SPECIFICATIONS FOR THE IDENTITY AND PURITY OF SOME FOOD COLOURS, EMULSIFIERS, STABILIZERS, ANTI-CAKING AGENTS AND CERTAIN OTHER FOOD ADDITIVES
SPECIFICATIONS FOR THE IDENTITY AND PURITY
OF SOME FOOD COLOURS,
EMULSIFIERS, STABILIZERS, ANTI-CAKING AGENTS
AND CERTAIN OTHER FOOD ADDITIVES

Issued jointly by FAO and WHO

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INTRODUCTION

The specifications and identity tests appearing in this publication were developed at the Thirteenth Session of the Joint FAO/WHO Expert Committee on Food Additives for the substances for which, in the consideration of the experts, adequate data was available. Readers are requested to consider these specifications only in conjunction with the Report of the above mentioned meeting, FAO Nutrition Meetings Report Series No.46; World Health Organization Technical Report Series 1970, No.445. In particular attention is drawn to the following paragraphs, namely, the principles governing establishment of chemical specifications (Section 1), and comments on substances on the agenda (Section 3). Here are explained the reasons for the absence of specifications for certain compounds which were on the agenda and the tentative nature of others or their presence as identification tests rather than full specifications. The test solutions mentioned in the specifications are those appearing in earlier publications on specifications listed in the General References.

For detailed monographs on toxicological evaluation of these substances, a reference is invited to the publication "Toxicological Evaluation of Some Food Colours, Emulsifiers, Stabilizers, Anti-caking Agents and Certain Other Substances" FAO Nutrition Meetings Report Series No.46A; WHO/Food Add/70.36.
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SOME FOOD COLOURS
ANNATTO EXTRACTS

Synonyms: C. I. Natural Orange 4; Lebensmittel Orange No. 3; Rocou; Orlean; Terre Orellana

Code (Index) Numbers: C. I. (1956) No. 75120
Schultz (1931) No. 1387

Definition: Annatto extracts are obtained by extraction of the colour of the pericarp of the fruit of Bixa orellana.

1. Annatto extract in oil is prepared by heating the finely divided pericarp with edible vegetable oil without addition of other solvents or reagents under vacuum of about 20 mm Hg at temperatures no higher than 130°C and filtering the mixture to obtain the extract. It contains several carotenoids, of which bixin is the principal colouring matter. Bixin is the monomethyl ester of norbixin:

![Bixin Structure]

Annatto extracts in oil contain 0.2 up to 2.6% of carotenoids expressed as bixin. (see Assay) At least 30% of the total carotenoid content is bixin. It is probably present in the α-form.
Annatto extract in oil is a red oily solution - or suspension.

2. Aqueous annatto extract is obtained by heating the finely divided pericarp with a solution of sodium hydroxide or potassium hydroxide at temperatures not higher than 70°C and filtering off the aqueous solution. In this process bixin being an ester is hydrolysed to norbixin, a symmetrical dicarboxylic acid.

![Norbixin Diagram]

The principal colouring matter of aqueous annatto extract is the alkali salt of norbixin. Aqueous annatto extract is a red alkaline solution.

**Functional Use in Food: Colour**

**Identification**

A. **Absorption spectrophotometry**

1. Annatto extract in oil, diluted with chloroform, shows an absorption curve as in Fig. 1, a and b.

2. Aqueous annatto extract, diluted with water, shows an absorption curve as in Fig. 1, c.
B. Column Chromatography and Carr-Price Reaction:

1. Annatto extract in oil: Dissolve enough of the extract 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 (App. A, 1, under a and b) 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 with 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 T.S. on the top of the column. The bixin-zone becomes immediately blue-green (differentiates bixin from crocetin).

After separation discharge the water layer and wash the benzene phase with water until the elimination of the acid reaction. Centrifuge for 10 minutes at 2500 rpm the generally emulsified solution of norbixin in benzene. Decant the clear norbixin solution and dry with anhydrous sodium sulfate. Add 3-5 ml of this solution on the top of the alumina column (App. A, 1, under a and b). Norboxin forms an orange-red zone at the surface of the column and gives the same Carr-Price reaction as bixin does.

C. Paper Chromatography

1. **Annatto extract in oil:** Impregnate Whatman 3 MM filter paper or another paper having the same properties with a mixture of 1 part liquid paraffin and 9 parts of petroleum ether boiling range 40-60°C. Place an adequate quantity of the extract on the starting line and develop with a mixture acetone: water: diethylamine (49:49:2). The annatto extract in oil produces at least 3 or 4 red or yellow spots, two red spots being clearly more intensive than the other spots. Dissolve the red spots in benzene and identify that one of the spots is bixin by the method given in B.1.

2. **Aqueous annatto extract:** Prepare a chromatogram as indicated under C.1. At least 4 coloured 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 that one of the spots is norbixin.

**Specification**

**Assay:**

1. **Annatto extract in oil:** 0.2 to 2.6% of carotenoids expressed as bixin. Dissolve annatto extract in oil in chloroform. Determine the pigment content expressed as bixin by measuring the absorption at nm 500 using the value $E_{1% \text{ in CHCl}_3, 1 \text{ cm}} = 2826$ for bixin at nm 500.
2. **Aqueous annatto extract**: Proceed as indicated in 1, using glacial acetic acid as a solvent.

**Limits of Impurities**

Arsenic (as As): not more than 5 mg/kg
Lead (as Pb): not more than 20 mg/kg
CHLOROPHYLL

Synonyms and varieties: C. 1. Natural Green 3; Lebensmittel-Grün Nr. 1

Class: Phorbin (= dihydroporphin)

Colour: Green

Code Numbers: C. 1. (1956) No. 75810  
C. 1. (1924) No. 1249a  
Schultz (1931) No. 1403

Chemical Name: Chlorophyll a: Magnesium complex of 1,3,5,8-tetramethyl-4-ethyl-2-vinyl-9-keto-10-carbomethoxyphorbinphytyl-7-propionate

Chlorophyll b: Magnesium complex of 1,5,8-trimethyl-3-formyl-4-ethyl-2-vinyl-9-keto-10-carbomethoxyphorbinphytyl-7-propionate

Empirical Formula: Chlorophyll a: C_{55}H_{73}O_5N_4Mg  
Chlorophyll b: C_{55}H_{70}O_6N_4Mg
**Structural Formula:**

\[
\text{Chlorophyll a} \\
\text{Chlorophyll b}
\]

**Molecular Weight:**

- a 893.54
- b 907.52

**Definition:**

Chlorophyll is the green pigment of plants. The product to be used as a food colour should be produced from alfalfa, lucerne, clover or spinach. It is a mixture of three parts of chlorophyll a and one part of chlorophyll b and enzymatic degradation products thereof. It is the ester of chlorophyllin with phytol. By the action of the enzyme chlorophyllase phytol is split off. Chlorophyll a crystallizes in green hexagonal plates, chlorophyll b in dark-green needles.

**Description**

The chlorophyll of commerce is an intensely dark green, aqueous, ethanolic, or oily solution of chlorophyll degradation products. It is soluble in ethanol, ether, chloroform and benzene; insoluble in water.
Identification Tests

A. Comparison for blue intensity vs. yellow intensity in tintometer (Lovibond). **Note:** Method required.

B. A solution of chlorophyll in ethanol is blue-green with deep red fluorescence.

C. Brown-phase reaction may be useful for characterization of chlorophyll, when this has not been previously treated with alkalies. Treat green ether or petroleum ether solution of colouring matter with a small quantity of a 10% solution of potassium hydroxide in methanol. Colour becomes brown, quickly returning to green.

Purity Tests

Lead (as Pb): not more than 20 mg/kg.
Arsenic (as As): not more than 5 mg/kg.
CHLOROPHYLL COPPER COMPLEX

DEFINITION

**Functional Use in Foods:** Food Colour Class: Porphyrin
Colour: Green

**Synonym:** Lebensmittel-Grün No. 2

**Code (Index) Numbers:** C.I. (1956) No. 75810

**Chemical Name:** Chlorophyll copper complex

DESCRIPTION

**Source:** Chlorophyll copper complex is obtained from chlorophyll by partial replacement of magnesium by copper and usually contains between 4 and 6% total copper.

