)O\(_6\) \cdot x\(H_2O\) (x = 0 - 5)

Structural formula

\[
\begin{align*}
\text{CH}_3\text{CHCOO}^- \\
\text{OH}
\end{align*}
\]

\[\text{Ca}^{2+} \cdot 0 \sim 5\,\text{H}_2\text{O}\]

Molecular weight
218.22 (anhydrous)

Assay
Calcium Lactate, when dried at 120° for 4 h, contains not less than 98.0% of \(\text{Ca}_2\text{H}_6\text{O}_6\). It contains up to 5 molecules of water of crystallization.

DESCRIPTION
White to cream coloured, almost odourless, crystalline powder or granules. The pentahydrate is somewhat efflorescent.

FUNCTIONAL USES
Buffer, dough conditioner, yeast food.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in water. Practically insoluble in ethanol.

** B. Positive test for lactate
To 5 ml of a 1 in 20 solution add 5 ml of dilute sulfuric acid and 2 ml of potassium permanganate TS, and heat. The odour of acetaldehyde is evolved.

** C. Positive test for calcium
Passes test

* These specifications were prepared at the 18th session of JECFA (1974) and published in NMRS 54B (1975).

* PURITY TESTS

** Loss on drying
Not more than 30% (120°, 4 h)

** pH
6.0 - 8.0 (1 in 20 soln)

Acidity
Dissolve 1 g of the sample in 20 ml of water, add 3 drops of phenolphthalein TS, and titrate with 0.1 N sodium hydroxide. Not more than 0.5 ml should be required.

Magnesium and alkali salts
Not more than 1%
See description under TESTS

** Fluoride
Not more than 30 mg/kg

** Arsenic
Not more than 3 mg/kg (Method II)

** Lead
Not more than 10 mg/kg

** Heavy metals
Not more than 20 mg/kg
Test 1 g of the sample as directed in the Limit Test.

TESTS

PURITY TESTS

Magnesium and alkali salts
Dissolve 1 g of the sample in 40 ml of water, add 0.5 g of ammonium chloride, boil, and add about 20 ml of ammonium oxalate TS. Heat the solution on a water bath for 1 h, cool, add water to 100 ml, and filter. To 50 ml of the filtrate, add 0.5 ml of sulfuric acid, evaporate to dryness, and ignite to constant weight. The residue should not exceed 5 mg.

METHOD OF ASSAY

Dissolve about 350 mg of previously dried sample, accurately weighed, in 150 ml of water containing 2 ml of dilute hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 M disodium ethylenediaminetetraacetate from a 50-ml buret. Then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy-naphthol blue indicator, and continue the titration to a blue end-point. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 10.91 mg of C₆H₈O₆CaO₆.

---

* All purity tests (except loss on drying) are calculated on the dried basis.

CALCIUM DL-MALATE *

SYNONYMS
DL-Monocalcium malate; INS No.352(ii), EEC No.352

DEFINITION
Chemical names
Calcium DL-malate, calcium-α-hydroxy succinate, hydroxybutanedioic acid calcium salt

Chemical formula
\[ \text{C}_6\text{H}_5\text{CaO}_7 \]

Structural formula
\[
\begin{align*}
\text{OH} \\
\text{CHCOO} & \quad \text{Ca} \\
\text{CH}_2\text{COO} & \\
\end{align*}
\]

Molecular weight
172.14

Assay
Content not less than 97.5% on the dried basis

DESCRIPTION
White, colourless powder

FUNCTIONAL USES
Buffering agent, seasoning agent

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Slightly soluble in water. Insoluble in ethanol

** B. Positive test for malate
Passes test
See description under TESTS

** C. Positive test for calcium
Passes test

PURITY TESTS

** Loss on drying
Not more than 2% (110°, 3 h)

** Fluoride
Not more than 30 mg/kg (Method I or III)

** Maleic acid
Not more than 0.05%

** Arsenic
Not more than 3 mg/kg (Method II)

** Lead
Not more than 10 mg/kg

** Heavy metals
Not more than 20 mg/kg. Test 1 g of the sample as directed in the Limit Test (Method II).

* These specifications were prepared at the 27th session of JECFA (1983) and published in FNP 28 (1983).

TESTS

IDENTIFICATION TEST

B. Positive test for malate

To 100 ml of saturated solution of the sample add 10 mg of sulfanilic acid. Heat the solution on a water bath for a few min., add 5 ml of a 1 in 5 solution of sodium nitrite and heat slightly. Make alkaline with sodium hydroxide TS. A red colour is produced.

METHOD OF ASSAY

Weigh accurately about 0.4 g of the sample, previously dried at 110° for 3 h, dissolve in a mixture of 10 ml of water and 2 ml of dilute hydrochloric acid TS, and dilute to about 100 ml with water. While stirring (preferably with a magnetic stirrer) add about 30 ml of 0.05 M disodium ethylenediaminetetraacetate from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxynaphthol blue indicator, and continue the titration to a blue end-point. Each ml of 0.05 M disodium ethylenediaminetetaacetate is equivalent to 8.607 mg of C₄H₄CaO₄.

CALCIUM HYDROGEN PHOSPHATE*

SYNONYMS
Dibasic calcium phosphate, dicalcium phosphate
INS No. 341 (ii), EEC No. E341

DEFINITION
Chemical names
Calcium monohydrogen phosphate, calcium hydrogen orthophosphate, secondary calcium phosphate

C.A.S. number
7757-93-9

Chemical formula
Anhydrous: CaHPO₄; Dihydrate: CaHPO₄·2H₂O

Formula weight
Anhydrous: 136.06; Dihydrate: 172.09

Assay
Calcium Hydrogen Phosphate, after drying at 200° for 3 h, contains not less than 98.0% and not more than the equivalent of 102.0% of CaHPO₄.

DESCRIPTION
White crystals or granules, granular powder or powder

FUNCTIONAL USE
Dough conditioner, yeast food

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Sparingly soluble in water. Insoluble in ethanol.

B. Positive test for phosphate
Passes test
See description under TESTS

** C. Positive test for phosphate
To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate TS. A yellow precipitate forms.

** D. Positive test for orthophosphate
Wet the sample with silver nitrate TS. A yellow colour is produced

PURITY TESTS

** Loss on drying
Anhydrous: Not more than 2% (200°, 3 h)
Dihydrate: Not less than 18% and not more than 22% (200°, 3 h)

** Fluoride
Not more than 50 mg/kg (Method I or III)

** Arsenic
Not more than 3 mg/kg
See description under TESTS

** Lead
Not more than 10 mg/kg
See description under TESTS

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS (continued)

* **Heavy metals**
  Not more than 30 mg/kg
  See description under TESTS

**TESTS**

**IDENTIFICATION TESTS**

* **B. Positive test for calcium**
  Dissolve about 0.1 g of the sample by warming with a mixture of 5 ml of dilute hydrochloric acid TS and 5 ml of water. Add 2.5 ml of ammonia TS, dropwise, with shaking, and then add 5 ml of ammonium oxalate TS. A white precipitate forms.

**PURITY TESTS**

* **Arsenic**
  A solution of 1 g of the sample in 5 ml of dilute hydrochloric acid TS and 30 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

* **Lead**
  Dissolve 0.5 g of the sample in 5 ml of dilute hydrochloric acid TS and neutralize it to phenolphthalein TS, dilute to about 20 ml with water, and filter. This solution meets the requirements of the Limit Test for Lead.

* **Heavy metals**
  Warm 1.333 g of the sample with 5 ml of dilute hydrochloric acid TS until no more dissolves, dilute to 50 ml with water, and filter. A 25-ml portion of the filtrate meets the requirements of the Limit Test for Heavy Metals (Method I), using 20 µg of lead ion (Pb) in the control (Solution A).

**METHOD OF ASSAY**

Weigh accurately about 0.3 g of the sample, previously dried for 3 h at 200°C. Dissolve in 10 ml of dilute hydrochloric acid TS, add about 120 ml of water and a few drops of methyl orange TS, and boil for 5 min, keeping the volume and pH of the solution in the beaker constant during the boiling period by adding hydrochloric acid or water as necessary. Add 2 drops of methyl red TS and 30 ml of ammonium oxalate TS. Then add dropwise, with constant stirring, a mixture of equal volumes of ammonia TS and water until the pink colour of the indicator just disappears. Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through an asbestos mat in a Gooch crucible, using gentle suction. Swirl the precipitate in the beaker with about 30 ml of a cold (below 20°C) wash solution prepared by diluting 10 ml of ammonium oxalate TS to 1000 ml. Allow the precipitate to settle, and pass the supernatant through the filter. Repeat this washing by decantation three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter with 10 ml portions of cold (below 20°C) water. Place the Gooch crucible in the beaker, and add 100 ml of water and 50 ml of cold dilute sulfuric acid (1 in 6). Add from a buret 35 ml of 0.1 N potassium permanganate, and stir until the colour disappears. Heat to about 70°C, and complete the titration with 0.1 N potassium permanganate. Each ml of 0.1 N potassium permanganate is equivalent to 6.803 mg of CaHPO₄₂⁻.

CALCIUM OXIDE*

SYNONYMS
Lime; INS No.529, EEC No.529

DEFINITION

Chemical name Calcium oxide
C.A.S. number 1305-78-8
Chemical formula CaO
Molecular weight 56.08
Assay Calcium oxide, after heating at about 800° to constant weight, contains not less than 95.0% of CaO

DESCRIPTION
Odourless, hard, white or grayish white masses or granules, or white to grayish white powder (Caution: Protect eyes when adding water).

FUNCTIONAL USES
Alkali, dough conditioner, yeast food.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Slightly soluble in water. Insoluble in ethanol. Soluble in glycerol.

B. Reaction with water Moisten the sample with water. Heat is generated.

** C. Positive test for alkali The sample is alkaline to moistened litmus paper.

D. Positive test for calcium Passes test
See description under TESTS

PURITY TESTS

** Loss on ignition Not more than 10% by the following procedure. Heat 1 g of the sample in a tared platinum crucible at about 800° to constant weight.

Barium Not more than 0.03%
See description under TESTS

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS

**Magnesium and alkali salts**

Not more than 3.6%  
See description under TESTS

**Acid insoluble matter**

Not more than 1%  
See description under TESTS

* Arsenic

Not more than 3 mg/kg  
A solution of 1 g of the sample in 15 ml of dilute hydrochloric acid TS and 20 ml of water meets the requirements of the Limit Test for Arsenic (Method II).

* Fluorides

Not more than 50 mg/kg (Method I or III).

**Heavy metals**

Not more than 40 mg/kg  
See description under TESTS

* Lead

Not more than 10 mg/kg  
Dissolve 1 g of the sample in 15 ml of dilute hydrochloric acid TS and neutralize to phenolphthalein TS. This solution meets the requirements of the Limit Test for Lead.

TESTS

IDENTIFICATION TESTS

**D. Positive test for calcium**

Mix 1 g of the sample with 20 ml of water and add acetic acid until the sample is dissolved. To the solution add ammonium oxalate TS. The white precipitate formed is soluble in hydrochloric acid, but insoluble in acetic acid.

PURITY TESTS

**Barium**

Cautiously mix 1.5 g of the sample with 10 ml water, add 15 ml of dilute hydrochloric acid TS, dilute to 30 ml with water and filter. To 20 ml of the filtrate add 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS and allow to stand for 15 minutes. The turbidity of the solution is not greater than that of a control prepared by adding water to 0.3 ml of barium standard solution (1.779 g barium chloride in 1000 ml of water) to make to 20 ml, adding 2 g of sodium acetate, 1 ml of dilute acetic acid TS and 0.5 ml of potassium chromate TS and allowing to stand for 15 minutes.

**Magnesium and alkali salts**

Dissolve 500 mg of the sample in 30 ml of water and 15 ml of dilute hydrochloric acid TS. Heat the solution and boil for 1 min. Add rapidly 40 ml of oxalic acid TS and stir vigorously. Add 2 drops of methyl red TS and neutralize the solution with ammonia TS to precipitate the calcium completely. Heat the mixture on a steam bath for 1 h, cool, dilute to 100 ml with water, mix well and filter. To 50 ml of the filtrate carefully add 0.5 ml of concentrated sulfuric acid, evaporate to dryness and ignite to constant weight in a tared platinum crucible.

PURITY TESTS (continued)

* **Acid insoluble matter**
  Slake 5 g of the sample, mix with 100 ml of water and sufficient hydrochloric acid, added dropwise, to effect solution. Boil the solution, cool, add hydrochloric acid, if necessary, to make the solution distinctly acid, and filter through a tared crucible. Wash the residue with water until free of chlorides, dry at 105°C for 1 h, cool, and weigh.

* **Heavy metals**
  Mix 2 g of the sample with 25 ml of water, cautiously add 7 ml of hydrochloric acid, followed by 3 ml of nitric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of dilute hydrochloric acid TS and 25 ml of hot water, filter, wash with a few ml of water, and dilute the filtrate to 100 ml with water. A 25-ml portion of this solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

**METHOD OF ASSAY**
Ignite at approximately 800°C about 1 g of the sample to constant weight, accurately weigh the residue and dissolve it in 20 ml of dilute hydrochloric acid TS. Cool the solution, dilute with water to 500 ml and mix. Pipet 50 ml of this solution into a suitable container and add 50 ml of water, then add 15 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 2.804 mg of CaO.

CALCIUM PEROXIDE*  
(Tentative)

SYNONYM  
INS No. 930  
Sometimes incorrectly referred to as calcium dioxide

DEFINITION  
Chemical names  
Calcium peroxide, calcium superoxide

C.A.S. number  
1305-79-9

Chemical formula  
CaO₂  
(empirical)

Formula weight  
72.08

Assay  
The product contains not less than 60 % of CaO₂

DESCRIPTION  
Calcium peroxide occurs as a white or yellowish, odourless, almost tasteless powder or granular material

Caution: Powerful oxidizing substance

FUNCTIONAL USE  
Strengthening agent for flour

* These specifications were prepared at the 9th session of JECFA (1965) and published in NMRS 40ABC (1969).
CALCIUM POLYPHOSPHATE*

SYNONYMS

INS No. 452(iv), EEC No. 544

DEFINITION

Calcium Polyphosphate is a heterogeneous mixture of calcium salts of polyphosphoric acids of general formula \( \text{H}_n\text{P}_{2}\text{O}_{5+n} \).

Assay

Content not less than 50.0 and not more than 71.0% of \( \text{P}_2\text{O}_5 \) on the ignited basis

DESCRIPTION

Odourless, colourless crystals or powder

FUNCTIONAL USES

Emulsifier, moisture-retaining agent, sequestrant, texturizer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Usually incompletely soluble in water; soluble in acid medium

** B. Positive test for phosphate

Mix 0.5 g of the sample with 10 ml of nitric acid and 50 ml of water, boil for about 30 min and cool. The resulting solution gives positive tests for phosphate.