**Appearance:** Blue-green powders, pastes or viscous liquids, having a slight amino-like odour.

CHARACTERISTICS

**Identification**

A. Solubility: Water: insoluble
Ethanol, Ester, Chloroform: soluble

B. Dissolve 0.2 g of the sample in 100 ml ether and add 1 ml of a solution of 1 g of sodium hydroxide in 5 ml methanol, stir and allow to stand for 30 minutes. Extract the solution three times with 10 ml each of water, and dilute to 1 000 ml with water. This solution exhibits absorption maxima at the wavelengths of 405 nm and 630 nm, the ratio of the absorbances 405/630 being $3.7 \pm 0.3$. 
C. Dissolve the residue on ignition of Chlorophyll Copper complex in 10 ml of diluted hydrochloric acid by heating on a water bath, filter if the solution is not clear, and dilute to 10 ml with water. Use this solution as the test preparation for the following tests: (i) To 5 ml of this preparation add ammonia T.S. to make the solution alkaline: a blue colour appears; (ii) To 5 ml of this preparation add 0.5 ml of sodium diethyldithiocarbamate solution (1 to 1000): a brown precipitate is formed.

**Specification**

**Assay:** Accurately weigh about 10 mg of Chlorophyll Copper complex with the micro-chemical balance, dissolve in 50 ml of ether, add 1 ml of a solution of sodium hydroxide in methanol (1 to 5), stir, and heat for 30 minutes on a water bath with a reflux condenser. Cool, extract 3 times with 10 ml each of water, combine the extracts, and add the phosphate buffer (pH 7.5) to produce 100 ml solution: the absorbance $E_{1\%}^{1\text{cm}}$ is not less than 249 at the wavelength of 405 nm.

Additional criteria: The article of commerce can be further specified by loss on drying and residue on ignition and total copper content.

**LIMITS OF IMPURITIES AND ABSENCE OF ADULTERANTS**

**Arsenic (as As):** not more than 5 mg/kg.

**Lead (as Pb):** not more than 10 mg/kg.

**Basic Coal Tar Dyes:** To 5 ml of a 0.5% ethereal solution of Chlorophyll copper add 1 ml acetic acid and 5 ml water and stir. Filter by a gravity filter paper moistened with water, allowing the ether layer to remain in the paper filter; the filtrate is colourless.

**Free Ionizable Copper:** not more than 200 mg/kg (IUPAC Method for Copper on the aqueous extract).
CHLOROPHYLLIN COPPER COMPLEX,
SODIUM OR POTASSIUM SALT

(Tentative)

DEFINITION

Functional Use in Foods: Food Colour Class: Porphyrin Colour: Green

Synonym: Lebensmittel-Grün No. 2

Code (Index) Numbers: C.I. (1956) No. 75810

Chemical Name: Sodium or Potassium salt of Chlorophyllin copper complex.

DESCRIPTION

Source: Chlorophyllin copper complex salts are obtained from chlorophyll by partial replacement of magnesium by copper and replacing the methyl and phytol ester groups with alkali and usually contains between 4 and 6% total copper.

Appearance: Blue-black powders having a slight amino-like odour.

CHARACTERISTICS

Identification

A. Solubility: Water: soluble Ethanol, Ether, Chloroform: insoluble

B. Dissolve 0.1 g of product in water and dilute to 100 ml with water. This solution exhibits absorption maxima
at the wavelengths of 405 nm and 530 nm. The ratio of the absorbances 405/530 being 3.7 ± 0.3.

C. Dissolve the residue on ignition of this material in 10 ml of diluted hydrochloric acid by heating on a water bath, filter if the solution is not clear, and dilute to 10 ml with water. Use this solution as the test preparation for the following tests: (i) To 5 ml of this preparation add ammonia T.S. to make the solution alkaline: a blue colour appears. (ii) To 5 ml of this preparation add 0.5 ml of sodium diethyldithiocarbamate solution (1 to 1000): a brown precipitate is formed. (iii) Test in the usual manner for sodium and/or potassium.

**Specification**

**Assay:** Accurately weigh about 10 mg of this material dried at 105° for 1 h with the micro-chemical balance, dissolve in phosphate buffer (pH 7.5) to produce a 100 ml solution: the absorbance $E_{1\%}^{1cm}$ is not less than 249 at the wavelength of 405 nm.

Additional criteria: The article of commerce can be further specified by loss on drying and residue on ignition, nitrogen, pH, iron and total copper.

**LIMITS OF IMPURITIES**

Arsenic (as As): not more than 5 mg/kg
Lead (as Pb): not more than 20 mg/kg.

Basic Coal Tar Dyes: To 5 ml of a 0.5% aqueous solution of this material add 1 ml acetic acid and extract three times with 5 ml of ether. Filter by a gravity filter paper moistened with water, allowing the ether layer to remain in the paper filter; the filtrate is colourless.

Free Ionizable Copper: not more than 2500 mg/kg (IUPAC Method).

Note Information needed on level used for colouring foods and on actual free ionizable copper content.
ERYTHROSINE*

Synonyms: C.I. Food Red 14; FD and C Red No. 3; LB - Rot 1

Class: Xanthene

Colour: Red


Chemical Name: Disodium or dipotassium salt of tetra-iodofluorescein.

Empirical Formula: $C_{20}H_6O_5I_4Na_2$

* This supersedes the specification contained in, "Specifications, Identity and Purity of Food Additives" - Vol. II Food Colours 1963; published under the auspices of the FAO and the WHO.
**Structural Formula**

![Structural Formula Image]

**Molecular Weight:** 879.9

**Description**

Erythrosine is a water-soluble food colour conforming to the following specifications.

**Identification Tests**

See Appendix A, 1. of "Specifications for Identity and Purity of Food Additives - Vol. II - Food Colours."* The tests should not indicate any presence of fluorescein.

---

* Published in 1963 under the auspices of the Food and Agriculture Organization of the United Nations and the World Health Organization
Purity Tests

Dye content: not less than 85% by the method described below. Dissolve 1000 g of Erythrosine in 250 ml of water, transfer to a clean 500-ml beaker, add 8.0 ml 1.5 N nitric acid and stir well.

Filter through a sintered glass crucible (porosity 3, diameter 5 cm) which has been weighed containing a small glass stirring rod. Wash thoroughly with 0.5% nitric acid until the filtrate gives no turbidity with silver nitrate T.S. and then wash with 30 ml water. Dry to constant weight at 135° ± 5°, carefully breaking up the precipitate by means of the glass rod. Cool in desiccator and weigh.

\[
\text{% dye content} = \frac{\text{weight of residue} \times 105.3}{\text{weight of the sample}}
\]

Loss on drying at 135°

Chlorides and sulfates calculated as sodium salts) 15 %

Water-insoluble matter: not more than 0.2 %.

Ether-extractable matter (from alkaline solution only): not more than 0.2 %.

Lead (as Pb): not more than 10 mg/kg.

Arsenic (as As): not more than 3 mg/kg.

Inorganic iodides: not more than 1 000 mg/kg as sodium iodide.

Place 5000 g of Erythrosine in 400 ml beaker and add 150 ml of water; add just enough 10% NaOH solution to give complete solution. Heat nearly to boiling and add 5 ml of \( H_3PO_4 \). Digest solution until precipitation is well coagulated, cool to room temperature, transfer to 250 ml volumetric flask, and make to volume. Mix thoroughly, filter through dry fluted filter, and discard first few ml of filtrate. Transfer 100 ml of filtrate to 500 ml tall-form beaker, add 2.5 ml of 30% NaOH solution, few glass beads, and 15 ml of 7% KMnO4 solution. Cover with watchglass, boil 5 minutes, and remove from heat. When boiling ceases, add carefully 10 ml of \( HNO_3 \) and boil 5 minutes more.
Remove beaker from heat and wash down cover glass and sides (excess KMnO₄ must be present). Add 5 ml of 10% NaNO₂ solution quickly with swirling (KMnO₄ colour will be destroyed, and brown suspension of MnO₂ left). Continue addition of the NaNO₂ solution cautiously, allowing each drop to react before next is added. When solution appears colourless by transmitted light, but some particles of solid MnO₂ remain, do not attempt to destroy these, but immediately add 1% KMnO₄ solution in 1 ml portions until solution becomes pink.

If more than 2 ml is required or if brown colour appears, add at once 10 ml of the diluted KMnO₄ solution and again heat to boiling. Repeat dropwise addition of the NaNO₂ solution and again add the diluted KMnO₄ solution to pink colour.

Filter solution rapidly with suction through a sintered-glass filter (medium porosity) into wide-mouthed 500 ml flask. Wash beaker and filter thoroughly with water (solution must remain pink after filtration). Add the NaNO₂ solution dropwise with shaking until 1 drop has been added in excess of that required to decolourize solution. Add 5 ml of 10% sulfamic acid solution, wash down sides of flask, and swirl contents. Cool solution to room temperature, add 2-3 g of solid KI, and titrate liberated iodine with the standard sodium thiosulfate solution.

1 ml of 0.05 N Na₂S₂O₃ 0.002498 g of NaI.
Subsidiary dyes: not more than 3%.
Intermediates: not more than 0.5%.
CHEMICALLY TREATED STARCHES
CHEMICALLY TREATED STARCHES

DEFINITION

Functional Use in Foods

Thickening agents.

Structural Formula and Molecular Weight

Chemically treated starch is a macromolecule composed of anhydro glucose units in linear and branched form and modified as indicated below under "Chemical Description." Its molecular weight varies greatly depending upon botanical origin and is generally lower than that of the native starch from which it is obtained.

DESCRIPTION

Appearance

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

Chemical Description

Chemically treated starches are food starches which have one or more of their original characteristics altered by chemical treatment in accordance with good manufacturing practice with one or more of the agents listed in Table I. In the case of starches treated with heat in the presence of acid or with alkali, (1, 2 and 3 in Table I) the alteration is a minor fragmentation of the chains.

When the starch is bleached, (4 in Table I) the change is in the colour only. Oxidation (5 in Table I) involves the production of carboxyl groups.
The treatments indicated under 6 and 7 of Table I result in partial substitution in the 6, 3 or 2-position of the anhydro glucose unit unless the 6-position is occupied for branching.

In case of crossbonding, where a polyfunctional substituting agent as mentioned under 6 in Table I connects two chains, the structure can be represented by:

Starch-O-R-O-Starch,
where R = crossbonding group and Starch refers to the linear and/or branched structure.

CHARACTERISTICS

Identification

A. Solubility Water (cold): insoluble (if not pregelatinized)
   Water (hot): form typical colloid with viscous properties
   Ethanol: insoluble

B. Chemically treated starches which have not been pregelatinized retain their granular structure and can be identified as starches by macroscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under crossed nicols the typical polarization cross will be observed.

C. Add a few drops of 0.1 N I/KI solution to an aqueous suspension of the product. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red.

D. Boil 2.5 g of the treated starch with 100 ml of hydrochloric acid solution (3% w/w) under reflux for 3 hours. Glucose can be identified in the hydrolysate by any usual method.
E. The appropriate tests to differentiate between the various treatments of the starches appear in the section entitled "Analytical Methods for Chemically Treated Starches" appearing immediately below this specification.

Specifications

The article of commerce can be specified by the parameter specific for the particular type of modification indicated in Column 3 of Table 1, and further as to loss on drying, ash, chloride, pH, protein and fat.

LIMITS OF IMPURITIES

<table>
<thead>
<tr>
<th>Substance</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>not more than 3 mg/kg</td>
</tr>
<tr>
<td>Lead</td>
<td>not more than 5 mg/kg</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>not more than 40 mg/kg</td>
</tr>
<tr>
<td>Sulphur dioxide</td>
<td>not more than 80 mg/kg</td>
</tr>
<tr>
<td>Column 1</td>
<td>Column 2</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Treatment</td>
<td>Maximum amount of substance reasonably required to accomplish the intended physical or technical effect</td>
</tr>
<tr>
<td>1. Roasted starch with addition of acid</td>
<td>0.15 % acid, calculated as hydrochloric acid anhydrous (100%) and based on dry starch</td>
</tr>
<tr>
<td>2. Acid treatment in slurry</td>
<td>7% hydrochloric acid or 2.0% sulphuric acid</td>
</tr>
<tr>
<td>3. Alkaline treatment with</td>
<td>Sodium or potassium hydroxide not to exceed 1.0%</td>
</tr>
<tr>
<td>4. Bleached starch</td>
<td>Sodium hypochlorite, sodium chlorite, hydrogen peroxide, potassium permanganate, peracetic acid, ammonium persulphate, sulphur dioxide in amounts not more than that sufficient to bleach the material.</td>
</tr>
<tr>
<td>5. Oxidized by treatment with</td>
<td>Chlorine, as sodium hypochlorite, not to exceed 5.0% of chlorine, based on dry starch</td>
</tr>
<tr>
<td>Column 1</td>
<td>Column 2</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Treatment (may be combined)</td>
<td>Maximum amount of substance reasonably required to accomplish the intended physical or technical effect</td>
</tr>
<tr>
<td>6. Esterified by treatment with</td>
<td>Acetic anhydride, not to exceed 10%</td>
</tr>
<tr>
<td></td>
<td>Vinyl acetate, not to exceed 7.5%</td>
</tr>
<tr>
<td></td>
<td>Adipic anhydride, not to exceed 0.12%</td>
</tr>
<tr>
<td></td>
<td>Sodium tripolyphosphate and/or sodium trimetaphosphate and/or orthophosphoric acid and/or sodium or potassium salts thereof</td>
</tr>
<tr>
<td>7. Etherified by treatment with</td>
<td>Propylene oxide, not to exceed 5</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TENTATIVE</strong> due to lack of adequate data for toxicological evaluation</td>
<td>succinic anhydride, not to exceed 4%</td>
</tr>
<tr>
<td>Column 1</td>
<td>Column 2</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Treatment</td>
<td>Maximum amount of substance reasonably required to accomplish the intended physical or technical effect</td>
</tr>
<tr>
<td>(may be combined)</td>
<td>Phosphorus oxychloride, not to exceed 0.1%</td>
</tr>
<tr>
<td></td>
<td>Epichlorohydrin, not to exceed 0.3%</td>
</tr>
</tbody>
</table>

Identification tests for the various treatments are given following this specification.
ANNEX

ANALYTICAL METHODS FOR CHEMICALLY TREATED STARCHES

Tests to Differentiate, Identify and Assay the Treatment and/or Limits of Residues of the Treatment Agent left in the Product.

1. **Hypochlorite oxidized starch**
   (Not for slightly oxidized potato starch)

   Because of the carboxyl group content, hypochlorite-oxidized starch has anionic properties. It can be dyed with positively charged dyes such as methylene blue: 50 mg of hypochlorite-oxidized starch is kept in suspension for 5-10 minutes in 25 ml of a 1 percent aqueous dye solution and stirred occasionally. After decantation of the excess solution the starch is washed in distilled water. Microscopic inspection clearly shows the colouring. By this test hypochlorite-oxidized starch is distinguished from native and acid modified starch of the same botanical origin.