** C. Positive test for calcium

The solution of the above B gives positive tests for calcium

PURITY TESTS

** Loss on ignition

Not more than 2% after drying at 105° for 4 h followed by ignition at 550° for 30 min

** Cyclic phosphate

Not more than 8% calculated on \( \text{P}_2\text{O}_5 \) content

** Fluoride

Not more than 10 mg/kg

** Arsenic

Not more than 3 mg/kg

See description under TESTS

** Lead

Not more than 10 mg/kg

See description under TESTS

** Heavy metals

Not more than 20 mg/kg

See description under TESTS

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* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982).

TESTS

PURITY TESTS

- **Arsonic**
  Dissolve 1 g of the sample in 15 ml of dilute hydrochloric acid TS, add 20 ml of water. Test this solution as directed in the Limit Test (Method II).

- **Lead**
  Dissolve 0.5 g of the sample in 10 ml of dilute hydrochloric acid TS, add 10 ml of water and neutralize to phenolphthalein TS by the addition of strong ammonia TS. Test this solution as directed in the Limit Test.

- **Heavy metals**
  Warm 1 g of the sample with 10 ml of dilute hydrochloric acid TS until no more is dissolved, dilute with water to 25 ml, and filter. Test this solution as directed in the Limit Test (Method I).

METHOD OF ASSAY

Mix about 300 mg of the sample, accurately weighed, with 15 ml of nitric acid and 30 ml of water, boil for 30 min and dilute with water to about 100 ml. Heat at 60°, and add excess of ammonium molybdate TS, and heat at 50° for 30 min. Filter, and wash the precipitate with dilute nitric acid (1 in 36), followed by potassium nitrate solution (1 in 100) until the filtrate is no longer acid to litmus. Dissolve the precipitate in 50 ml of 1 N sodium hydroxide, add phenolphthalein TS, and titrate the excess of sodium hydroxide with 1 N sulfuric acid. Each ml of 1 N sodium hydroxide is equivalent to 3.086 mg of P₂O₅.

CALCIUM PROPIONATE*

SYNONYMS
INS No. 282, EEC No. E282

DEFINITION
Chemical names: Calcium propionate, calcium propanoate.
C.A.S. number: 4075-81-4
Chemical formula: C₈H₈CaO₄
Structural formula: \[\text{CH}_3\text{CH}_2\text{COO} \quad \text{Ca} \quad \text{CH}_3\text{CH}_2\text{COO}\]
Molecular weight: 186.22
Assay: After drying at 120° for 2 h, contains not less than 98% of C₆H₁₀CaO₄.

DESCRIPTION
A white crystalline solid with not more than a faint odour of propionic acid.

FUNCTIONAL USES
Antimould and antirope agent.

CHARACTERISTICS

IDENTIFICATION TESTS
** A. Solubility
Freely soluble in water, soluble in ethanol.

** B. Positive test for calcium
Passes test
To a solution of the sample add ammonium oxalate TS. A white precipitate forms that is soluble in hydrochloric acid, but insoluble in acetic acid.

C. Positive test for propionate
Warm the sample with sulfuric acid. The propionic acid evolved may be recognized by its odour.

D. Positive test for alkali salt of organic acid
Ignite the sample at a relatively low temperature. The alkaline organic acid residue effervescences with acids.

PURITY TESTS
** Loss on drying
Not more than 9.5% after drying at 120° for 2 h.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).
PURITY TESTS (cont’d)

* **pH** 6-9 (1 in 10 soln).

**Water insolubles**

Not more than 0.3%.

Proceed as directed in the specifications for Sodium Propionate

* **Fluoride**

Not more than 10 mg/kg.

Weigh 5 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method I or III).

* **Arsenic**

Not more than 3 mg/kg.

A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* **Iron**

Not more than 50 mg/kg.

Proceed as directed in the specifications for Sodium Propionate

* **Heavy metals**

Not more than 10 mg/kg.

A solution of 2 g of the sample, weighed to the nearest mg, in 25 ml of water meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

**Calcium content**

Chelatometry

Dissolve in a beaker 2.5 g of the sample, weighed to the nearest mg, in 5 ml of hot dilute hydrochloric acid TS. Cool, transfer to a 250-ml volumetric flask, dilute to volume with water, and mix. Transfer 50 ml of the solution to a 400-ml beaker, add 100 ml of water, 25 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation** and 3 ml of naphthol green TS. Titrate with 0.05 M disodium ethylene-diaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 9,311 mg of CaO.

Tare a clean, dry weighing bottle to an accuracy of ± 0.2 mg and weigh to the nearest mg, 0.4 g of the sample, previously dried at 120° for 2 h, into it. Slide the opened weighing bottle into a 200-ml wide-rimmed beaker containing 50 ml of glacial acetic acid and swirl to effect solution. Place a mechanical agitator and a glass-calomel electrode assembly into the beaker in such a manner that the weighing bottle is lodged behind the electrodes and does not move with the agitation. Set the pH meter on the + mV circuit and titrate the sample with 0.1 N perchloric acid. Add the perchloric acid in large increments until the mV-change with each addition shows that the titration is nearing the end point (the deflection of the needle becomes noticeable). Then reduce the added fractions to 0.1 ml and take mV-circuit readings until there is a further decrease in the mV-changes after each addition. Plot the curve obtained from the milliliters added against the mV-readings and determine the quantity of the titrant corresponding to half-way up the steepest gradient.


** An alternative indicator is hydroxynaphthol blue, of which 0.25 g is used. In this case the naphthol green TS is omitted.
Calcium propionate
content (continued)

\[ \text{% calcium propionate} = \frac{V \times N \times 0.09311}{W} \times 100 \]

in which

- \( V \) = ml of the perchloric acid
- \( N \) = exact normality of the perchloric acid, and
- \( W \) = weight (in grams) of the sample

Acid content

Neutralization titration

Weigh, to the nearest mg, 3 g of the sample, previously dried at 120° for 2 h, into a distillation flask and add 200 ml of 50% phosphoric acid. Heat to boiling for 2 h and collect the distillate. During distillation keep the volume in the flask at about 200 ml by adding water using a dropping funnel. Titrate the distillate with \( N \) sodium hydroxide using phenolphthalein TS as indicator. Each ml of \( N \) sodium hydroxide is equivalent to 93.11 mg of \( \text{C}_2\text{H}_4\text{CaO}_4 \).

Acid content

Ion-exchange method

Half fill a chromatographic column* (1.5 cm in diameter, 20 cm long) with a strong cation-exchange resin (Amberlite IR 120, Amberlite IR 100, Duolit C III, Dorvex 50, Lewatit KS, Ion Exchanger I Merck). Add 0.1 \( N \) hydrochloric acid through the top of the column, with the outflow orifice closed, until the resin is completely covered and let stand 1-2 h. Drain the acid and rinse the column with water (about 1 L) until 20 ml of eluate forms a red colour when one drop each of 0.02 \( N \) sodium hydroxide and phenolphthalein TS is added. Weigh, to the nearest mg 0.05 g of the sample, previously dried at 120° for 2 h, into a flask. Dissolve in 15 ml of water and pour slowly into the column. Wash the flask and the column with about 200 ml of water and collect the total filtrate in a conical flask. Add two drops of phenolphthalein TS and titrate with 0.1 \( N \) sodium hydroxide using a micro-burette. Each ml of 0.1 \( N \) sodium hydroxide is equivalent to 9.311 mg of \( \text{C}_4\text{H}_6\text{CaO}_4 \).

* The column may be used for at least four tests without regeneration.
CALCIUM 5'-RIBONUCLEOTIDES*

SYNONYMS

Calcium ribonucleotides
INS No. 634, EEC No. 634

DEFINITION

Chemical name (Mixture of) calcium inosine-5'-monophosphate and calcium guanosine-5'-monophosphate

Chemical formula

\[ C_{10}H_{10}CaN_{3}O_{7}P \cdot xH_2O \]

Structural formula See Structural formula for Calcium 5'-Guanylate and Calcium 5'-Inosinate.

Assay Content not less than 97% and not more than the equivalent of 102% of \( C_{10}H_{12}CaN_{4}O_{7}P \) and \( C_{10}H_{12}CaN_{3}O_{7}P \), calculated on the anhydrous basis. The proportion of \( C_{10}H_{11}CaN_{4}O_{7}P \) or \( C_{10}H_{12}CaN_{3}O_{7}P \) to the sum of them is between 47% and 53%.

DESCRIPTION Odourless, white or off-white crystals or powder having a characteristic taste

FUNCTIONAL USE Flavour enhancer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Sparsely soluble in water

** B. Positive test for ribose Passes test See description under TESTS

** C. Positive test for organic phosphate Passes test See description under TESTS

** D. Positive test for inosinic acid Passes test See description under TESTS

** E. Positive test for guanylic acid Passes test See description under TESTS

** F. Positive test for calcium Passes test

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* These specifications were prepared at the 18th session of JECFA (1974) and published in NMRS 54B (1975).

PURITY TESTS

* pH  
7.0 - 8.0 (1 in 2,000 soln)

* Water  
Not more than 23% (Karl Fisher Method)

* Arsenic  
Not more than 3 mg/kg  
Proceed as directed in the specifications for Glutamic Acid

* Lead  
Not more than 10 mg/kg  
Proceed as directed in the specifications for Glutamic Acid

* Heavy metals  
Not more than 20 mg/kg  
Proceed as directed in the specifications for Glutamic Acid

Water soluble matter  
Passes test  
See description under TESTS

Amino acid  
Not detected by the following test. To 5 ml of a 1 in 2,000 solution add 1 ml of ninhydrin TS and heat for 3 min. No colour is produced.

Related foreign substances  
Chromatographically not detectable  
See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Positive test for ribose  
To 3 ml of a 3 in 10,000 solution add 0.2 ml of a 1 in 10 alcohol solution of orcinol and 3 ml of a 1 in 1,000 hydrochloric acid solution of ferric ammonium sulfate. Heat in a water bath for 10 min. A green colour is produced.

C. Positive test for organic phosphate  
To 5 ml of a 1 in 2,000 solution add 2 ml of magnesia mixture TS. No precipitate is formed. To 5 ml of a 1 in 50 solution add 5 ml of nitric acid, boil for 10 min, and neutralize with sodium hydroxide TS, add ammonium molybdate TS and warm. A yellow precipitate is formed, which dissolves in sodium hydroxide TS or ammonia TS.

D. Positive test for inosinic acid  
To 2 ml of a 1 in 2,000 solution add 2 ml of 10% hydrochloric acid and 0.1 g of zinc powder, heat in a water bath for 10 min, and filter. Cool the filtrate in ice water, add 1 ml of a 3 in 1,000 sodium nitrite solution, shake well, and allow to stand for 10 min. Add 1 ml of a 1 in 200 ammonium sulfamate solution, shake well, and allow to stand for 5 min. Add 1 ml of a 1 in 500 N-(1-naphthyl)-ethylenediamine dihydrochloride solution. A violet red colour is produced.

E. Positive test for guanylic acid  
To 1 ml of a 1 in 5,000 solution add 1 ml of 10% hydrochloric acid, heat in a water bath for 10 min, cool, and add 0.5 ml of Folin-Ciocalteu TS and 2 ml of saturated sodium carbonate solution. A blue colour is produced.

PURITY TESTS

**Water soluble matter**

To 1 g of the sample, add 50 ml of water, allow to stand for 10 min with occasional shaking, filter through analytical grade filter paper (Whatman No. 42 or equivalent). Evaporate a 25 ml portion of the solution to dryness on a water bath and dry the residue at 105° for 1 h. Residue weighs less than 80 mg.

**Related foreign substances**

Proceed as directed under "Thin-layer chromatography" in the General Methods*, using 5 μl of a 1 in 1,000 solution as the sample solution, a mixture of 80 volumes of a saturated solution of ammonium sulfate, 18 volumes of a 13.6% w/v solution of sodium acetate and 2 volumes of isopropyl alcohol as the developing solvent, and microcrystalline cellulose as the adsorbent. Stop the development when the solvent front has advanced about 10 cm from the point of the application, dry the plate in air, and observe under ultraviolet light (about 254 nm) in a dark place. No spot other than those of 5'-inosinic acid and 5'-guanylic acid is detected.

**METHOD OF ASSAY**

Calculate the contents of calcium inosine-5'-monophosphate (I) and calcium guanosine-5'-monophosphate (G) in the sample by the following equation, using values for I₀ and G₀ obtained as described below:

\[
\text{Content (\%)} = \frac{\text{I}_0 + \text{G}_0}{100 - \text{water (\%)}} \times 100
\]

Weigh accurately about 650 mg of the sample, and dissolve in water to make 500 ml (Solution A).

To determine I₀ (calcium inosine-5'-monophosphate), take a 1-ml portion of Solution A, add 4 ml of 6 N hydrochloric acid and water to make to 10 ml. Heat in a water bath for 40 min, cool, add 0.4 g of zinc powder, allow to stand for 50 min, shaking occasionally and vigorously, and add water to make to 20 ml. Filter through filter paper. To a 10-ml portion of the filtrate add 1 ml of 6 N hydrochloric acid, and add 1 ml of a 1 in 1,000 sodium nitrite solution, cooling in an ice-water bath. Shake well, allow to stand for 10 min, add 1 ml of a 1 in 200 ammonium sulfamate solution, shake well, and allow to stand for 5 min. Add 1 ml of a 1 in 500 N-(1-naphthyl)-ethylenediamine dihydrochloride solution, shake well, allow to stand for 15 min at room temperature, and add water to make to 20 ml (Sample solution). For the control, prepare in the same manner as the sample, using 1 ml of water instead of Solution A. Determine the absorbance of the sample solution at 515 nm against the control solution.

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To prepare calibration curves, weigh accurately about 3 mg each of disodium 5'-inosinate and disodium 5'-guanylate, and dissolve respectively in 100 ml of 0.01 N hydrochloric acid. Determine the absorbance at 250 nm on the solution of disodium 5'-inosinate and at 260 nm on the solution of disodium 5'-guanylate, using 0.01 N hydrochloric acid as the control. Determine the molecular extinction coefficients $E_1$ and $E_0$, and calculate the contents of (I) disodium inosine-5'-monophosphate and (G) disodium guanosine-5'-monophosphate by the equations:

Content (%) of I = \( \frac{E_1}{12,160} \times 100 \)

Content (%) of G = \( \frac{E_0}{11,800} \times 100 \)

Weigh accurately a quantity of each which is equivalent to about 50 mg, combine and dissolve in water to make 200 ml (Solution B). To 1-ml, 2-ml and 3-ml portions of Solution B add 4 ml of 6 N hydrochloric acid and make each to 10 ml with water. Prepare Standard Solutions in the same manner as directed for preparing Sample Solution from Solution A. Determine the absorbance of each Standard Solution at 515 nm and prepare the calibration curve. For the control, use the control solution used for Sample Solution. Calculate the content of I (disodium inosine-5'-monophosphate) from the calibration curve and the absorbance of Sample Solution.

From the content of I, calculate the content of $I_{\text{ca}}$ (calcium inosine 5'-monophosphate) as 0.985 x I.

To determine $G_{\text{ca}}$ (calcium guanosine-5'-monophosphate), take 1 ml of Solution A, add 4 ml of 2 N hydrochloric acid and water to make to 10 ml. Heat in a water bath for 30 min, cool, add 2 ml of Folin-Ciocalteu TS and 5 ml of a 4 in 5 sodium carbonate solution. Allow to stand for 15 min, and add water to make to 50 ml. Centrifuge if necessary, and use the supernatant for the test (Sample Solution).

Prepare the control in the same manner as the Sample Solution, using 1 ml of water instead of Solution A. Determine the absorbance of the Sample Solution at 750 nm.

To 1-ml, 2-ml and 3-ml portions of Solution B, add 4 ml of 2 N hydrochloric acid and make each to 10 ml with water. Prepare Standard Solutions in the same manner as directed in preparing Sample Solution. Determine the absorbance of each Standard Solution at 750 nm, and prepare the calibration curve. For the control, use the control solution used for Sample Solution. Calculate the content of G (disodium guanosine-5'-monophosphate) from the calibration curve and the absorbance of Sample Solution.

From the content of G calculate the content of $G_{\text{ca}}$ (calcium guanosine-5'-monophosphate) as 0.986 x G.
CALCIUM SACCHARIN*  

SYNONYMS
INS No. 954

DEFINITION
Chemical names
Calcium salt hydrate (2:7) of 1,2-benzisothiazole-3-one-1,1-dioxide, 3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide, 2,3-dihydro-3-oxobenzisulfonazole; calcium o-benzosulfimide.

Chemical formula
C_{14}H_8CaN_2O_6S_2 · 3\frac{1}{2}H_2O

Structural formula
\[
\begin{array}{c}
\text{N} \\
\text{SO}_2
\end{array}
\]
Ca · 3\frac{1}{2}H_2O

Molecular weight
467.48

Assay
Content not less than 99% C_{14}H_8CaN_2O_6S_2 on the dried basis

DESCRIPTION
White crystals or a white, crystalline powder, odourless or with a faint, aromatic odour having a sweet taste even in very dilute solutions. About 500 times as sweet as sucrose in dilute solutions.

FUNCTIONAL USE
Sweetening agent

CHARACTERISTICS

IDENTIFICATION TESTS

**A. Solubility**
Freely soluble in water, soluble in ethanol

**B. Melting range**
Passes test
Proceed as directed in the specifications for Sodium Saccharin

**C. Derivation to salicylic acid**
Passes test
Proceed as directed in the specifications for Saccharin

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* These specifications were prepared at the 24th session of JECFA (1980) and published in FNP 17 (1980).

IDENTIFICATION TESTS (continued)

D. Derivation to fluorescent substance
   Passes test
   Proceed as directed in the specifications for Saccharin

* E. Positive test for calcium
   Passes test

PURITY TESTS

* Loss on drying
   Not more than 15% (120°, 4 h)

* Arsenic
   Not more than 3 mg/kg (Method II)

* Selenium
   Not more than 30 mg/kg

* Heavy metals
   Not more than 10 mg/kg
   Test 2 g of the sample as directed in the Limit Test (Method II).

  Benzoic and salicylic acid
   Add ferric chloride TS dropwise to 10 ml of a hot, saturated solution of the sample. No precipitate or violet colour appears.

* Readily carbonizable substances
   Dissolve 0.2 g of the sample in 5 ml of sulfuric acid TS. Keep at 48° to 50° for 10 min. The colour should not be darker than a very light brownish-yellow (Matching Fluid A).

  Toluenesulfonamides
   Not more than 25 mg/kg
   Proceed as directed in the specifications for Saccharin

METHOD OF ASSAY

Weigh accurately about 0.5 g of the sample and transfer quantitatively to a separator with the aid of 10 ml of water. Add 2 ml of dilute hydrochloric acid TS, and extract the precipitated saccharin first with 30 ml, then with five 20 ml portions, of a liquid composed of 9 volumes of chloroform and 1 volume of ethanol. Filter each extract through a small filter paper moistened with the solvent mixture. Evaporate the combined filtrates on a steam bath to dryness with the aid of a current of air. Dissolve the residue in 75 ml of hot water, cool, add phenolphthalein TS, and titrate with 0.1 N sodium hydroxide. Perform a blank determination, and make any necessary correction. Each ml of 0.1 N sodium hydroxide is equivalent to 20.22 mg of C12H4CaN2O6S2.

CALCİUM SILİCATE*

SYNONYMS
INS No.552, EEC No.552

DEFINITION
Calcium Silicate is a synthetic hydrous calcium silicate or polysilicate prepared by a variety of reactions between siliceous material (e.g. diatomaceous earth) and natural calcium compounds (e.g. lime with varying proportions of other elements, such as magnesium, etc). The article of commerce may be further specified as to calcium and silicon dioxide contents, loss on drying, loss on ignition, pH of a 10% water slurry, bulk density, moisture, sulfate and chloride.

Chemical name  Calcium silicate
C.A.S. number  1344-95-2

DESCRIPTION
Calcium Silicate is a very fine, white or off-white powder with low bulk density and high physical water absorption.

FUNCTIONAL USE
Anticaking agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Insoluble in water and ethanol.

B. Positive test for silicate
Passes test.
Proceed as directed in the identification test for silicate under Aluminium Silicate.

C. Positive test for calcium
Passes test.
See description under TESTS.

PURITY TESTS

** Fluoride
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 I or II).

Asbestos
Absent as determined by the Method given in the specifications for Talc.

Arsenic
Not more than 3 mg/kg.
See description under TESTS.

Lead
Not more than 10 mg/kg.
See description under TESTS.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

PURITY TESTS (continued)

Heavy metals

Not more than 40 mg/kg.
See description under TESTS.

TESTS

IDENTIFICATION TESTS

* C. Positive test for calcium

Neutralize the filtrate obtained in Test B above with ammonia TS using 2 drops methyl red TS as indicator. Then add dilute hydrochloric acid TS dropwise until the solution is acid. Upon the addition of ammonium oxalate TS a white granular precipitate of calcium oxalate forms. This precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

PURITY TESTS

* Arsenic

A 10 ml portion of the sample solution (see below) diluted to 35 ml with water meets the requirements of the Limit Test for Arsenic (Method II).

* Lead

Neutralize a 10 ml portion of the sample solution (see below) with ammonia TS, using phenolphthalein as indicator, and dilute to 20 ml with water. This solution meets the requirements of the Limit Test for Lead, using 10 μg of lead ion (Pb) in the control.

* Heavy metals

A 5 ml portion of the sample solution (see below) diluted to 25 ml with water meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

Sample solution for the determination of arsenic, lead and heavy metals:

Weigh 10 g of the sample to the nearest mg, and transfer into a 250 ml beaker. Add 50 ml of 0.5 N hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool and let the undissolved material settle. Decant the supernatant liquid through Whatman No.4 or equivalent filter paper into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portion of hot water, decanting each washing through the filter paper into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water and mix.

CALCIUM SORBATE*

SYNONYMS

INS No. 203, EEC No. E203

DEFINITION

Chemical names
C A S. number
Chemical formula
Structural formula
Molecular weight
Assay

CA LCIUM SO RBATE; calcium salt of trans, trans 2,4-hexadienoic acid.
7492-55-9
C_{12}H_{14}CaO_{4}

After drying for 4 h in a desiccator over sulfuric acid, contains not less than 98% and not more than the equivalent of 102% of C_{12}H_{14}CaO_{4}.

DESCRIPTION

A fine white crystalline powder not showing any change in colour after heating at 105° for 90 min.

FUNCTIONAL USES

Antimicrobial preservative, fungistatic agent.

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility

Soluble in water; practically insoluble in ethanol.

** B. Positive test for calcium

Passes test
To a saturated solution of the sample add ammonium oxalate TS. A white precipitate forms that is soluble in hydrochloric acid, but insoluble in acetic acid.

C. Positive test for sorbate

Melting range of sorbic acid derived from the sample is between 130° to 135°. Proceed as directed in the specifications for Potassium Sorbate.

* These specifications were prepared at the 17th session of JECFA (1973) and published in FNP 4 (1978).

IDENTIFICATION TESTS (continued)

D. Positive test for double bond

To 2 ml of a 1 in 10 solution of the sample add a few drops of bromine TS. The colour of the bromine disappears.

PURITY TESTS

* Loss on drying

Not more than 3% after drying for 4 h over sulfuric acid in a vacuum desiccator.

* Fluoride

Not more than 10 mg/kg.

Weigh 5 g of the sample to the nearest mg and proceed as directed in the Fluoride Limit Test (Method I or III).

* Arsenic

Not more than 3 mg/kg.

A sample solution prepared as directed for organic compounds meets the requirements of the Limit Test for Arsenic (Method II).

* Heavy metals

Not more than 10 mg/kg.

Test 2 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 μg of lead ion (Pb) in the control (Solution A).

Aldehydes

Not more than 0.1% (as formaldehyde)

Proceed as directed in the specifications for Potassium Sorbate.

METHOD OF ASSAY

Neutralization titration

Weigh to the nearest mg, 0.25 g of the sample, previously dried for 4 h in a desiccator over sulfuric acid. Dissolve in 35 ml of glacial acetic acid and 4 ml of acetic anhydride in a 250-ml glass stoppered flask, warming to effect solution. Cool to room temperature, add 2 drops of crystal violet TS and titrate with 0.1 N perchloric acid in glacial acetic acid to a blue-green end point which persists for at least 30 sec. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 13.12 mg of C₁₂H₁₀CaO₄.

CALCIUM STEAROYL LACTYLATE*
(Tentative)**

SYNONYMS
Calcium stearoyl lactate
INS No. 482(i), EEC No. E482

DEFINITION
Calcium Stearoyl Lactate is a mixture of calcium salts of the reaction products formed by combining fatty acids with lactic acid. The mixture contains fatty acid salts, salts of fatty acid esters of lactic acid and salts of fatty acid esters of polymerized lactic acid. The component in primary abundance is calcium stearoyl lactate, the calcium salt of the stearic acid ester of lactic acid. The fatty acid component is primarily stearic acid with related food fatty acids present in lesser abundance.

Chemical name
Calcium bis[2-(2-stearoyloxy-propionyloxy)]propionate, calcium di-(2-stearoyloxy)-propionate

C.A.S. number
5793-94-2

Structural formula
\[
\text{CH}_3
\]
\[
(C_{17}H_{35}-\text{COO}-\text{CH}-\text{COO})_2\text{Ca}
\]

DESCRIPTION
White or slightly yellowish powder or brittle solid with a characteristic odour

FUNCTIONAL USES
Emulsifier, stabilizer

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility
Slightly soluble in hot water

B. Positive test for calcium
Passes test
See description under TESTS

C. Positive test for fatty acid
Passes test
See description under TESTS

*** D. Positive test for lactate
Passes test

* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/2 (1984).

** Information required on nomenclature including principal name and synonyms, composition on items in commerce and limit value of purity tests as listed in the next page.

PURITY TESTS

* Calcium content
  Information required

* Arsenic
  Not more than 3 mg/kg (Method II)

* Heavy metals
  Not more than 10 mg/kg
  Test 2 g of the sample as directed in the Limit Test (Method II)

* Total lactic acid
  Information required

* Acid value
  Information required

* Ester value
  Information required

TESTS

IDENTIFICATION TESTS

B. Positive test for calcium

Add 10 ml of dilute hydrochloric acid TS to 2 g of the lactate sample, heat for 5 min in a water bath, filter and neutralize the filtrate with ammonia TS. Add 5 ml of ammonium oxalate TS. A white precipitate is formed, soluble in dilute hydrochloric acid TS, but insoluble in dilute acetic acid TS.

C. Positive test for fatty acid

Take the residue from the filter in test B, add 30 ml of sodium hydroxide TS, sodium hydroxide TS, heat for 30 min on a steam bath and filter. Add 20 ml of dilute hydrochloric acid TS to the filtrate after cooling, extract twice with 30 ml of ether, wash the ether solution with 20 ml of water, dehydrate with anhydrous sodium sulfate and evaporate the ether. The residue melts between 54° and 69°.

CALCIUM SULFATE

SYNONYMS
INS No. 516, EEC No. 516

DEFINITION
Chemical name: Calcium sulfate
C.A.S. number: 7778-18-9
Chemical formula:

- Anhydrous: CaSO₄
- Dihydrate: CaSO₄·2H₂O

Formula weight:

- Anhydrous: 136.14
- Dihydrate: 172.18

Assay: Calcium Sulfate contains not less than 99.0% of CaSO₄, calculated on the dried basis.

DESCRIPTION
Fine, white to slightly yellow-white, odourless powder

FUNCTIONAL USES
Yeast food, dough conditioner, firming agent, sequestrant

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Slightly soluble in water
Insoluble in ethanol

B. Positive test for calcium
Passes test
See description under TESTS

C. Positive test for sulfate
Passes test
See description under TESTS

PURITY TESTS

** Loss on drying
Anhydrous: Not more than 1.5% (250° to constant weight)
Dihydrate: Between 19 and 23% (250° to constant weight)

** Fluoride
Not more than 30 mg/kg (Method I or III)

** Arsenic
Not more than 3 mg/kg
See description under TESTS

** Selenium
Not more than 30 mg/kg
0.2 g of the sample meets the requirements of the Limit Test for Selenium (Method II)

* These specifications were prepared at the 19th session of JECFA (1975) and published in NMRS 55B (1976).

PURITY TESTS (continued)

**Lead**

Not more than 10 mg/kg

See description under TESTS

**Heavy metals**

Not more than 20 mg/kg

See description under TESTS

TESTS

IDENTIFICATION TESTS

B. Positive test for calcium

Warm 0.2 g of the sample with 4 ml of dilute hydrochloric acid TS and 16 ml of water (Solution A). To 10 ml of Solution A add 5 ml of ammonium oxalate TS. A white precipitate is formed.

C. Positive test for sulfate

To the remaining 10 ml of Solution A (see "B" above) add barium chloride TS. A white precipitate is formed which is insoluble in hydrochloric and nitric acid.

PURITY TESTS

" Arsenic

Mix 1 g of the sample with 10 ml of water, add 12 ml of dilute hydrochloric acid TS, and heat to boiling to dissolve the sample. Cool, filter, and dilute the filtrate to 35 ml with water. This solution meets the requirements of the Limit Test for Arsenic (Method II).