2. **Specific reaction of acetyl groups**

   Acetate is liberated upon saponification of acetylated starch. After concentration the acetate is converted to acetone by heating with calcium hydroxide. The acetone thus produced strains blue with 0-nitro-benzaldehyde. About 10 g of the test substance is suspended in 25 ml water to which is added 20 ml 0.4 N NaOH. After shaking for 1 hour the starch is filtered off and the filtrate evaporated in an oven at 110°C. The residue is dissolved in a few drops of water and transferred to a test tube. Calcium hydroxide is added and the tube heated thereby giving off acetone vapours. These produce a blue colour on a paper strip soaked in a fresh saturated solution of 0-nitrobenzaldehyde in 2 N NaOH. The blue colour is more distinct when the original yellow colour of the reagent is removed with 1 drop of HCl 1: 10.
3. **Test for Ester Groups**

The infrared spectrum of a thin film gives a typical absorption band at about 1720 cm\(^{-1}\) which is an indication for ester groups. The limit of detection is about 0.5 percent acetyl odipyl & succinyl groups in the product.

4. **Assay: Determination of Acetyl Groups and Succinyl Groups**


For succinyl the calculation of the degree of substitution of the sodium salt is following:

\[
\%\text{succinyl} = \frac{(\text{Blank titer} - \text{Sample titer}) \times \text{acid norm} \times 0.123 \times 100}{\text{sample weight in grams (dry basis)}}
\]

\[
\text{DS} = \frac{162 \times A}{12300-122 \times A} \quad (\text{where } A = \text{succinyl})
\]

5. **Phosphorous**

**Method 1**

**Principle**

The sample is ignited in the presence of an added electrolyte to convert phosphorous to a stable form which is not volatilized during ignition. The residual phosphate is taken up in acid and determined spectrophotometrically as the molybdovanadophosphoric acid complex (Note 1).

**Scope**

The procedure is applicable to determination of phosphorus in unmodified and modified corn starches (Note 2).
Special Apparatus

1. Platinum or Silica Dishes: About 100-ml capacity.

2. Muffle Furnace: Equipped with a pyrometer and capable of operating at controlled temperatures up to 650°C.

3. Spectrophotometer: The method requires an instrument capable of continuously-variable wavelengths in the visible spectrum and equipped with matching cuvettes having a cell depth not over 2.0 cm (Note 2).

Reagents

1. Calcium Acetate solution, 2 percent: Dissolve 20 g of reagent grade calcium acetate monohydrate (Ca(C₂H₃O₂)₂·H₂O) in 980 ml of distilled water.

2. Ammonium Vanadate solution, 0.25 percent: Dissolve 2.5 g of ammonium metavanadate (NH₄VO₃) in 600 ml of boiling water. Cool to 60-70°C and add 20 ml of concentrated nitric acid. Cool to room temperature and dilute to 1-liter volume with distilled water.

3. Ammonium Molybdate solution, 5 percent: Dissolve 50 g of ammonium molybdate tetrahydrate ((NH₄)₆Mo₇O₂₄·₄H₂O) in 900 ml of warm water. Cool to room temperature and dilute to 1-liter volume with distilled water.

4. Standard Phosphorus solution, 0.1 mg P/ml: Dissolve 0.4387 g of reagent grade potassium dihydrogen phosphate (KH₂PO₄) in water and dilute to 1-liter volume with distilled water.

5. Nitric Acid solution, 29 percent: Add 300 ml of concentrated nitric acid (sp.gr. 1.42) to 600 ml of distilled water, and mix.

Procedure

Standardization Curve: Pipet 5.0, 10.0, and 15.0 ml of standard phosphorus solution into respective 100 ml
volumetric flasks, and use another flask for a blank. To each flask add, in order, 10 ml of 29 percent nitric acid, 10 ml of 0.25 percent ammonium vanadate, and 10 ml of 5 percent ammonium molybdate, mixing thoroughly after addition of each reagent (Note 3). Dilute to volume with distilled water, mix thoroughly, and allow to stand for 10 minutes. Using the blank as a reference solution at 100 percent transmission, determine the transmission of each standard at 460 m\(\mu\). Plot log % transmission versus mg of phosphorus per 100 ml (Note 4).

Analysis of Corn Starch: Weigh accurately 10 g of corn starch into a platinum or silica dish; add 10 ml of 2 percent calcium acetate solution in a fine stream, distributing the solution uniformly in the sample (Note 5). Place the dish on a hot plate and carefully evaporate to dryness, then increase heat and carbonize the sample on the hot plate or over a gas flame. Place the dish in a muffle furnace at 600-650°C until the ash is free of carbon (1-2 hours, Note 6).

Cool to room temperature and wet the ash with 15 ml of water. Slowly wash down the sides of the dish with 5 ml of 29 percent nitric acid; quantitatively transfer to a 200-ml volumetric flask, rinsing the dish with three 20-ml portions of distilled water. Dilute to volume with distilled water and mix thoroughly. (If not clear, gravity filter through a retentive paper.). Transfer an aliquot selected to contain not more than 1.5 mg of phosphorus to a 100-ml volumetric flask, and add 50 ml of water to another flask to serve as a blank. To each flask add, in order, 10 ml of 29 percent nitric acid, 10 ml of 0.25 percent ammonium vanadate, and 10 ml of 5 percent ammonium molybdate, mixing thoroughly after addition of each reagent (Note 3). Dilute to volume with water, mix thoroughly, and allow to stand for 10 minutes. Determine % transmission of the sample at 460 m\(\mu\), using the blank as a reference solution at 100 percent transmission (Note 7). Read mg of phosphorus in the aliquot from the standardization curve.
Calculation

Total Phosphorus, ppm (as is) = 

\[ \frac{mg \text{ Phosphorus (From Graph)} \times 200 \times 1000}{\text{Aliquot Volume} \times \text{Sample Wt. (g)}} \]

Notes and Precautions

1. The chemistry of the chromophore is in dispute; for a discussion of some of the possibilities, see Kitson and Mellon, Anal. Chem. 16, 379 (1944).

2. The procedure as written is not recommended for starches having phosphorus contents below 40 ppm. For phosphorus levels in such a low range, adequate precision can be attained by tenfold dilution of the phosphorus standard and use of 10-cm cuvettes.

3. To avoid interference from precipitation and side reactions, reagents must be added in the order stated. Since the vanadate and molybdate are present in large excess, volumes of reagents need not be controlled more closely than ± 1 ml.

4. The standardization curve is reproducible and need be checked only when fresh reagents are prepared.

5. If desired, 3 ml of a saturated solution of magnesium nitrate and 7 ml of water may be substituted for the calcium acetate. The two systems give comparable results; calcium acetate is recommended principally because it yields a higher-density ash and because an ignited magnesium nitrate blank must be included when using that salt.

6. If difficulty is experienced in obtaining a carbon-free ash, the dish may be removed from the muffle, cooled, and the residue moistened with several drops of 29 percent nitric acid. Heating is then continued.
7. Calcium acetate has not been observed to contribute apparent phosphorus to the system; however, it would be advisable to check an ignited calcium acetate blank against a water blank whenever a new lot of calcium acetate hydrate is opened.

**Method 2**

**Principle**

The sample is digested with a mixture of sulphuric and nitric acids, and the phosphorus content is determined spectrophotometrically by the metol method.

**Scope**

The procedure is applicable to determination of phosphorus in unmodified and modified starches.

**Special Apparatus:**

**Spectrophotometer:** The method requires an instrument with good susceptibility in the red part of the visible spectrum.

**Reagents**

a. Sulphuric acid 96 percent.

b. Nitric acid 65 percent.

\( \text{P}_2\text{O}_5 \) content must be less than 0.1 mg per 50 ml.

**I. Metol solution:**

Dissolve 1 g of monomethyl-p-aminophenolsulphate (metol), 5 g sodiumsulphite 7 aq. and 150 g sodium bisulphite in about 700 ml of distilled water and dilute to 1 000 ml.

Filter if the solution is not clear.

This solution is stable for several months.
II. **Molybdate solution:**  
Dissolve 50 g ammonium molybdate pro analysis in about 450 ml of distilled water.

Pour this solution gradually in 500 ml 10 N sulphuric acid while stirring.