" Lead

Mix 1 g of the sample with 10 ml of water, add 15 ml of dilute hydrochloric acid TS, and heat to boiling to dissolve the sample. Cool, and neutralize to phenolphthalein TS. Filter, evaporate to about 20 ml, cool, and refilter if necessary to obtain a clear solution. This solution meets the requirements of the Limit Test for Lead.

" Heavy metals

Mix 1 g of the sample with 10 ml of water, add 15 ml of dilute hydrochloric acid TS, and heat to boiling to dissolve the sample. Cool, and add ammonium hydroxide to pH 7. Filter, evaporate to a volume of about 25 ml, and refilter if necessary to obtain a clear solution. This solution meets the requirements of the Limit Test for Heavy Metals (Method I) using 20 μg of lead ion (Pb) in the control (Solution A).

METHOD OF ASSAY

Chelatometry

Dissolve about 250 mg of the sample, accurately weighed, in 100 ml of water and 4 ml of dilute hydrochloric acid TS, boil if necessary to effect solution, and cool. Add 15 ml of sodium hydroxide TS, 40 mg of murexide indicator preparation and 3 ml of naphthol green TS, and titrate with 0.05 M disodium ethylenediaminetetraacetate until the solution is deep blue in colour. Each ml of 0.05 M disodium ethylenediaminetetraacetate is equivalent to 6.807 mg of CaSO₄.

CANTHAZANTHIN*

SYNONYMS
Cl Food Orange 8; INS No. 161g, EEC No. E161

DEFINITION
These specifications apply to predominantly all trans (Z)isomers of canthaxanthin together with minor amounts of other carotenoids. Diluted and stabilized forms are prepared from canthaxanthin meeting these specifications and include solutions or suspensions of canthaxanthin in edible fats or oils, emulsions and water dispersible powders. These preparations may have different cis/trans isomer ratios.**

Class
Carotenoid

Code numbers
Cl (1975) No 40850
CAS No 514-78-3

Chemical names
β-Carotene-4,4'-dione, canthaxanthin, 4,4'-dioxo-β-carotene

Chemical formula
C₃₀H₆₂O₂

Structural formula

Molecular weight
564.86

Assay
Not less than 96% of total colouring matters (expressed as canthaxanthin).

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

---

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).

These specifications define only synthetic canthaxanthin. The Committee was aware of naturally occurring canthaxanthin but had no indication of a commercial available food colour obtained from natural sources.

** The analytical methods described for the parent colour are not necessarily suitable for the assay of or determination of impurities in the stabilized forms. (Appropriate methods should be available from the manufacturer).
CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility

** B. Spectrophotometry
   A solution of canthaxanthin in cyclohexane has an absorbance maximum between 468 and 472 nm.

C. Positive test for carotenoid
   The colour of a solution of canthaxanthin in acetone disappears after successive additions of a 5% solution of sodium nitrite and 1N sulfuric acid.

D. Carr-Price reaction
   A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price reagent TS.

PURITY TESTS

* Sulfated ash
   Not more than 0.1%
   Proceed as directed under the test for Ash (Sulfated ash, Method I) using 2 g of sample.

*** Arsenic
   Not more than 3 mg/kg

*** Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 40 mg/kg
   Test 1 g of the sample as directed in the Heavy Metals Limit Test (Method II).

   Subsidiary colouring matters
   Carotenoids other than canthaxanthin:
   Not more than 5% of total colouring matters.
   Proceed as directed in the specifications for β-Carotene, Synthetic except that the absorbance is determined at the wavelength maximum of Canthaxanthin, about 485 nm.

METHOD OF ASSAY

Proceed as directed in Method of Assay for Oil-Soluble Food Colours** using the following conditions:

\[
W = 0.1 \text{ g} \\
V_1 = V_2 = V_3 = 100 \text{ ml} \\
v_1 = v_2 = 5 \text{ ml} \\
A^2 = 2200 \\
\lambda_{\text{max}} = \text{about } 470 \text{ nm}
\]


CARAMEL COLOURS

SYNONYMS

Class I: Plain caramel, caustic caramel
Class II: Caustic sulfite caramel
Class III: Ammonia caramel,
Class IV: Sulfite ammonia caramel
INS No. 150a,b,c,d; EEC No. E150a,b,c,d

DEFINITION

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

Class I - prepared by heating carbohydrates with or without acids or alkalis; no ammonium or sulfite compounds are used.

Class II - prepared by heating carbohydrates with or without acids or alkalis in the presence of sulfite compounds; no ammonium compounds are used.

Class III - prepared by heating carbohydrates with or without acids or alkalis in the presence of ammonium compounds; no sulfite compounds are used.

Class IV - prepared by heating carbohydrates with or without acids or alkalis in the presence of both sulfite and ammonium compounds.

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

Where ammonium compounds are used they are one or any of the following:

- ammonium hydroxide
- ammonium carbonate and ammonium hydrogen carbonate
- ammonium phosphate
- ammonium sulfate
- ammonium sulfite and ammonium hydrogen sulfite

Where sulfite compounds are used they are one or any of the following:

- sulfurous acid
- potassium, sodium and ammonium sulfites and hydrogen sulfites

Food-grade antifoams may be used as processing aids during manufacture.

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988).
DESCRIPTION
Dark brown to black liquids or solids having an odour of burnt sugar and a somewhat bitter taste.

FUNCTIONAL USE
Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
  Miscible with water

B. Classification
  Caramel Colour I: Not more than 50% of the colour is bound by DEAE Cellulose and not more than 50% of the colour is bound by Phosphoryl Cellulose.

  Caramel Colour II: More than 50% of the colour is bound by DEAE Cellulose and it exhibits an Absorbance Ratio of more than 50.

  Caramel Colour III: Not more than 50% of the colour is bound by DEAE Cellulose and more than 50% of the colour is bound by Phosphoryl Cellulose.

  Caramel Colour IV: More than 50% of the colour is bound by DEAE Cellulose and it exhibits an Absorbance Ratio of not more than 50.

See description under TESTS

PURITY TESTS

The following limits and ranges apply to the individual classes of Caramel Colours as indicated and, unless otherwise stated, are expressed on a solids basis.

For methods see descriptions under TESTS

<table>
<thead>
<tr>
<th></th>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>Class IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid content</td>
<td>62-77%</td>
<td>65-72%</td>
<td>53-83%</td>
<td>40-75%</td>
</tr>
<tr>
<td>Colour intensity</td>
<td>0.01-0.12</td>
<td>0.06-0.10</td>
<td>0.08-0.36</td>
<td>0.10-0.60</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>max 0.1%</td>
<td>max 0.2%</td>
<td>1.3 -6.8%</td>
<td>0.5-7.5%</td>
</tr>
<tr>
<td>Total sulfur</td>
<td>max 0.3%</td>
<td>1.3 -2.5%</td>
<td>max. 0.3%</td>
<td>1.4-10.0%</td>
</tr>
<tr>
<td>Sulfur dioxide</td>
<td>-</td>
<td>max 0.2%</td>
<td>-</td>
<td>max 0.5%</td>
</tr>
<tr>
<td>Ammoniacal nitrogen</td>
<td>-</td>
<td>-</td>
<td>max. 0.4%</td>
<td>max 2.8%</td>
</tr>
<tr>
<td>4-Methylimidazole (MEDI)</td>
<td>-</td>
<td>-</td>
<td>max 300 mg/kg &amp; max 200 mg/kg on an equivalent colour basis</td>
<td>max 1000 mg/kg &amp; max 250 mg/kg on an equivalent colour basis</td>
</tr>
<tr>
<td>2-Acetyl-4-tetrahydroxybutylimidazole (THI)</td>
<td>-</td>
<td>-</td>
<td>max 40 mg/kg &amp; max 25 mg/kg on an equivalent colour basis</td>
<td>-</td>
</tr>
</tbody>
</table>

PURITY TESTS (continued)

The following limits apply to all classes of caramel and are expressed on the basis of the product as is:

* Arsenic
  Not more than 1 mg/kg

* Lead
  Not more than 2 mg/kg

** Heavy Metals
  Not more than 25 mg/kg
  Prepare and test an 800 mg sample as directed in Method II under the Limit Test for Heavy Metals, using 20 μg of lead ion (Pb) in the control (Solution A).

IDENTIFICATION TESTS

B. Classification

Colour bound by DEAE Cellulose

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

Special Reagent

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

Procedure

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

\[
\frac{(X_1 - X_2)}{X_1} \times 100
\]

in which \(X_1\) is the absorbance of the caramel colour solution at 560 nm, and \(X_2\) is the absorbance of the supernatant after DEAE Cellulose treatment at 560 nm.


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

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

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

\[
\frac{(X_1 - X_2)}{X_1} \times 100
\]

in which \(X_1\) is the absorbance of the caramel colour solution at 560 nm, and \(X_2\) is the absorbance of the supernatant after Phosphoryl Cellulose treatment at 560 nm.

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

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

PURITY TESTS

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

\[
\% \text{ solids} = \frac{w_p - w_s}{w_c} \times 100
\]

Where

- \( w_p \) = final weight of sand plus caramel
- \( w_s \) = weight of sand
- \( w_c \) = weight of caramel initially added

The contents of Total Nitrogen, Total sulfur, Ammoniacal nitrogen, sulfur dioxide, 4-MEI and THI are expressed on a solids basis. The concentration \( C_1 \) of each impurity is determined on an "as is" basis; the concentration \( C_2 \) on a solid basis is then calculated using the formula:

\[
C_2 = \frac{C_1 \times 100}{\% \text{ solids}}
\]

Colour Intensity

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

Procedure

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

\[
\text{Colour Intensity} = \frac{A_{610} \times 100}{\% \text{ solids}}
\]

Where \% solids is determined as described in the section under Solids content.

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

\[
C_s = \frac{C_2}{\text{Equivalent colour basis}} \times 0.1
\]

Equivalent colour basis = Colour intensity

where \( C_s \) = concentration on a solids basis.

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

* **Total Nitrogen**

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

**Total sulfur**

In the largest available casserole that fits in an electric muffle furnace, place 1-3 g MgO or equivalent quantity of Mg(NO$_3$)$_2$, 6H$_2$O (6.4 - 19.2 g), 1 g powdered sucrose, and 50 ml HNO$_3$. Add 5-10 g caramel colour. Place same quantities of reagents in another casserole for blank. Evaporate on steam bath to paste. Place casserole in cold electric muffle (25°) and gradually heat until all NO$_2$ fumes are driven off. Cool, dissolve and neutralize with HCl (1+2.5), adding excess of 5 ml. Filter, heat to boiling, and add 5 ml 10% BaCl$_2$ 2H$_2$O solution dropwise. Evaporate to 100 ml, let stand overnight, filter, wash, ignite, and weigh the BaSO$_4$. Correct result for BaSO$_4$ obtained in blank and report as mg S/100 g.

Commercial instruments that analyse for total sulfur such as, the Leco-Combustion/Titration procedure can also be used and are recommended for sample amounts of about 200 mg.

**Sulfur dioxide**

**Apparatus**

Use a modified Monier-Williams apparatus* for the determination of sulfurous acid, or construct the apparatus as shown in the figure. The assembly consists of a 1000-ml three-neck round-bottom distillation flask having 24/40 standard-taper ground-glass joints. A 30-cm Allihn condenser is attached in the reflux position to an outer neck of the flask, and the other end of the condenser is connected with 1/4-inch Tygon or silicon tubing (preboiled with 1 in 20 hydrochloric acid solution and rinsed with water) to the absorption tube assembly (having 35/20 ball joints or the equivalent). Connect the centre neck of the flask with a 125-ml cylindrical separator, and attach a piece of tubing to a short U-tube inserted through a rubber stopper in the neck of the separator. Attach a curved glass inlet tube, reaching nearly to the bottom of the flask, to the other outer neck of the flask, and connect the inlet tube to a 250-ml gas-washing bottle with a piece of the tubing. The gas-washing bottle, in turn, is connected by tubing to a nitrogen cylinder.

Grind 4.5 g of pyrogallol (pyrogallic acid) with 5 ml of water in a small mortar, and transfer the slurry to the gas-washing bottle. Grind the residue again, and transfer

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* Available from 5GA Scientific, Inc., Bloomfield, N.J., USA.
Sulfur dioxide
(continued)

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

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

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

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

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

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

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

\[
\text{Sulfur dioxide } S_0 \% = \frac{(S - B) \times 0.0032 \times 100}{W}
\]

in which W is the weight of the sample taken, in g.
**Ammonium nitrogen**

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

\[
\text{Ammoniacal nitrogen} = (B - S) \times 0.0014 \times 100/W
\]

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

**4-Methylimidazole**

The following materials and reagents are required (the reagents should be ACS grade or equivalent where applicable).

**Materials**
- Pyrex glasswool, 22 x 300 mm chromatography column with PTFE stopcock (e.g. Kimax 17800);
- 150 ml polypropylene beaker (e.g. Nalge 1201);
- 250 ml round-bottom flask (e.g., Pyrex 4320);
- 75 mm powder funnel;
- 5 cm spatula;
- rotary vacuum evaporator;
- hot plate;
- pan for water bath;
- disposable Pasteur pipets;
- 5 ml volumetric flask.

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

**Procedure**

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

* Information on an improved method is sought.
* 4-Methylimidazole (continued)

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

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

The bulk of the solvent is then removed from the eluate on a rotary vacuum evaporator operated at 45-50 kPa and with the round-bottom flask maintained at 35° in a water bath. The extracted residue is transferred quantitatively to a 5 ml volumetric flask with a disposable Pasteur pipet, by rinsing the round-bottom flask several times with small (ca. 0.75 ml) portions of either tetrahydrofuran or acetone. Both solvents have been used with equal success. After mixing the contents thoroughly by several inversions of the flask, the extract is ready for GLC analysis. The extracts should be analysed as soon as possible after their preparation, because stability problems have occasionally been encountered with extracts more than 1 day old.

The GLC analysis is carried out using a gas chromatograph equipped with a hydrogen flame detector. The column is glass, 1 mm x 6 mm o.d. x 4 mm i.d., filled with 7.5% Carbowax 20M + 2% KOH on 90/100 mesh Anakrom ABS. The GLC parameters are as follows: carrier, nitrogen, 50 ml/min; hydrogen, 50 ml/min; oxygen, 80 ml/min; injection port, 200°; column isothermal, 180°; detector, 250°; sample size, 5 μl. All quantitation is done by using the internal standard technique.

* 2-Acetyl-4-tetrahydroxybutylimidazole (THI)

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

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

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

The solution is concentrated to dryness at 40°C and 15 torr. The syrup residue is dissolved in carbonyl-free methanol (250 µl) and the 2,4-dinitrophenylhydrazine reagent (250 µl) is added. The reaction mixture is transferred to a septum-capped vial and stored for 5 h at room temperature.

A volume of 5 µl (but also from 1 to 25 µl) is injected onto a LiChrosorb RP-8 (10 µm) HPLC column. The mobile phase is MeOH/0.1 M H₃PO₄ 50/50 (v/v). Adjustments in mobile phase composition may be needed as column characteristics vary, depending upon the manufacturer. (Use of LiChrosorb RP-8, 10 µm, 250 x 4 mm "Vertex" column manufactured by Knauer, Bad Homburg, F.R.G. is strongly recommended). At a mobile phase flow rate of 2 ml/min and column dimensions of 250 x 4.6 mm, THI-DNPH is eluted at about 6.3 ± 0.1 min. It is detected at 385 nm and the peak height is measured. The amount is calculated from a calibration curve prepared with THI-DNPH in methanol.

Materials
- 2,4-Dinitrophenylhydrazine hydrochloride reagent:
  Commercial 2,4-dinitrophenylhydrazine (5 g) is added to concentrated hydrochloric acid (10 ml) in a 100-ml erlenmeyer flask, and the latter is gently shaken until the free base (red) is converted to the hydrochloride (yellow). Ethanol (100 ml) is added and the mixture is heated on a steam bath until all the solid has dissolved. After crystallization at room temperature, the hydrochloride is filtered off, washed with ether, dried at room temperature and stored in a desiccator. On storage the hydrochloride is slowly converted to the free base. The latter can be removed by washing with dimethoxyethane. The reagent is prepared by mixing 0.5 g of 2,4-dinitrophenylhydrazine hydrochloride in 15 ml of 5% methanol in dimethoxyethane for 30 min. It should be stored in the refrigerator and be checked periodically by HPLC.
- Cation-exchange resin (strong): Dowex 50 AG x 8, H⁺, 100-200 mesh.
- Cation-exchange resin (weak): Amberlite CG AG 50 1, H⁺, (100-200 mesh). (Sediment two or three times prior to use).

* Information on an improved method is sought.
* 2-Acetyl-4-tetrahydroxybutylimidazole (THI)

(continued)

- Dimethoxyethane: If impure, dimethoxyethane is purified by distillation from 2,4-dinitrophenylhydrazine in the presence of acid and redistilled from sodium hydroxide. Immediately prior to use it is passed through a column of neutral aluminium to remove peroxides.

Instrumental

Combination Columns

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

HPLC

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

Calibration

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

* Information on an improved method is sought.
CARBOHYDRASE FROM ASPERGILLUS NIGER, VAR.*

(Experimental)

SOURCES
Commercial enzyme preparations are produced by the controlled fermentation of Aspergillus niger, var.

ACTIVE PRINCIPLES
1. α-Amylase (Glycogenase)
2. Pectinase: usually mixture of following two enzymes
   2-a. Polygalacturonase (Pectin depolymerase)
   2-b. Pectin methyl esterase
3. Cellulase
4. Glucoamylase (Amyloglucosidase, Glucan 1,4-α-glucosidase
5. β-Galactosidase (Lactase)

SYSTEMATIC NAMES AND NUMBERS
1. 1,4-α-α-D-Glucan glucanohydrolase - EC 3.2.1.1
2-a. Poly (1,4-α-D-galactouronide) glycanohydrolase - EC 3.2.1.15
2-b. Pectin pectylhydrolase - EC 3.1.1.11
3. 1,4-(1,3; 1,4)-β-D-Glucan-4-glucanohydrolase - EC 3.2.1.4
4. 1,4-α-β-D-Glucan glucohydrolase - EC 3.2.1.3
5. Lactose galactohydrolase - EC 3.2.1.108

REACTIONS CATALYZED
1. Hydrolysis of 1,4-α-glucosidic linkages in polysaccharides (starch, glycogen) yielding dextrins and oligo- and mono-saccharides.
2-a. Hydrolysis of 1,4-α-galactouronide linkages in pectin.
2-b. Demethylation of pectin.
3. Hydrolysis of 1,4-β-glucosidic linkages in cellulose yielding β-dextrins.
4. Hydrolysis of 1,4-α and 1,6-α-glucosidic linkages in polysaccharides (starch, glycogen) yielding glucose.
5. Hydrolysis of lactose yielding glucose and galactose.

DESCRIPTION
The products occur as off-white to tan amorphous powders. They also occur as liquid preparations, the aqueous solutions usually being tan to dark brown in colour. They are practically insoluble in ethanol, chloroform and ether.

FUNCTIONAL USES
Preparation of starch syrups, alcohol, beer ale, fruit juices, chocolate syrup, bakery products, liquid coffee, wine, glucose and dairy products.

* These specifications were prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).
GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"

CHARACTERISTICS

IDENTIFICATION TESTS

1. α-Amylase activity
   The sample shows fungal α-amylase activity

2. Pectinase activity
   The sample shows pectinase activity

3. Cellulase activity
   The sample shows cellulase activity

4. Glucoamylase activity
   The sample shows glucoamylase activity

5. β-Galactosidase activity
   The sample shows β-galactosidase activity
   See description under TESTS

TESTS

DETERMINATION OF β-GALACTOSIDASE (LACTASE) ACTIVITY

Principle of method
The procedure is based on the hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG), by β-Galactosidase derived from Aspergillus sp. o-Nitrophenol is liberated from the substrate and its absorbance at 400-420 nm is measured under alkaline conditions. Lactase (β-Galactosidase) exhibits maximal activity on ONPG substrate at pH 4-5, at 37°C. Activity on ONPG is correlated with activity on lactose though the pH optimum on the latter substrate is at pH 3-4, at 37°C.

Apparatus
- Colorimeter: Klett-Summerson, Filter No. 42
- Constant, 37°C water bath
- 15 x 150 mm test tubes
- Assorted pipettes

Solutions
Stock buffer
- 0.1 M citric acid: Dissolve 21 g of citric acid monohydrate in 1 L
- 0.2 M dibasic sodium phosphate - Dissolve 28.4 g anhydrous Na₂HPO₄ in 1 L

Mix 400 ml of the Na₂HPO₄ solution with sufficient citric acid solution to give pH 4.3

Buffer for assay
Dilute above 1 to 20. Final pH 4.4

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* See Annex 1 at the end of this compendium


*** Based on Lederberg, J., J. Bact., 60, 381 (1950).
Solutions (continued)

Alkaline reagent to stop hydrolysis to develop ONP colour
- 1.1 M Na₂CO₃
- 0.01 M EDTA (Disodium ethylenediaminetetra-acetate)

Dissolve 11.6 g of anhydrous sodium carbonate and 0.37 g of EDTA (disodium salt) in a final volume of 100 ml of water. Reagent is stable for several weeks.

Substrate
Dissolve ONPG (o-nitrophenyl-β-D-galactopyranoside) in water at a concentration of 3.75 mg/ml. This will give a solution of 0.0125 M substrate. Prepare fresh daily before use.

Enzyme to be tested
Dilute the enzyme in water to yield 1 - 3 lactase units/ml. If the activity of the sample is unknown, make several dilutions.

Procedure
1. Pipette into a 15 x 150 mm test tube in the following order:
   - 2 ml of diluted buffer
   - enzyme solution containing 1 - 3 lactase units
   - water to a final volume of 6 ml
2. Attemperate at 37°C for 5 min
3. Start the reaction with substrate. Pipette 4 ml of substrate into the test solution. The substrate should also be attemperated for 5 min at 37°C before addition
4. Incubate the reaction mixture for 20 min at 37°C
5. At exactly 20 min, add 1 ml of the alkaline solution of Na₂CO₃ - EDTA to stop the reaction and develop the colour
6. Read in a Klett-Summerson Filter No. 42.

Blanks
(run with each assay)

Substrate
Pipette into a 15 x 150 mm test tube, 2 ml of diluted buffer and 4 ml of water. Attemperate for 5 min at 37°C. Add 4 ml of attemperated substrate at 0 time. After 20 min add 1 ml of alkaline Na₂CO₃ - EDTA solution and read in a Klett-Summerson Filter No. 42.

Enzyme
Add to 15 x 150 mm test tubes in the following order 2 ml of diluted buffer, enzyme solution and water to 10 ml. After 20 min at 37°C add 1 ml of alkaline Na₂CO₃ - EDTA solution and treat as above. Enzyme blanks need not be run unless enzyme solutions used in the test are highly coloured.

Note: Alkaline ONP tends to coat glass, therefore, frequent rinsing of Klett tube with detergent is required.

Definition of Lactase unit
One Lactase unit (LU) is defined as that quantity of enzyme responsible for the production of 10-8 moles o-nitrophenol per minute at pH 4.4 and 37°C.

Activity
To obtain the net Klett reading, subtract the blank values from the experimental values. Lactase units are found by referring the net Klett value to the a lactase standard curve (see next page)
Calculation

Units determined from standard curve x total dilution = total units/g or ml

Example
- Enzyme diluted 0.2 g to 200 ml and 1 ml of that solution to an additional 200 ml. Total dilution used 200,000.
- 1 ml for assay
- No. 42 Net Klett: 245 = 2 units from the curve
- 2 units x 200,000 dilution = 400,000 units/g

LACTASE STANDARD CURVE
CARBOHYDRASE FROM ASPERGILLUS AWAMORI, VAR.*
(Tentative)

SOURCES
Commercial enzyme preparations are produced by the controlled fermentation of species
of Aspergillus awamori, var.

ACTIVE PRINCIPLE
Glucan 1,4-α-glucosidase (glucoamylase, amyloglucosidase)

SYSTEMATIC NAME AND NUMBER
1,4-α-D-Glucan glucohydrolase - EC 3.2.1.3

REACTION CATALYZED
Glucoamylase hydrolyzesterminal 1,4-linked (and also 1,6-linked when the next bonds
in sequence is 1,4) α-D-glucoosidic residues successively from non-reducing
ends of polysaccharides (starch, glycogen, etc.) yielding β-D-glucose (dextrose).

SECONDARY ENZYME ACTIVITY
α-Amylase - EC 3.2.1.1

DESCRIPTION
The purified enzymes are typically offered as clear, tan to brown liquids. They are
practically insoluble in ethanol, chloroform and ether.

FUNCTIONAL USE
Preparation of glucose

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in
Food Processing"**

CHARACTERISTICS
IDENTIFICATION TESTS
Glucoamylase The sample shows glucoamylase activity***

* These specifications were prepared at the 22nd session of JECFA (1978) and published in FNP 7 (1978).

** See Annex 1 at the end this Compendium.

*** See General Methods for Enzyme Preparations under Glucoamylase Activity in the Guide to JECFA
CARBOHYDRASE FROM BACILLUS LICHENIFORMIS

SYNONYMS
Diastase, ptyalin, glycogenase;
INS No. 1100

SOURCES
Commercial enzyme preparations are produced by the controlled fermentation of Bacillus licheniformis.

ACTIVE PRINCIPLE
α-Amylase

SYSTEMATIC NAME AND NUMBER
1,4-α-D-Glucan glucohydrolase - EC 3.2.1.1

REATIONS CATALYZED
The enzyme preparations hydrolyze 1,4-α-glucosidic linkages in polysaccharides, yielding dextrins and oligo- and monosaccharides.

SECONDARY ENZYME ACTIVITY
Microbial serine proteinase - EC 3.4.21.14

DESCRIPTION
The products are typically offered as off-white to tan amorphous powders or as brown liquids. They are soluble in water, but practically insoluble in ethanol, chloroform and ether.

FUNCTIONAL USES
Preparation of and/or use in cereal and starch, fruits and vegetables, beverages, sugar and honey, confectionery and bakery.

GENERAL SPECIFICATIONS
Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"**

CHARACTERISTICS

IDENTIFICATION TESTS

α-Amylase activity

The sample shows bacterial α-amylase activity***

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* These specifications were prepared at the 29th session of JECFA (1986) and published in FNP 34 (1986).

** See Annex 1 at the end of this Compendium.

CARBOHYDRASE FROM RHIZOPUS ORYZAE, VAR.*

SOURCES Commercial enzyme preparations are produced by the controlled fermentation of Rhizopus oryzae, var.

ACTIVE PRINCIPLES
1. α-Amylase (Glycogenase)
2. Pectinase
3. Glucoamylase (Amyloglucosidase, Glucan 1,4-α-glucosidase)

SYSTEMATIC NAMES AND NUMBERS
1. 1,4-α-D-Glucan glucohydrolase - EC 3.2.1.3
2. Poly (1,4-α-D-galactouronide) glycanohydrolase - EC 3.2.1.15
3. 1,4-α-D-Glucan glucohydrolase - EC 3.2.1.1

REATIONS CATALYZED
1. Hydrolysis of 1,4-α-glucosidic linkages in polysaccharides (starch, glycogen) yielding dextrins and oligo- and mono- saccharides.
2. Hydrolysis of 1,4-α-galactouronide linkages in pectin.
3. Hydrolysis of 1,4-α and 1,6-α-glucosidic linkages in poly- saccharides (starch, glycogen) yielding glucose.

DESCRIPTION The products occur as off-white to tan amorphous powders. They also occur as liquid preparations, the aqueous solutions usually being tan to dark brown in colour. They are practically insoluble in ethanol, chloroform and ether.

FUNCTIONAL USES Preparation of starch syrups and fruit juices, and manufacture of glucose.

GENERAL SPECIFICATIONS Must conform to the *General Specifications for Enzyme Preparations used in Food Processing**

CHARACTERISTICS

IDENTIFICATION TESTS
1. α-Amylase activity The sample shows fungal α-amylase activity***
2. Pectinase activity The sample shows pectinase activity
3. Glucoamylase activity The sample shows glucoamylase activity***

* These specifications were prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this Compendium.

CARBOHYDRASE FROM SACCHAROMYCES SPECIES

SOURCES

Commercial enzyme preparations of carbohydrases (Saccharomyces) are produced by the controlled fermentation of a number of species of Saccharomyces traditionally used in the manufacture of food.

ACTIVE PRINCIPLES

1. β-Fructofuranosidase (invertase, saccharase)
2. β-Galactosidase (lactase)

SYSTEMATIC NAMES AND NUMBERS

1. β-D-Fructofuranoside fructohydrolase - EC 3.2.1.26
2. β-D-Galactoside galactohydrolase - EC 3.2.1.23

REACTIONS CATALYSED

1. Hydrolyzes sucrose to a mixture of glucose and fructose.
2. Hydrolyzes lactose to a mixture of glucose and galactose.

DESCRIPTION

The purified enzymes occur as white to tan amorphous powders. They are soluble in water, the solutions usually being light yellow in colour. They are practically insoluble in alcohol, chloroform and ether.

FUNCTIONAL USES

Manufacture of candy and ice cream and modification of dairy products.