Dilute to 1 000 ml.

III. **Sodium acetate solution:**  
Neutralize 1 000 ml 5 N sodium hydroxide solution with acetic acid.

Dilute to 2 000 ml.

IV. **Standard Phosphorus solution:**  
Dissolve 1.9166 g of reagent grade potassium dihydrogen phosphate (dried over sulphuric acid if necessary) in distilled water and dilute to 1 000 ml.

If the solution has to be stored for a prolonged period of time, add a few drops of chloroform.

1 ml contains 1 mg $P_2O_5$.

For measurement dilute 50 ml to 1 000 ml with distilled water.

10 ml of this solution contains 0.5 mg $P_2O_5$.

**Procedure**

Weigh:

- Potato starch : 2 grams
- Other starches : 5 grams
- Starch with monostarch phosphate : 1 gram

Bring in 500 ml. Kohlrausch flask 10 ml of the concentrated sulphuric acid and 10 ml of the concentrated nitric acid solution.
Add the starch. Heat mixture until development of nitrous fumes becomes violent, then stop heating. If development of brown fumes diminishes, repeat the heating procedure, and add as long as contents of the flask obtain a dark colour, a few drops of nitric acid. This procedure is continued until the solution becomes colourless. (A faint yellow or green colour sometimes disappears on cooling). Dilute after cooling with 20 ml of distilled water, bring to the boil to remove nitrous fumes. Bring contents in calibrated flask of 200 ml, and dilute to mark.

Pipette 10 ml in calibrated flask of 100 ml, and add successively 35 ml distilled water, 10 ml of solution I, and 10 ml of solution II and shake.

After 15 minutes add 20 ml of solution III and dilute to mark. Determine extinction at 600-610 nm. For comparison a blank and a standard are measured.

a. Blank

Take for every series of determinations a Kohlrausch flask of 500 ml, with 10 ml sulphuric and 10 ml nitric acid, without starch addition. Treat by same procedure as the sample.

b. Also determine extinction of a standard, in which in a calibrated flask of 100 ml, 10 ml of the blank solution under a., and 10 ml of the phosphorus solution (0.5 mg P₂O₅) are treated with solutions I - III (Eb).

Calculation:

Subtract from extinction of the solution obtained from the samples extinction of the blank.

Potato starch: \( \frac{E}{Eb} \times 500 = \text{mg P}_2\text{O}_5 \) per 100 gram of starch

Other starches: \( \frac{E}{Eb} \times 200 = \text{mg P}_2\text{O}_5 \)

Starch with monostarch: \( \frac{E}{Eb} \times 1000 = \text{mg P}_2\text{O}_5 \)
Note: If solutions I - III are made correctly, pH before addition of solution III is about 1.0, after addition slightly above 3.0. If the latter value is too low the intensity of the colour will continue to increase rapidly.

6. Propylene Oxide, Epichlorohydrins

7. Propylene Chlorohydrins, Glycerol monochlorhydrin and dichlorhydrin.

Determined by gas chromatography according to Ragelis, E. P., Fishes, B. S., Klimeck, B. A. and Johnson, C., J. A.O. A.C., 51, 709 (1968) after extraction of the product with acetone-water (5+1, V/V) according to Heuser, S. G. and Scudamore, K. A. Analyst 93, 252-258 (1968).

8. Sulphur Dioxide

Principle

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

Scope

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

Special Apparatus

"Monier-Williams" apparatus for the determination of sulfurous acid, constructed with standard-taper glass connections, can be obtained from Scientific Glass Apparatus Company, Bloomfield, N.J. It is customary however to
construct the apparatus with regular laboratory glassware using rubber stopper connexions (see sketch).

SULFUR DIOXIDE - Apparatus


The "Monier-Williams" sulfurous acid apparatus is constructed from standard laboratory glassware and connected with rubber stoppers and rubber tubing in accordance with diagram. In operation, carbon dioxide is passed through the scrubber and bubbled through the heated reaction mixture, sweeping sulfur dioxide through the condenser and into the receivers where it is absorbed quantitatively.
The assembly consists of a 1,000-ml two-neck round-bottom boiling flask to which a gas-inlet tube, 60-ml dropping funnel having a 2-mm bore stopcock, and sloping Allihn reflux condenser are attached. A delivery tube connects upper end of condenser to bottom of a 250-ml Erlenmeyer receiving flask, which is followed by a Peligot tube.

Reagents

1. Sodium Carbonate solution: Dissolve approximately 15 g of Na₂CO₃ or 40 g of Na₂CO₃·10H₂O in distilled water, and dilute to 100-ml volume.

2. Hydrogen Peroxide, 3 percent: Dilute 10 ml of C.P. neutral 30 percent hydrogen peroxide (H₂O₂) with distilled water to 100-ml volume.

3. Sodium Hydroxide, 0.1 N: Standardize using bromphenol blue indicator.

4. Hydrochloric Acid, concentrated: Reagent grade.

5. Bromphenol Blue Indicator.

Procedure

Pass carbon dioxide from a generator or cylinder through a sodium carbonate scrubber solution to remove chlorine, hence into the gas-inlet tube of the boiling flask. Place 15 ml of 3 percent hydrogen peroxide in the receiving flask and 5 ml in the Peligot tube. Connect the apparatus and introduce into the boiling flask, by means of the dropping funnel, 300 ml of distilled water and 20 ml of concentrated hydrochloric acid. Boil contents approximately 10 minutes in a current of carbon dioxide.

Weigh 100 g (± 1 g) of starch sample and disperse in approximately 300 ml of recently-boiled distilled water. Transfer slurry to boiling flask by means of the dropping funnel, regulating sample-addition rate and gas flow rate through the apparatus to prevent drawback of hydrogen peroxide, inclusion of air, or burning of sample. Boil mixture gently for 1 hour in a slow current of carbon dioxide.
Stop flow of water in condenser just before end of distillation. When delivery tube just above receiving flask becomes hot, remove tube from condenser immediately. Wash delivery tube and Peligot tube contents into receiving flask, and titrate with 0.1 N sodium hydroxide, using bromphenol blue indicator (see Note).

Determine a blank on the reagents, and correct results accordingly.

**Calculation**

\[
\text{Sulfur Dioxide (as is)} = \\
= \frac{(\text{ml} \times 0.1 \text{ N NaOH} - \text{Blank}) \times 0.0032 \times 100}{100 \text{ g}}
\]

**Note:**

A gravimetric determination may be made after titration. Acidify with HC1, precipitate with BaCl2, settle, filter, wash, ignite, and weigh as BaSO4.
SOME EMULSIFIERS AND STABILIZERS
CARRAGHEENAN

Synonyms

Carragheenan, Irish moss gelose, chondrus extract.

Chemical Description

Carragheenan consists chiefly of the calcium, potassium, sodium, ammonium and magnesium salts of polysaccharide sulphate esters which on hydrolysis yield galactose and anhydrogalactose.

Definition

Carragheenan is obtained by extraction with water of members of the Gigartinaceae and Solieriaceae families of the class Rhodophyceae (red seaweed). Members of these families used in the production of carragheenan include Chondrus crispus; C. ocellatus; Eucheuma cottonii; E. spinosum; Gigartina acicularis; G. pistillata; G. radula; and G. stellata.

Description

Carragheenan occurs as a yellowish to colourless, coarse to fine, powder which is practically odourless.

Uses

Thickening agent and stabilizer.

Identification

A. Solubility

Water: soluble

Ethanol: insoluble
B. Add 4 g to 200 ml of water and heat the mixture in a water bath at a temperature of about 80°, with constant stirring, until a viscous solution results. Replace any water lost by evaporation and allow it to cool to room temperature. It becomes more viscous and may form a gel.
To 50 ml of the solution or gel add 100 mg of potassium chloride and 50 mg of sodium chloride; mix well, reheat and cool. A short-textured gel forms.

C. To a solution of 100 mg of sample in 20 ml water add 3 ml IN barium chloride and 5 ml 2 N hydrochloric acid and filter if there is a precipitate. Boil filtrate for 5 minutes. A white, crystalline precipitate is formed.

D. Identify galactose and anhydrogalactose as indicated in Annex.

Purity Tests

Ash (Total): Between 20 to 37 percent on the dry weight basis. Transfer about 2 g, accurately weighed, into a previously ignited, tared silica or platinum crucible. Heat the sample with a suitable infrared heat lamp, increasing the intensity gradually, until it is completely charred and then continue for an additional 30 minutes. Transfer the crucible and charred sample into a muffle furnace and ignite at about 550° for 1 hour, then cool in a dessicator 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 infrared heat lamp before igniting again.
Ash (Acid-insoluble): Not more than 2 percent.
Sulphate (as SO₄): Between 20 and 40 percent on the dry weight basis. The sulphates are precipitated as barium sulphate in hydrochloric acid, after hydrolysis under reflux. Weigh accurately 1 g of sample in 100 ml long neck round bottom flask. Add 50 ml N/5 hydrochloric acid. Fit a cooler consisting preferably of 5 round bulbs and heat to boiling under reflux for 1 hour. Add 25 ml 110-volume hydrogen peroxide solution and continue boiling under reflux for 5 hours when the solution becomes completely clear. Transfer the solution to 600 ml beaker. Bring to boil and stir in dropwise 10 ml of 10 percent solution of barium chloride. Let stand for 2 hours 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 till the filtrate is free from chloride. Dry the filter paper in a drying oven and ash at 1000°C in a tared silica crucible. Let cool when the ash is white. Weigh and calculate the weight (P) of the barium sulphate obtained.

\[ \text{P} \times 0.04116 \]

% sulphate:

Arsenic: Not more than 3 mg/kg.
Heavy metals: Not more than 40 mg/kg.
Lead: Not more than 10 mg/kg.

**Additional criteria:** The article of commerce can be further specified by viscosity and loss on drying.
FURCELLARAN

Synonym

Danish agar, Furcellaran

Chemical Description

Furcellaran consists chiefly of salts of polysaccharide sulphate esters which on hydrolysis yield galactose and anhydro galactose.

Definition

Furcellaran is obtained by extraction with water of the red alga Furcellaria fastigata (Fam. Florideae) and is precipitated from the extract with potassium chloride. Potassium chloride may be added to the gelling properties of the product.

Description

Furcellaran occurs as a yellowish to colourless, coarse to fine, powder, which is practically odourless and has a mild, salty taste due to the potassium chloride.

Uses

As thickening agent and stabilizer.

Identification

A. Solubility: Water: Soluble
   Ethanol: Insoluble

B. Add four grams to 200 ml of water and heat the mixture in a water bath at a temperature of about 80°, with constant stirring, until a viscous solution results. Replace any water lost by evaporation and allow it to cool to room temperature. It forms a gel.
C. To a solution of 100 mg of sample in 20 ml water add 3 ml 1N barium chloride and 5 ml 2N hydrochloric acid, filter if precipitate is formed. Boil filtrate for 5 minutes. A white crystalline precipitate is formed.

D. Identify galactose, glucose and xylose as indicated in the Annex.

Purity Tests

Ash (Total): As: Not more than 3 mg/kg.
            Heavy metals: not more than 30 mg/kg.
            Ph: not more than 10 mg/kg.

Sulphate (as SO₄): Between 14 and 18 percent on the dry weight basis. Method as indicated under carrageenan.

Additional criteria: The article of commerce can be further specified by viscosity, loss on drying, and potassium chloride content.
ARABIC GUM

Synonym

Acacia gum

Chemical Description

Gum arabic consists chiefly of a high molecular weight polysaccharides and their calcium, potassium and magnesium salts which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid.

Definition

Gum arabic is a dried gummy exudation obtained from the stems and branches of Acacia Sengal (L) Willdenow or of related species of Acacia (Fam. Leguminosae).

Description

Unground gum arabic occurs as white or yellowish white spheroidal tears of varying size or in angular fragments and is sometimes mixed with darker fragments and pieces of bark. This crude material must be cleaned before use in foods. It is also available commercially in the form of white to yellowish white flakes, granules or as a powder.

Uses

Thickening agent and stabilizer.

Identification

A. Solubility of the powder:

Water: soluble. One gram dissolves in 2 ml of water forming a solution which flows readily and is acid to litmus.

Ethanol: insoluble.
B. A 1 in 10 solution filtered through diatomaceous earth is slightly levorotatory.

C. Identify arabinose, rhamnose, galactose and glucuronic acid as indicated in the Annex.

Purity Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash (Total)</td>
<td>Not more than 4 percent.</td>
</tr>
<tr>
<td>Ash ( Acid-insoluble)</td>
<td>Not more than 0.5 percent.</td>
</tr>
<tr>
<td>Acid (Insoluble matter)</td>
<td>Not more than 1 percent.</td>
</tr>
<tr>
<td>Starch, or dextrin</td>
<td>Boil a 1 in 50 solution of the gum, cool and add a few drops of iodine TS. No bluish or reddish colour is produced.</td>
</tr>
<tr>
<td>Tannin-bearing gums</td>
<td>To 10 ml of a 1 in 50 solution add about 0.1 ml of ferric chloride TS. No blackish colouration or blackish precipitate is formed.</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Not more than 3 mg/kg.</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Not more than 40 mg/kg.</td>
</tr>
<tr>
<td>Lead</td>
<td>Not more than 10 mg/kg.</td>
</tr>
</tbody>
</table>

Additional criteria: The article of commerce can be further specified by loss on drying and viscosity.
CAROB BEAN GUM

Synonyms

Carob, Locust bean gum, St. John's bread, Algaroba.

CHEMICAL DESCRIPTION

Carob bean gum consists chiefly of high molecular weight polysaccharides composed mainly of galactomannans.

Definition

Carob bean gum is obtained from the ground endosperms of Ceratonia siliqua (L.) Taub., (Fam. Leguminosae).

Description

Carob bean gum is a white to yellowish white, nearly odourless powder.

Uses

Thickening agent and stabilizer.

Identification

A. Solubility: Water: Forms a solution in hot water. Ethanol: Insoluble

B. A water solution of carob bean gum may be converted to a gel by the addition of small amounts of sodium borate.

C. Transfer a 2 gram sample into a 400 ml beaker, moisten it 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 the solution prepared as above into another 400 ml beaker, heat the mixture in a boiling water bath for about 10 minutes and then cool to room temperature. There is an appreciable increase in viscosity (differentiating from guar gum).

D. Identify sugars as indicated in the Annex. Only mannose and galactose are present.

E. Place some ground carob bean gum in an aqueous solution containing 0.5 percent iodine and 1 percent potassium iodide on a glass slide for microscopic examination. Carob bean meal contains long stretched tubiform cells, separate or slightly interspaced; their brown contents are much less regularly formed than in guar gum. (Guar gum shows close groups of round to pear formed cells; their contents are yellow to brown).

**Purity Tests**

- **Ash (Total):** Not more than 1.2 percent.
- **Acid-insoluble matter:** Not more than 5 percent. See the Annex for method.
- **Protein:** Not more than 8 percent. Determine nitrogen by Kjeldahl method. The percent of nitrogen determined multiplied by 5.7 gives the percent of protein in the sample.
- **Starch:** To a 1 in 10 solution of the gum add a few drops of iodine T.S. No blue colour is produced.
- **Arsenic:** Not more than 3 mg/kg.
- **Heavy metals:** Not more than 20 mg/kg.
- **Lead:** Not more than 10 mg/kg.

**Additional criteria:** The article of commerce can be further specified by viscosity and loss on drying.
GUAR GUM

Synonyms

Gum cyamopsis, guar meal, guar flour.

Chemical Description

Guar gum consists chiefly of polysaccharides of high molecular weight composed mainly of galactomannans.