GENERAL SPECIFICATIONS

Must conform to the "General Specifications for Enzyme Preparations used in Food Processing"**

CHARACTERISTICS

IDENTIFICATION TESTS

1. Invertase activity
   The sample shows invertase activity***

2. β-Galactosidase activity
   The sample shows β-galactosidase activity

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* These specifications were prepared at the 15th session of JECFA (1971) and published in NMRS 50B (1972).

** See Annex 1 at the end of this compendium.

CARBON DIOXIDE*  
(Tentative)**

| SYNONYMS          | Carbonic acid anhydride, dry ice (solid form)  
|                   | INS No. 290, EEC No. E290  

| DEFINITION         |  
|                   | Chemical name: Carbon dioxide  
|                   | C.A.S. No.: 124-38-9  
|                   | Chemical formula: CO₂  
|                   | Molecular weight: 44.01  
|                   | Assay: Content not less than 99% v/v of CO₂ on the gaseous basis  

| DESCRIPTION        | A colourless gas under normal environmental conditions with a slight pungent odour. Commercial carbon dioxide is shipped and handled as a liquid in pressurized cylinders or bulk storage systems, or in compressed solid blocks of "dry ice". Solid (dry ice) forms usually contain added substances, such as propylene glycol or mineral oil, as binders.  

| FUNCTIONAL USES    | Carbonating agent, packaging gas, preservative, freezing agent, extraction solvents.  

| CHARACTERISTICS    | The following specifications apply to gaseous carbon dioxide as produced from its condensed liquid or solid phase by evolution to the gas phase at normal environmental conditions. Additional specifications may be applied to liquid or solid forms of carbon dioxide (such as non-condensible hydrocarbons content or residue on evaporation) by vendors or by specific users of commercial carbon dioxide products.  

| IDENTIFICATION TESTS |  
| A. Precipitate formation | When a stream of the sample is passed through a solution of barium hydroxide, a white precipitate is produced which dissolves with effervescence in dilute acetic acid.  
| B. Extinction of flame | A flame is extinguished in an atmosphere of the sample.  

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNP 34 (1986).  
** Information required on the adequacy of the test method of oil content.  
PURITY TESTS

**Acidity**
- Passes test.
  See description under TESTS.

**Oil**
- Passes test.
  See description under TESTS.

**Phosphine, hydrogen sulfide and other organic reducing substances**
- Passes test.
  See description under TESTS.

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

TESTS

PURITY TESTS

**Acidity**
Transfer 50 ml of water, previously boiled and cooled to room temperature, into a Nessler tube. Introduce 1,000 ml of the sample into the water through a tube (1 mm internal diameter) keeping the opening of the tube within 2 mm from the bottom of the vessel. Add 0.1 ml of methyl orange TS. The red colour produced is not darker than the colour of an identical control solution to which has been added 1.0 ml of 0.01 N hydrochloric acid instead of the carbon dioxide.

**Principle**
Carbon dioxide gas is bubbled through Carbon tetrachloride. The oil content of the carbon tetrachloride is determined by infrared spectroscopy at the wavelength for the C-H stretching frequency, 2700 cm⁻¹.

**Apparatus**
- Scrubber bottle (dreschel type) containing 150 ml carbon tetrachloride. (Carbon tetrachloride should be of a purity such that there is no absorption peak at 2700 cm⁻¹).
- Double beam infrared spectrometer and accessories, to measure at 2700 cm⁻¹.
- Mineral oil (liquid paraffin) of known purity, for use as a standard.

**Procedure**
Using appropriate sampling procedures for evolution of gaseous samples, pass 5.0 l of sample through the scrubber apparatus. Measure infrared absorbance of the carbon tetrachloride solution from the apparatus at 2700 cm⁻¹, using pure carbon tetrachloride in the reference cell. The absorbance at 2700 cm⁻¹ must not exceed that of a standard solution containing 0.1 mg/l mineral oil in carbon tetrachloride.

* Information required on the adequacy of this test.
Phosphine, hydrogen sulfide and other organic reducing substances

Carbon monoxide

Transfer 25 ml of silver ammonium nitrate TS and 3 ml of ammonia TS into a Nessler tube. In the absence of light, introduce 1,000 ml of the sample in the same manner as in the test of Acidity. No brown colour is produced.

Principle
Carry out the test on the first portion of gas issuing from the cylinder. Use 5.0 l of the sample mixed with an equal volume of carbon monoxide-free nitrogen and 10 l of carbon monoxide-free nitrogen as the control. The difference between the volumes of 0.002 N sodium thiosulfate used in the two titrations is not greater than 0.5 ml.

Apparatus
The apparatus consists of the following parts connected in series:
- U-tube containing anhydrous silica gel impregnated with chromium trioxide.
- Scrubber bottle (dreschel type) containing 100 ml of a 40% w/v solution of potassium hydroxide.
- U-tube containing phosphorus pentoxide dispersed on previously granulated, fused pumice.
- Tube containing recrystallized iodic anhydride (IO\textsubscript{3}) in granules, previously dried at 200° and kept at a temperature of 120°. The iodic anhydride is packed in the tube in 1 cm columns separated by 1 cm columns of glass wool to give an effective length of 5 cm.
- Flask containing 2.0 ml of potassium iodide TS and 3 drops of starch solution TS.

Procedure
Flush the apparatus with 5.0 l of carbon dioxide-free air and, if necessary, discharge the blue colour in the iodide solution by adding the smallest necessary quantity of freshly prepared 0.002 N sodium thiosulfate. Continue flushing until not more than 0.045 ml of 0.002 N sodium thiosulfate is required after passing 5.0 l of carbon dioxide-free air. Pass the gas from the cylinder through the apparatus.

Flush the last traces of liberated iodine into the reaction flask by passing through the apparatus 1.0 l of carbon monoxide-free air. Titrate the liberated iodine with 0.002 N sodium thiosulfate. Carry out a blank assay using 10 l of carbon monoxide-free nitrogen. The difference between the volumes of 0.002 N sodium thiosulfate solution used in the two titrations should not be more than 0.5 ml (equivalent to 10 µl/l carbon monoxide).

METHOD OF ASSAY
Transfer a 1 in 3 potassium hydroxide solution into a gas pipette of adequate volume. Measure accurately about 1,000 ml of the sample into a gas burette containing a 1 in 10 sodium chloride solution. Transfer the sample into the gas pipette and shake well. When the volume of gas remaining unabsorbed is constant (V ml), the content of carbon dioxide is calculated by:

\[
\text{Content (v/v %)} = \frac{\text{vol of sample (ml)} - V \text{ ml}}{\text{vol of sample (ml)}} \times 100
\]
CARMINES*

SYNONYMS

Cochineal carmine, carmine, CI Natural Red 4;
INS No. 120, EEC No. E120

DEFINITION

Carmines are obtained from an aqueous extract of Cochineal, which consists of the dried bodies of the female insect Dactylopius coccus Costa.

The colouring principle is a hydrated aluminium chelate of carminic acid in which aluminium and carminic acid are thought to be present in the molar ratio 1:2.

In commercial products the colouring principle is present in association with ammonium, calcium, potassium or sodium cations, singly or in combination, and these cations may also be present in excess.

Commercial products also contain proteinaceous material derived from the source insect, and may also contain free carminate or a small excess of aluminium cations.

Class

Anthraquinone

Code numbers

CI (1975) No. 75470
CAS No. 1390-65-4

Chemical name

Hydrated aluminium chelate of carminic acid (7-α-D-glucopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2-anthracenecarboxylic acid)

Chemical formula

C_{28}H_{30}O_{13}

Structural formula

Carminic acid

* These specifications were prepared at the 26th session of JECFA (1982) and published in FNP 25 (1982).
Possible structural formula of the aluminium complex of carminic acid:

\[
\begin{array}{c}
\text{β-D-Gluc} \\
\text{O} \\
\text{H} \\
\text{O} \\
\text{CH}_3 \\
\text{COOH} \\
\text{O} \\
\text{OH} \\
\text{H}_2\text{O} \\
\text{Al(OH)}_3 \\
\text{O} \\
\text{OH} \\
\text{CH}_3 \\
\text{COOH} \\
\end{array}
\]

\[\times M^+\]

\[\text{H}_2\text{O} \]

\[\text{H}_2\text{O}\]

M\(^+\): cation Ca\(^{++}\), Na\(^{+}\), K\(^{+}\), NH\(_4\)\(^{+}\)

Molecular weight 492.39

Assay Content not less than 42% of C\(_{22}\)H\(_{34}\)O\(_{13}\)

DESCRIPTION Red to dark red, friable, solid or powder

FUNCTIONAL USE Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility Ammonium carmine: Freely soluble in water at pH 3.0 and pH 8.5.
Calcium carmine: Very slightly soluble in water at pH 3.0, freely soluble in water at pH 8.5.

B. Colour reaction Make the solution slightly alkaline by adding 1 drop of 10% sodium hydroxide or potassium hydroxide solution. A violet colour is produced.

C. Colour reaction Add a small sodium dithionite (Na\(_2\)S\(_2\)O\(_4\)) crystal; the acid, neutral or alkaline solutions of Cochineal do not decolourize (difference from Orchil).

IDENTIFICATION TESTS (continued)

D. Colour reaction
Dry a small quantity of Cochineal in a porcelain dish. Cool thoroughly and treat the dry residue with 1 or 2 drops of cold sulfuric acid. No colour change occurs.

E. Colour reaction
Passes test
See description under TESTS

PURITY TESTS

* Loss on drying
Not less than 20% (135°C, 3 h)

* Total ash
Not more than 12%
Test 1 g of the sample as directed in the test for Ash (Total Ash)

Matter insoluble in dilute ammonia
Not more than 1%
See description under TESTS

* Arsenic
Not more than 3 mg/kg (Method II)

* Lead
Not more than 10 mg/kg

* Heavy metals
Not more than 20 mg/kg
Test 1 g of the sample as directed in the Limit Test for Heavy Metals (Method II).

* Protein
Not more than 25% (non ammonia N x 6.25)
Proceed as directed under Nitrogen Determination in the General Methods.

* Microbiological criteria
Salmonella: absent

TESTS

IDENTIFICATION TESTS

E. Colour reaction
Acidify a solution of Cochineal with 1/3rd its volume of hydrochloric acid and shake with amyl alcohol. Wash the amyl alcohol solution of Cochineal 2-4 times with an equal volume of water to remove hydrochloric acid, etc. Dilute amyl alcohol with 1-2 volumes of gasoline and shake with a few small portions of water to remove colour. Add, dropwise, 5% uranium acetate, shaking thoroughly after each addition. In the presence of Cochineal a characteristic emerald-green colour is produced.

PURITY TESTS

Matter insoluble in dilute ammonia
Dissolve about 0.25 g of the sample, previously dried and accurately weighed, in 2.5 ml of dilute ammonia solution (160 ml of strong ammonia TS, made up to 500 ml) and dilute to 100 ml with water: the solution is clear. Filter through sintered glass filter (British Standard Grade No. 3). Wash with a solution containing 0.1% of NH₃ and dry to constant weight at 105°C.

METHOD OF ASSAY

Add about 30 mg of the sample, accurately weighed in 30 ml of 2 N hydrochloric acid, boil to dissolve and cool. Transfer quantitatively to a 1000 ml volumetric flask, dilute to volume with water, and mix. Determine the absorbance of the solution in a 1 cm cell at the wavelength of maximum absorbance (about 494 nm) using 3 ml of 2 N hydrochloric acid per 100 ml aqueous solution as the blank. If the measured absorbance of the solution is not within the range 0.20 to 0.25, then the weight of sample taken should be adjusted accordingly. Calculate the percentage of carminic acid in the sample taken for analysis by the formula:

\[
\frac{15 \times A \times 100}{0.262 \times X}
\]

in which

- \( A \) = absorbance of the sample solution;
- \( X \) = weight in mg of the sample taken; and
- 0.262 = absorbance of a solution of carminic acid having a concentration of 15 mg per litre.
CAROB BEAN GUM

SYNONYMS
Locust bean gum, algaroba, carob gum,
INS No. 410, EEC No. E410

DEFINITION
Carob Bean Gum is primarily the endosperm of *Ceratonia siliqua* (L.) Taub, (Fam. Leguminosae). It consists mainly of high molecular weight polysaccharides composed of galactomannans. It may be purified by washing only with ethanol or isopropanol. The article of commerce may be further specified as to viscosity.

C.A.S. number
9000-40-2

DESCRIPTION
A white to yellowish white, nearly odourless powder.

FUNCTIONAL USES
Thickening agent, stabilizer

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
Soluble in hot water, insoluble in ethanol.

** B. Gel formation
To a water solution of the sample add small amounts of sodium borate TS. A gel is formed.

C. Viscosity
Passes test
See description under TESTS

D. Positive test for galactose and mannose
Passes test
See description under TESTS

E. Microscopic examination
Passes test
See description under TESTS

PURITY TESTS

** Loss on drying
Not more than 14% (105°, 5 h)

** Total ash
Not more than 1.2% (800° for 3-4 h)

Acid-insoluble matter
Not more than 4%
See description under TESTS

** Arsenic
Not more than 3 mg/kg (Method II)

* These specifications were prepared at the 35th session of JECFA (1989) and published in FNP 49 (1989).

PURITY TESTS (continued)

* Lead
   Not more than 10 mg/kg

* Heavy metals
   Not more than 20 mg/kg
   Test 1 g of the sample as directed in the Limit Test (Method II) using 20 
   μg of lead ion (Pb) in the control (Solution A).

Proteins
   Not more than 7%
   See description under TESTS

Starch
   Not detectable by the Method described below.
   To a 1 in 10 solution of the sample add a few drops of iodine TS. No blue
   colour is produced.

Ethanol and isopropanol
   Not more than 1%, singly or in combination
   See description under TESTS

TESTS

IDENTIFICATION TESTS

C. Viscosity
   Transfer 2 g of the sample into a 400-ml beaker and moisten thoroughly with
   about 4 ml of isopropanol. Add, with vigorous stirring, 200 ml of water and
   continue the stirring until the gum is completely and uniformly dispersed. An
   opalescent, slightly viscous solution is formed. Transfer 100 ml of this solution
   into another 400-ml beaker. Heat the mixture in a boiling water bath for about
   10 min and cool to room temperature. There is an appreciable increase in
   viscosity (differentiating carob bean gum from guar gum).

D. Positive test for
   galactose and
   mannose
   Boil a mixture of 100 mg of the sample and 20 ml of 10% sulfuric acid for 3 h.
   Allow to cool and add excess barium carbonate mixing with a magnetic stirrer
   until the solution is of pH 7, and filter. Evaporate the filtrate in a rotary
   evaporator at 30-50°C in vacuum until a crystallized (or syrupy) residue is
   obtained. Dissolve in 10 ml of 40% methanol. This is the hydrolysate. Place
   1 to 10 μl spots of hydrolysate on the starting line of two chromatoplates of silica
   gel and spots containing 1 to 10 μg of galactose and mannose expected to be
   present in the hydrolysate. Use two solvent systems, one for each plate: A. a
   mixture of formic acid, methyl ethyl ketone, tertiary butanol and water
   (15:30:40:15 by vol.) and B. a mixture of isopropanol, pyridine, acetic acid and
   water (40:40:5:20 by vol.) to develop the plates. After development, spray with
   a solution of 1.23 g anisidine and 1.66 g phthalic acid in 100 ml ethanol and heat
   the plates at 100°C for 10 min. A greenish yellow colour is produced with
   hexoses, a red colour with pentoses and a brown colour with uronic acids.
   Compare sample with those for the solution of galactose and mannose.

E. Microscopic
   examination
   Place some ground sample in an aqueous solution containing 0.5% iodine and
   1% potassium iodide on a glass slide and examine under microscope. Carob bean
   gum contains long stretched tubiform cells, separated or slightly interspaced.

E. Microscopic examination (continued)

Their brown contents are much less regularly formed than in Guar gum. (Guar gum shows close groups of round to pear shaped cells. Their contents are yellow to brown).

PURITY TESTS

Acid-insoluble matter

Transfer 1.5 g of the sample, accurately weighed, into a 250-ml beaker containing 150 ml of water and 1.5 ml of concentrated sulfuric acid. Cover the beaker with a watch glass and heat the mixture on a steam bath for 6 h rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation.

Next add about 500 mg of a suitable filter aid, accurately weighed, and filter through a tared Gooch crucible provided with an asbestos pad. Wash the residue several times with hot water, dry the crucible and its contents at 105° for 3 h, cool in a desiccator and weigh. The difference between the total weight and weight of the filter aid plus crucible and pad is the weight of the acid-insoluble matter. Calculate the acid-insoluble matter content as a percentage.

Determine nitrogen by Kjeldahl method. The percentage of nitrogen determined multiplied by 6.25 gives the percent of protein in the sample.

Principle

The alcohols are converted to the corresponding nitrite esters and determined by Headspace Gas Chromatography.*

Sample preparation

Dissolve 100 mg of sample in 10 ml of water using sodium chloride as a dispersing agent if necessary.

Internal standard solution

Prepare an aqueous solution containing 50 mg/l of n-propanol.

Standard alcohol solution

Prepare an aqueous solution containing 50 mg/l each of ethanol and isopropanol.

Procedure

Weigh 200 mg of urea into a 25-ml "dark vial" (Reacti-flasks, Pierce, Rockford 3, USA or equivalent). Purge with nitrogen for 5 min. and then add 1 ml of saturated oxalic acid solution, close with a rubber stopper and swirl. Add 1 ml of sample solution, 1 ml of internal standard solution and simultaneously start a stop watch (T=0). Swirl the vial and recap with an open screw cap fitted with a silicone rubber septum. Swirl until T=30 sec. At T=45 sec inject through the septum 0.5 ml of an aqueous solution of sodium nitrite (250 g/L). Swirl until T=70 and at T=150 sec withdraw through the septum 1 ml of the headspace using a pressure lock syringe (Precision Sampling Corp, Baton Rouge, Louisiana, USA or equivalent).

Gas chromatography

Insert syringe needle in the injection port; precompress the sample, then open the syringe and inject the sample.

Ethanol and isopropanol (continued)

**GC conditions**
- **Column:** glass, 90 cm x 4 mm id
- **Column packing:** first 15 cm packed with chrompack (or equivalent) and the remainder with Poropak R 120-150 mesh (or equivalent).
- **Column temperature:** 150° isothermal
- **Injection port temperature:** 250°
- **Carrier gas:** nitrogen
- **Flow rate:** 80 ml/min.
- **Detector:** flame ionization

**Calculation**
Quantify the ethanol and isopropanol present in the sample by comparing the peak areas with the corresponding peaks obtained by chromatographing the headspace produced by substituting in the procedure 1 ml of Standard alcohol solution for 1 ml of Sample solution.
CAROTENES (algae)*
(Tentative)**

SYNONYMS
Natural β-carotene; INS No. 160a(ii)

DEFINITION
Carotene (algae) is obtained by extraction of *Dunaliella salina*, *bardawil* or *kona* algae. The main colouring principle is β-carotene together with minor amounts of other carotenoids. Besides the colour pigments, carotenes (algae) may contain lipids, and tocopherols, added or naturally occurring in the source material and food grade vegetable oil, added to retard oxidation of the pigment.

The only solvents used for the extraction are hexane, ethanol and vegetable oil.

The main articles of commerce are solutions or suspensions in food grade vegetable/plant oil and water dispersible powders formulated using approved food additives. This is for ease of use and to improve stability as β-carotene easily oxidises.

Class Carotenoid

Code numbers CI (1975) No. 75130
β-Carotene: CI (1975) No. 40800
CAS No 7235-40-7

Chemical formula β-carotene: C₅₀H₉₀

Structural formula

Molecular weight β-Carotene: 536.88

Assay Content of β-carotene is not less than declared

FUNCTIONAL USE Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

*** A. Solubility Insoluble in water

* These specifications were prepared at the 35th session of JECFA (1989) and published in FNP 49 (1990).

** Information required on the source algae, e.g. difference of species, difference of components of resulting products, etc., influence of manufacturing process such as spray-drying to the quality of finished powder preparations, and technological justification for the amount of ethanol residue.

IDENTIFICATION TESTS (continued)

* B. Spectrophotometry
   A cyclohexane solution of the sample (1 in 200,000) shows maximum absorption at the wave length of 453-457 nm and 482-486 nm.

C. Carr-Price test
   A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price TS.

PURITY TESTS

** Arsenic
   Not more than 3 mg/kg

** Lead
   Not more than 10 mg/kg

*** Heavy metals
   Not more than 40 mg/kg
   Test 0.5 g of the sample as directed in the Heavy Metals Limit Test (Method II)

*** Residual solvent
   Ethanol
   Not more than 10%
   Information required on the technological justification for the amount of ethanol residue.

   Hexane
   Not more than 50 mg/kg

   Tocopherols
   Not more than 0.5%
   Information required on the method for determination of tocopherols content

METHOD OF ASSAY
   Proceed as directed in Method of Assay for Oil-Soluble Food Colours in General Methods* using the following conditions:

\[ W(g) = \text{amount to obtain adequate absorbance} \]

\[ V_1 = V_2 = V_3 = 100 \text{ ml} \]

\[ v_1 = v_2 = 5 \text{ ml} \]

\[ A^{1/2} = 2450 \]

\[ \lambda_{\text{max}} = 455-457 \text{ nm} \]

---


CAROTENES (Vegetable)*
(Tentative)**

SYNONYMS
Caroten-es-natural, Cl Food Orange 5;
INS No. 160 a(ii), EEC No. E160a

DEFINITION
Carotenes (vegetable) are obtained by solvent extraction of carrots, grass alfalfa
and vegetable oil with subsequent removal of the solvent. The main colouring
principle consists of carotenoids of which beta-carotene accounts for the major
part. Minor amounts of alpha, gamma-carotene and other pigments may be present.
Besides the colour pigments, this substance may contain oils, fats and waxes
naturally occurring in the source material. Only the following solvents may be
used in the extraction: acetone, methanol, ethanol, propan-2-ol, hexane and
dichloromethane.***

Class
Carotenoid

Code numbers
CI (1975) No. 75130
beta-Carotene: CI (1975) No. 40800
CAS No 7235-40-7

Chemical formula
beta-carotene: C_{40}H_{56}

Structural formula

Molecular weight
beta-Carotene: 536.88

Assay
Content of carotenes (calculated as beta-carotene) is not less than declared.

FUNCTIONAL USE
Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

**** A. Solubility
Insoluble in water

---

* These specifications were prepared at the 35th session of JECFA (1989) and published in FNP 49 (1990).

** Information required on composition of commercial products, and method(s) to distinguish between carotenes
(vegetable) and synthetic colours.

*** Edible vegetable oil may be added to articles of commerce for standardizing purposes.

IDENTIFICATION TESTS (continued)

* B. Spectrophotometry  
A cyclohexane solution of the sample (1 in 200,000) shows maximum absorption at the wavelength of 453-457 nm and 482-486 nm.

C. Carr-Price test  
A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price TS.

PURITY TESTS

** Arsenic  
Not more than 3 mg/kg

** Lead  
Not more than 10 mg/kg

*** Heavy metals  
Not more than 40 mg/kg
Test 0.5 g of the sample as directed in the Heavy Metals Limit Test (Method II).

*** Residual solvent

Acetone
Methanol
Propan-2-ol
Hexane
Ethanol

} Not more than 50 mg/kg singly ethanol or in combination

Dichloromethane
Not more than 10 mg/kg****

METHOD OF ASSAY

Proceed as directed in Method of Assay for Oil-Soluble Food Colours* using the following conditions:

\[ W(g) = \text{amount to obtain adequate absorbance} \]

\[
V_1 = V_2 = V_3 = 100 \text{ ml} \\
v_1 = v_2 = 5 \text{ ml} \\
A_{15m}^{15} = 2450 \\
\lambda_{\text{max}} = 455-457 \text{ nm} \\
\]

---


**** Information required on the actual amount of residue, technological justification for such chlorinated hydrocarbon and minimum residue level technologically achievable.
**BETA-CAROTENE, SYNTHETIC**

**SYNONYMS**

CI Food Orange 5;
INS No. 160a(i), EEC No. E160a

**DEFINITION**

These specifications apply to predominantly all trans (Z) isomer of β-carotene together with minor amounts of other carotenoids. Diluted and stabilized forms are prepared from β-carotene meeting these specifications and include solutions or suspensions of β-carotene in edible fats or oils, emulsions and water dispersible powders. These preparations may have different cis/trans isomer ratios.**

Class

Carotenoid

Code numbers

CI (1975) No. 40800
CAS No 7235-40-7

Chemical names

β-Carotene, β,β-carotene

Chemical formula

C₄₀H₅₆

Structural formula

![Structural formula of β-carotene](image)

Molecular weight

536.88

Assay

Not less than 96% total colouring matters (expressed as β-carotene).

**FUNCTIONAL USE**

Food colour

**DESCRIPTION**

Red to brownish-red crystals or crystalline powder.

β-Carotene is sensitive to oxygen and light and should therefore be kept in a light-resistant container under inert gas.

**CHARACTERISTICS**

**IDENTIFICATION TESTS**

*** A. Solubility

Insoluble in water. Practically insoluble in ethanol.
Slightly soluble in vegetable oils. Soluble in chloroform

---

* These specifications were prepared at the 31st session of JECFA (1987) and published in FNP 38 (1988)

** The analytical methods described for the parent colour are not necessarily suitable for the assay of or determination of impurities in the stabilized forms. Appropriate methods should be available from the manufacturer).

IDENTIFICATION TESTS (continued)

* B. Spectrophotometry Determine the absorbance of the sample solution C (See Method of Assay) at 455 nm and 483 nm. The ratio $A_{455}/A_{483}$ is between 1.14 and 1.19.

* C. Spectrophotometry Determine the absorbance of the sample solution C at 455 nm and that of sample Solution B (See Method of Assay) at 340 nm. The ratio $A_{455}/A_{340}$ is not lower than 15.

D. Positive test for carotenoid The colour of a solution of β-carotene in acetone disappears after successive additions of a 5% solution of sodium nitrite and 1 N sulfuric acid.

E. Carr-Price reaction A solution of the sample in chloroform turns blue on addition of an excess of Carr-Price reagent TS.

PURITY TESTS

** Sulfated ash Not more than 0.1%
Proceed as directed under the test for Ash (Sulfated ash, Method I) using 2 g of sample.

*** Arsenic Not more than 3 mg/kg

*** Lead Not more than 10 mg/kg

** Heavy metals Not more than 40 mg/kg
Test 1 g of the sample as directed in the Limit Tests (method II).

Subsidiary colouring matters Carotenoids other than β-carotene:
Not more than 3% of total colouring matters.
See description under TESTS

TESTS

PURITY TESTS

* Subsidiary colouring matters Carotenoids other than β-carotene:
Dissolve about 80 mg of sample in 100 ml chloroform. Apply 400 μl of this solution as a streak 2 cm from the bottom of a TLC-plate (Silicagel 0.25 mm).

Immediately develop the chromatogram with a solvent mixture of 95 parts dichloromethane and 5 parts diethyl ether in a saturated chamber, suitably protected from light, until the solvent front has moved 15 cm above the initial streak. Remove the plate, allow the main part of the solvent to evaporate at room temperature and mark the principal band as well as the


bands corresponding to other carotenoids. Remove the silicagel adsorbent that contains the principal band, transfer it to a glass-stoppered 100 ml centrifuge tube and add 40.0 ml chloroform (solution 1).

Remove the silicagel adsorbent that contains the combined bands corresponding to the other carotenoids, transfer it to a glass-stoppered, 50 ml centrifuge tube and add 20.0 ml chloroform (solution 2).

Shake the centrifuge tubes by mechanical means for 10 minutes and centrifuge for 5 min. Dilute 10.0 ml of Solution 1 to 50.0 ml with chloroform (solution 3).

Determine, with a suitable spectrophotometer, the absorbances of Solutions 2 and 3 in 1-cm cells at the wavelength maximum about 464 nm, using chloroform as blank.

Calculation

Carotenoids other than β-carotene (%) = \[
\frac{A_2 \times 100}{10A_3 + A_2}
\]

Where

\(A_2\) = absorbance of Solution 2

\(A_3\) = absorbance of Solution 3

**METHOD OF ASSAY**

Proceed as directed in Method of Assay for Oil-Soluble Food Colours* using the following conditions:

\[
\begin{align*}
W &= 0.08 \text{ g} \\
V_1 &= V_2 = V_3 = 100 \text{ ml} \\
v_1 &= v_2 = 5 \text{ ml} \\
A_{1\text{max}}^\text{\%} &= 2500 \\
\lambda_{\text{max}} &= \text{about } 455 \text{ nm}
\end{align*}
\]

CARRAGEENAN*

SYNONYMS

INS No. 407, EEC No. E407

Products of commerce are sold under different names such as:

- Irish moss gelose
- Carrageenan (from *Chondrus* and *Gigartina* spp.)
- Eucheumen (from *Eucheuma* spp.)
- Iridophycan (from *Iridaea* spp.)
- Hypnean (from *Hypnea* spp.)
- Furcellaran or Danish agar (from *Furcellaria fastigiata*)

DEFINITION

Carrageenan is a generic term for chemically similar hydrocolloids obtained by aqueous extraction from certain members of the class *Rhodophyceae* (red seaweeds). Specific national legislation may limit the sources for manufacture of carrageenan. The purity and identity criteria in this monograph apply to the chemical substance(s) which are generically carrageenan hydrocolloids according to the Description.