Definition

Guar gum is obtained by grinding the endosperm of *Cyamopsis tetragonolobus* (L) Taub., (Fam. Leguminosae).

Uses

Thickening agent and stabilizer.

Identification

A. Solubility: Forms a solution in cold or hot water.

B. A water solution of guar gum may be converted to a gel by the addition of small amounts of sodium borate.

C. Transfer a 2 gram sample into a 400 ml beaker, moisten it 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, viscous solution is formed. Transfer 100 ml of the solution into another 400 ml beaker, heat the mixture in a boiling water bath for about 10 minutes and then cool to room temperature. There is no substantial increase in viscosity (differentiating guar from locust bean gum).

D. Identify sugars as indicated in Annex. Only mannose and galactose are present.
E. Place some ground guar gum in an aqueous solution containing 0.5 percent iodine and 1 percent potassium iodide on a glass slide for microscopic examination. Guar gum shows close groups of round to pear formed cells; their contents are yellow to brown. (Locust bean meal contains long stretched tubiform cells, separate or slightly interspaced; their brown contents are much less regularly formed than in guar gum.)

**Purity Tests**

- **Ash (Total):** Not more than 1.5 percent.
- **Acid-insoluble matter:** Not more than 7 percent. See Annex for method.
- **Protein:** Not more than 10 percent. Determine nitrogen by Kjeldahl method. The percent of nitrogen in the sample multiplied by 5.7 gives the percent of protein in the sample.
- **Starch:** To a 1 in 10 solution of the sample add a few drops of iodine T.S. No blue colour is produced.
- **Arsenic:** Not more than 3 mg/kg.
- **Heavy metals:** Not more than 20 mg/kg.
- **Lead:** Not more than 10 mg/kg.

**Additional criteria:** The article of commerce can be further specified by viscosity and loss on drying.
KARAYA GUM

Synonyms

Sterculia, Kadaya, Katilo, Kullo, Kuteera, muscara miscalled Indian tragacanth.

Chemical Description

Karaya gum consists chiefly of high molecular weight polysaccharides which on hydrolysis yield galactose, rhamnose and galacturonic acid.

Definition

Karaya gum is a dried gummy exudation obtained from Sterculia urens Roxburgh and other species of Sterculia (Fam. Sterculiaceae), or from Cochlospermum gossypium A. P. De Condolle, or other species of Cochlospermum Kunth (Fam. Bixaceae).

Description

Unground gum karaya occurs in tears of variable size or in broken irregular pieces having a somewhat crystalline appearance. It is pale yellow to pinkish brown, translucent and horny and is sometimes admixed with darker fragments and pieces of bark. This crude material must be cleaned before use in foods. Powdered karaya gum is light grey to pinkish grey. The gum has a slight odour and taste of acetic acid.

Uses

Thickening agent and stabilizer.

Identification

A. Solubility of the powder:

Water: Two grams added to 50 ml of water swells to form a granular, stiff, slightly opalescent gel which is acid to litmus.
Ethanol: Insoluble

B. The gum swells in 60 percent ethanol as distinct from other gums.

C. Boil 1 gram with 20 ml of water until a mucilage is formed, add 5 ml of hydrochloric acid and again boil the mixture for 5 minutes. A permanent pink or red colour develops.

D. Identify rhamnose, galactose and galacturonic acid as indicated in the Annex.

**Purity Tests**

Ash (Acid, Insoluble): Not more than 1 percent.
Acid-insoluble matter: Not more than 3 percent. See Annex for the method.

Starch: To a 1 in 10 solution of the gum add a few drops of iodine T.S. No blue colour is produced.

Arsenic: Not more than 3 mg/kg.
Heavy metals: Not more than 40 mg/kg.
Lead: Not more than 10 mg/kg.

Additional criteria: The article of commerce can be further specified by colour, size, viscosity and loss on drying.
TRAGACANTH GUM

Synonym

Tragacanth

Chemical Description

Tragacanth gum consists chiefly of high molecular weight polysaccharides composed of galacto-arabans and acidic polysaccharides containing galacturonic acid groups.

Definition

Tragacanth gum is a dried gummy exudation obtained from Astragalus gummifer Labillardiere, or other Asiatic species of Astragalus (Fam. Leguminosae).

Description

Unground Tragacanth occurs as flattened, lamellated, frequently curved fragments or straight or spirally twisted linear pieces from 0.5 to 2.5 mm in thickness. It is white to pale yellow in colour, translucent, horny in texture and having a short fracture. It is odourless and has an insipid, mucilaginous taste. It is rendered more easily pulverizable if heated to a temperature of 50°. Powdered Tragacanth is white to yellowish white in colour.

Uses

Thickening agent and stabilizer.

Identification

A. Solubility of the powder: Water: One gram in 50 ml of water swells to form a smooth, stiff, opalescent mucilage free from cellular fragments.

Ethanol: Insoluble
B. Examine microscopically as a suspension in water. Numerous angular fragments with circular or irregular lamellae and starch grains up to 25 μm in diameter are visible and there should be very few or no fragments of lignified tissues.

C. Identify arabinose, xylose, fucose, galactose and galacturonic acid as indicated in the Annex.

**Purity Tests**

Ash: (Total) Not more than 3 percent.
Ash (Acid-insoluble): Not more than 0.5 percent.
Arsenic: Not more than 3 mg/kg.
Heavy metals: Not more than 40 mg/kg.
Lead: Not more than 10 mg/kg.

**Additional criteria:** The article of commerce can be further specified by viscosity and loss on drying.
ANNEX

IDENTIFICATION AND OTHER TESTS FOR STABILIZERS AND THICKENERS

1. Chromatographic Identification of Sugars in the Hydrolysate from gums.

Boil a mixture of 100 mg of the sample and 20 ml of 10 percent sulphuric acid for 3 hours. Allow to cool and add excess barium carbonate (about 10 mg). Mix with a magnetic stirrer until the solution is of pH 7, and filter. Evaporate the filtrate in a rotary evaporator at 30°-50° in vacuum until a crystalline (or syrupy) or residue is obtained. Dissolve it in 10 ml 40 percent methanol. This is the hydrolysate used below.

Prepare thin layer chromatoplates with a mixture of 15 g cellulose powder (e.g. Camag Cellulose-D) and 90 ml water and dry them for 10 minutes at 100°.

Place 1 to 10 microlitre spots of hydrolysate on the starting line of two chromatoplates and spots containing 1 to 10 micrograms of the sugars which could be present in the hydrolysate. Use two solvents: A. a mixture or formic acid, methyl ethyl ketone, tertiary butanol and water (15:30:40:15 by volume) and B. a mixture of isopropanol, pyridine, acetic acid and water (40:40:5:20 by volume) 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° for 10 minutes. A greenish yellow colour is produced with hexoses, a red colour with pentoses and a brown colour with uronic acids.

2. Method for Acid Insoluble Matter: Transfer a 2 g sample, accurately weighed, into a 250 ml beaker containing 150 ml of water and 15 ml of 1 percent sulphuric acid. Cover the beaker with a watch glass and heat the mixture on a steam bath for 6 hours rubbing down the wall of the
beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. Then 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°C for 3 hours, cool in a desiccator and weigh. The difference between the weight of the filter aid plus crucible and pad and the total weight is the weight of the Acid Insoluble Matter. Calculate as percentage.
POLYGLYCEROL ESTERS OF INTERESTERIFIED RICINOLEIC ACID

DEFINITION

Functional Use in Foods: Emulsifier

Synonyms: Glyceran esters of condensed castor oil fatty acids

Structural formula:

The major components have the general structure

\[
\begin{align*}
\text{OR} \\
\text{RO} - (\text{CH}_2 - \text{CH} - \text{CH}_2\text{O})_n - \text{R}
\end{align*}
\]

Where the average value of \( n \) is about 3 and \( R \) is hydrogen or a condensation polymer of ricinoleic acid with itself (average 5 to 8 units) thus:

\[
\begin{align*}
0 & \quad (\text{CH}_2)_5\text{CH}_3 \\
\text{R'} - \text{C} - \text{O} - \text{CH} - \text{CH}_2 - \text{CH} = \text{CH} - (\text{CH}_2)_7\text{C} - \\
\end{align*}
\]

Description

Polyglycerol esters of interesterified ricinoleic acid are prepared by the esterification of polyglycerol with condensed castor oil fatty acids. The product is a highly viscous liquid.