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

- *Furcellariaceae* such as *Furcellaria*
- *Gigartinaceae* such as *Chondrus*, *Gigartina*, *Iridaea*
- *Hypneaceae* such as *Hypnea*
- *Phyllophoraceae* such as *Phyllophora*, *Gymnogongrus*, *Ahnfeltia*
- *Solieriaceae* such as *Eucheuma*, *Anatheca*, *Meristotheca*.

C.A.S. number

9000-07-1

DESCRIPTION

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

The prevalent copolymers in food-grade carrageenan hydrocolloid are designated as *kappa*, *iota*, and *lambda*-carrageenan. *Kappa*-carrageenan (C.A.S. number, 11114-20-8) is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; *iota*-carrageenan is similar, except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between *kappa*-carrageenan and *iota*-carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In *lambda*-carrageenan (C.A.S. number, 9062-07-1), the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked).

---

* These specifications were prepared at the 29th session of JECFA (1985) and published in FNP 34 (1986).

DESCRIPTION (continued)

Carrageenan hydrocolloids are obtained by extraction from seaweeds into water or aqueous dilute alkali. The hydrocolloid may be recovered by alcohol precipitation, by drum drying or by precipitation in aqueous potassium chloride and freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol and isopropanol. Articles of commerce may be diluted with sugars for standardization purposes, mixed with salts to obtain specific gelling or thickening characteristics or may contain emulsifiers carried over from drum drying processes. Carrageenan is a yellowish or tan to white, coarse to fine powder that is practically odourless and has a mucilaginous taste.

FUNCTIONAL USE

Thickener/gelling agent and stabilizer

CHARACTERISTICS

IDENTIFICATION TESTS

* A. Solubility
Soluble in water. Insoluble in ethanol
See description under Tests for solubility in water

B. Infra-red absorption spectra
Passes test
See description under TESTS

C. Identification of hydrocolloid and predominant type of copolymer
Passes tests
See description under TESTS

D. Test for sulfate groups
Passes tests
See description under TESTS

E. Test for galactose and anhydrogalactose
Passes tests
See description under TESTS

PURITY TESTS

* Loss on drying
Not more than 12%. Dry at 105\(^\circ\)C to constant weight (approximately 4 h).

Viscosity of a 1.5% solution
Not less than 5 centipoises at 75\(^\circ\)C by the method specified under TESTS

Sulfate (as SO\(_4^2\))
Not less than 15% and not more than 40% on the basis of dry hydrocolloid content of commercial product.**
See description under TESTS


** The dry hydrocolloid content of commercial products is defined as the sample minus percent loss on drying and percent of added substances (i.e., sugars, salts or emulsifiers).
PURITY TESTS (continued)

<table>
<thead>
<tr>
<th>Test</th>
<th>Requirement</th>
<th>Description</th>
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<tbody>
<tr>
<td>Total ash</td>
<td>Not less than 15% and not more than 40% on dry weight basis.</td>
<td>See description under TESTS. Retain ash for the Acid-insoluble ash test.</td>
</tr>
<tr>
<td>Acid-insoluble ash</td>
<td>Not more than 1%</td>
<td>See description under TESTS</td>
</tr>
<tr>
<td>Acid insoluble matter</td>
<td>Not more than 2%</td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>Not more than 3 mg/kg</td>
<td>A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Arsenic (Method II).</td>
</tr>
<tr>
<td>Lead</td>
<td>Not more than 10 mg/kg</td>
<td>A sample solution prepared as directed for organic compounds will meet the requirements of the Limit Test for Lead.</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Not more than 40 mg/kg</td>
<td>Test 0.5 g of the sample as directed in Method II under the Limit Test for Heavy Metals using 20 μg of lead ion (Pb) in the control.</td>
</tr>
<tr>
<td>Residual solvents</td>
<td>Not more than 0.1% of ethanol, isopropanol or methanol, singly or in combination.</td>
<td>See description under TESTS.</td>
</tr>
</tbody>
</table>

TESTS

IDENTIFICATION TESTS

* A. Solubility
Carrageenan is soluble in water at a temperature of about 80°, forming a viscous, clear or slightly opalescent solution that flows readily. It disperses in water more readily if first moistened with alcohol, glycerin, or a saturated solution of sucrose in water.

* B. Infra-red absorption spectra
Obtain infra-red absorption spectra on the gelling and non-gelling fractions of the sample by the following procedure: Disperse 2 g of the sample in 200 ml of 2.5% potassium chloride solution, and stir for 1 h. Let stand overnight, stir again for 1 h, and transfer into a centrifuge tube. (If the transfer cannot be made because the dispersion is too viscous, dilute with up to 200 ml of the potassium chloride solution.) Centrifuge for 15 min at approximately 1000 g.

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

B. Infra-red absorption spectra (continued)

<table>
<thead>
<tr>
<th>Wave Number (cm(^{-1}))</th>
<th>Molecular Assignment</th>
<th>Absorbance Relative to 1050 cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Kappa</td>
</tr>
<tr>
<td>1220-1260</td>
<td>ester sulfate</td>
<td>0.2-1.2</td>
</tr>
<tr>
<td>928-933</td>
<td>3,6-anhydrogalactose</td>
<td>0.2-0.6</td>
</tr>
<tr>
<td>840-850</td>
<td>galactose-4-sulfate</td>
<td>0.1-0.5</td>
</tr>
<tr>
<td>825-830</td>
<td>galactose-2-sulfate</td>
<td>-</td>
</tr>
<tr>
<td>810-820</td>
<td>galactose-6-sulfate</td>
<td>-</td>
</tr>
<tr>
<td>800-805</td>
<td>3,6-anhydrogalactose-2-sulfate</td>
<td>0-0.2</td>
</tr>
</tbody>
</table>

C. Identification of hydrocolloid and predominant type of copolymer

Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel.

To 50 ml of the solution or gel add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ("brittle") gel indicates a carrageenan of a predominantly kappa type, a compliant ("elastic") gel indicates a predominantly iota type. If the solution does not gel, the carrageenan is of a predominantly lambda type.

D. Test for sulfate groups

To a solution of 100 mg of sample in 20 ml of water add 3 ml of N barium chloride and 5 ml of 2 N hydrochloric acid and filter if there is precipitate. Boil the filtrate for 5 minutes. A white, crystalline precipitate is formed.

E. Test for galactose and anhydrogalactose

Proceed as directed under Identification of gum constituents in the the General Methods*, using the following as reference standards: galactose, rhamnose, galacturonic acid, 3,6-anhydrogalactose, mannose, arabinose and xylose. Galactose and 3,6-anhydrogalactose should be present.

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PURITY TESTS

**Viscosity of a 1.5% solution**

Transfer 7.5 g of the sample into a tared, 600-ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g, and heat in water bath, with continuous agitation, until a temperature of 80° is reached (20 - 30 min). Add water to adjust for loss by evaporation, cool to 76-77°, and heat in a constant temperature bath at 75°. Pre-heat the bob and guard of a Brookfield LVF or LVT viscometer to approximately 75° in water, then dry the bob and guard, and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm and, after six complete revolutions of the viscometer, take the viscometer reading on the 0-100 scale.

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

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

**Sulfate (as SO₄)**

**Principle:**
Hydrolysed sulfate groups are precipitated as barium sulfate.

**Procedure**

Weigh 1 g of sample to the nearest 0.1 mg in a 100-ml long-neck bottom round flask. Add 50 ml 0.2N hydrochloric acid. Fit a cooler consisting preferably of 5 round bulbs and heat to boiling under reflux for 1 h. Add 25 ml 10% (by volume) hydrogen peroxide solution and continue boiling under reflux per 5 h when the solution becomes completely clear. Transfer the solution to a 600-ml beaker. Bring to boil and stir in dropwise 10 ml of a 10% solution of barium chloride. Let stand for 2 h on a boiling water bath. Filter the precipitate through ash-free filter paper meant for slow filtration (blue ribbon) and wash it with boiling distilled water until the filtrate is free from chloride. Dry the filter paper in a drying oven, gently combust the filter and ash at 1,000° in a tared silica crucible. Let cool when the ash is white. Weigh and calculate percentage sulfate from the weight in mg (P) of the barium sulfate obtained as P x 0.04116.
* **Total ash**

Weigh 2 g of the sample to the nearest 0.1 mg and transfer into a previously ignited, tared silica or platinum crucible. Heat the sample with a suitable infra-red heat lamp, increasing the intensity gradually, until it is completely charred and then continue for an additional 30 min. Transfer the crucible and charred sample into a muffle furnace and ignite at about 550° for 1 h, then cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight is attained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spot with a 1-in-10 solution of ammonium nitrate and dry under an infra-red heat lamp before igniting again. Calculate the percentage of total ash from the dry-weight of the sample.

* **Acid-insoluble ash**

Boil the ash obtained as directed under Total ash, above, with 25 ml of dilute hydrochloric acid TS for 5 min, collect the insoluble matter on a tared crucible or ashless filter, wash with hot water, ignite and weigh. Calculate the percentage of acid-insoluble ash from the weight of sample taken for the Total ash test.

* **Residual solvents**

**Standard Alcohol Solution**

Transfer 500.0 mg each of chromatographic quality methanol, ethanol and isopropanol into a 50 ml volumetric flask, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix.

**TBA Standard Solution**

Transfer 500.0 mg of chromatographic quality tertiary-butyl alcohol into a 50-ml volumetric flask, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix.

**Mixed Standard Solution**

Pipet 4 ml each of the Standard Alcohol Solution and of the TBA Standard Solution into a 125-ml graduated Erlenmeyer flask, dilute to about 100 ml with water, and mix. This solution contains approximately 40 µg of each alcohol and of tertiary-butyl alcohol per ml. Sample Preparation. Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distil about 100 ml, adjusting the heat so that foam does not enter the column. Add 4.0 ml of TBA Standard Solution to the distillate to obtain the Sample Preparation.

Residual solvents
(continued)

Procedure
Inject about 5 μl of the Mixed Standard Solution into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m x 3.2-mm stainless steel column packed with 80/100-mesh Porapak QS or equivalent. The carrier is helium flowing at 80 ml per min. The injection port temperature is 200°, the column temperature is 165°, and the detector temperature is 200°. The retention time of isopropanol is about 2 min, and that of tertiary-butyl alcohol about 3 min.

Determine the areas of the methanol, ethanol, isopropanol and TBA peaks, and calculate each response factor, f, by the formula A / A TBA, in which A is the area of each alcohol peak.

Similarly, inject about 5 μl of the Sample Preparation, and determine the peak areas, recording the area of each alcohol peak as a, and that of the tertiary-butyl alcohol peak as a TBA. Calculate each alcohol content, in mg/kg, in the sample taken by the formula:

\[
\frac{(a \times 4000)}{(f \times a \text{ TBA} \times W)}
\]

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

CARTHAMUS RED *
(Tentative)

SYNONYMS
Carthamin, carthamic acid, CI Natural Red 26

DEFINITION
Carthamus Red is obtained from the dried petals of *Carthamus Tinctorius* L. The Carthamus Yellow is extracted from the petals with water and the residue treated with aqueous sodium hydroxide to extract the Carthamus Red. It is precipitated from the extract by addition of acid, separated by filtration and dried.

Code numbers
CI (1975) No. 75140

Chemical name
Principally carthamin: 6-6-D-glucopyranosyl-2-[[3-8-D-glucopyranosyl-2,3,4-trihydroxy-5-[3-(4-hydroxyphenyl)-1-oxo-2-propenyl]-6-oxo-1,4-cyclohexadien-1-yl]methylene]-5,6-dihydroxy-4-[3-(4-hydroxyphenyl)-1-oxo-2-propenyl]-4-cyclohexene-1,3-dione

Chemical formula
*C*<sub>49</sub>*H*<sub>38</sub>*O*<sub>22</sub>

Structural formula
Carthamin

Molecular weight
910.81

Assay
Content not less than 80% total colouring matters on a volatile matter-free basis.

DESCRIPTION
Dark red to red-brown powder with a characteristic slight odour.

FUNCTIONAL USE
Food colour

CHARACTERISTICS

IDENTIFICATION TESTS

** A. Solubility
- Very slightly soluble in water
- Very slightly soluble in ethanol
- Practically insoluble in ether

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* These specifications were prepared at the 28th session of JECFA (1984) and published in FNP 31/1 (1984).

IDENTIFICATION TESTS (cont'd)

* B. Spectrophotometry

In dimethyl formamide

$\lambda_{\text{max}} = 530 \text{ nm}$

C. Thin layer chromatography

$R_f = 0.54 - 0.55$

See description under TESTS

D. Colour reaction

Passes test

See description under TESTS

E. Colour reaction

Passes test

See description under TESTS

PURITY TESTS

* Loss on drying

Not more than 5% (vacuo, over P$_2$O$_5$, 24 h)

Information required on temperature

** Arsenic

Not more than 3 mg/kg

** Lead

Not more than 10 mg/kg

*** Heavy metals

Not more than 40 mg/kg

Proceed as directed in the Limit Test for Heavy Metals

Synthetic dyes

Absent

Information required on method of analysis

TESTS

IDENTIFICATION TESTS

* C. Thin layer chromatography

Activate some silica gel (Kiesel Gel G) for 1 h at 100°C and prepare a TLC plate. Prepare an 0.02% solution of the sample in methanol and apply 0.02 ml to the plate. Allow to dry and develop using a mixture of n-butanol, acetic acid and water (4:1:2 by volume) until the solvent front has ascended about 10 cm. Allow to dry and measure the Rf of the red spot.

D. Colour reaction

Dissolve 10 mg of the sample in 50 ml water. The colour of the solution is red. Add alkali to raise the pH to above 7. The colour changes to orange-yellow.

E. Colour reaction

To 0.05 g of the sample add 2 ml of 5% phosphoric acid, heat for 1 h on a water bath. After cooling, filter and wash the residue with 3 ml of water. Combine the filtrate and the washings. Neutralize the combined solution with sodium hydroxide; add 5 ml of Fehling's TS and heat on a water bath for 10 min. A red precipitate is produced.


METHOD OF ASSAY

Place about 10 mg of the sample, previously dried, and accurately weighed, in 300-ml ground stoppered flask, add 150 ml of dimethyl-formamide, dissolve by shaking occasionally, and allow to stand for 2 hours. Filter this solution through a glass filter into a 200-ml volumetric flask. Wash the bottle and filter with two 25-ml portions of dimethylformamide, combine the filtrate and the washings, add dimethylformamide to volume, and mix. Determine the absorbance at 530 nm using 1-cm cells. Calculate the content using the absorptivity for Carthamus Red.*