Characteristics

Identification

A. Solubility

Water: insoluble
Ethanol: insoluble
Hydrocarbons, ethers, halogenated hydrocarbons: soluble

B. Hydrolyse the material as indicated in the Annex.

C. Test for ricinoleic acid. The fatty acids liberated in B should have a hydroxyl value corresponding to that for castor oil fatty acids (about 150 to 170).

D. Spot 5 to 20 µl of the residue obtained in B alongside control spots of glycerol on paper such as Whatman No. 3 and develop using descending chromatography for 36 h with isopropanol: water 90:10. The glycerol spot moves 40 cm and the polyglycerols are revealed in succession below that for glycerol when the paper is sprayed with either permanganate in acetone or ammoniacal silver nitrate.

**Specification**

Not less than three-quarters of the polyols should be glycerol, diglycerol and triglycerol as determined by the gas chromatography method referred to below 1/. The article of commerce may be specified further as to saponification value, solidification point of the free fatty acids, iodine value, acid value, hydroxyl value and ash content.

**Limits of Impurities**

Other polyols: Polyols other than polyglycerol and glycerol should be absent by the tests indicated in the Annex.

Arsenic: not more than 3 mg/kg

Heavy metals: not more than 10 mg/kg

---

1/ Sen, Keating and Barrett, J. Gas Chromatography 1967, 5, 269.
**PROPYLENE GLYCOL ESTERS OF FATTY ACIDS**

**DEFINITION**

**Functional Use in Foods:** Emulsifier

**Structural Formula:**

\[
\begin{align*}
\text{CH}_3\text{-CH-OR} \\
\text{CH}_2\text{-OR}
\end{align*}
\]

Where \( R \) or \( R' \) represents the fatty acid moiety; \( R \) or \( R' \) is hydrogen in the mono-esters.

**DESCRIPTION**

Propylene glycol esters of fatty acids are mixtures of propylene glycol mono- and di-esters of fatty acids of food fats. They are mainly the mono-esters with some di-esters. The commercial products contain mono- and di-glycerides when fats are used for transesterification with propylene glycol and are white to yellowish white beads, or flakes having a bland odour and taste.

**CHARACTERISTICS**

**Identification**

A. **Solubility:**

- Water: Insoluble
- Ethanol: Soluble

B. Identify fatty acids and propylene glycol and glycerol as in Annex.

**Specification**

Propylene glycol: The total propylene glycol content in the commercial product must be indicated.

* Published previously as Food Additive Specification FAS/IV/35 through the Joint FAO/WHO Food Standards Programme.
Additional criteria for the commercial product:
The composition of the article of commerce can be further specified by saponification value, iodine value, solidification point, free propylene glycol, soap content, hydroxyl

LIMITS OF IMPURITIES

Acids other than fatty acids and polyols other than propylene glycol and glycerol must be absent (See Methods in Annex).

Arsenic: Not more than 3 mg/kg
Heavy metals: Not more than 10 mg/kg

EXAMINATION

The following is an international referee method to be used in cases of dispute:

Assay for propylene glycol and glycerol:

Transfer about 15 g of sample, accurately weighed into a 500-ml flask, add 250 ml of ethanol and 7.5 of potassium hydroxide and mix. Reflux the solution for 2 hours, transfer into an 800-ml beaker rinsing the flask with about 100 ml of water and adding the rinse water to the beaker. Heat on a steam or water bath, adding water occasionally to replace the ethanol and evaporate until the odour of ethanol can no longer be detected. Adjust the volume to about 250 ml with hot water, neutralize with dilute sulphuric acid (1 in 2), add a slight excess of acid, heat with gentle stirring until the fatty acid layer separates. Transfer the fatty acids into a warm 500-ml separatory funnel, wash with four 20 ml portions of hot water and combine the washings with the original aqueous layer from the saponification. Extract the combined aqueous layer with three 20 ml portions of petroleum ether. Neutralize the aqueous layer with sodium hydroxide T.S. to pH 7. Transfer the solution to a 500-ml volumetric flask and dilute to the mark with water.
Determination of propylene glycol:

Pipette 5.0 ml of the solution into a 125 ml Erlenmeyer flask, add 5.0 ml of 1 M periodic acid, swirl and let stand 15 minutes. Add 10 ml of a saturated solution of sodium bicarbonate, followed by 15.0 ml of 0.1 N sodium arsenite and 1 ml of potassium iodide solution (1 in 20) and mix. Add enough sodium bicarbonate so that at the end point some remains undissolved, and titrate with 0.1 N iodine, using a 10-ml microburet and continuing the titration to a faint yellow colour. Perform a blank determination and make the appropriate corrections. Each ml of 0.1 N iodine is equivalent to 3.805 mg of propylene glycol.

\[
g \text{propylene glycol/100 g ester} = \frac{38.05 \times \text{ml } 0.1 \text{ N Sodium sol.}}{\text{sample weight in g}}
\]

If the qualitative test for polyols included under Identification showed the product to be a mixture of propylene glycol and glycerol esters of fatty acids it becomes necessary to determine the glycerol content of the polyol solution obtained after saponification and separation of liberated fatty acids.

Determination of glycerol:

Pipette 50 ml of the solution into a 600-ml beaker, add bromothymol blue T.S. and acidify with 0.2 N H₂SO₄ to a definite greenish-yellow colour. Neutralize with 0.05 N sodium hydroxide to a definite blue end-point free of green colour. Prepare a blank containing 50 ml of water and neutralize in the same manner. Pipette 50 ml of sodium periodate solution T.S. (see Annex) into each beaker, mix by swirling, cover with a watch glass and allow to stand for 30 minutes at room temperature (not above 35°) in the dark or in subdued light. Add 10 ml of a mixture of equal volumes of ethylene glycol and water and allow to stand 20 minutes. Dilute each solution to about 300 ml and titrate with 0.1 N sodium hydroxide to pH 8.1 ± 0.1 for the sample and 6.5 ± 0.1 for the blank using a calibrated pH meter. Each ml of 0.1 N sodium hydroxide after correction for the blank is equivalent to 9.210 mg. of glycerol.
g glycerol/100 g of esters = \frac{9.210 \times \text{ml } 0.1 \text{ N NaOH}}{\text{sample weight in g}}

The true propylene glycol content in g/100 g of ester is equal to the apparent propylene glycol content in g/100 of ester - 1.65 x g glycerine/100 g of ester.
CALCIUM STEAROYL LACTYLATE
and
SODIUM STEAROYL LACTYLATE

(Tentative)

DEFINITION

Functional Use in Foods: Emulsifier

Structural Formula:

\[
\text{lactate:} \quad \text{lactylate:}
\]

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

Where R is the hydrocarbon chain of the fatty acid moiety.

DESCRIPTION

Calcium or sodium stearoyl lactylate consists mainly of the calcium or sodium salts of lactic acid and its dimer which have been esterified with the fatty acids of food fats such as commercial stearic acid. Some esters of other fatty acids such as myristic acid may be present and there may also be some free fatty acids.

Appearance: Calcium or sodium stearoyl lactylate as a white or slight yellowish white powder or brittle solid depending on lactic/fatty acid ratio. It has a characteristic odour.

CHARACTERISTICS

Identification

A. Solubility: Water: insoluble
        Ethanol: soluble
B. Add 10 ml of dilute hydrochloric acid to 2 g of sample, heat for 5 minutes in a water bath, filter and neutralize the filtrate with ammonia T.S.

(a) **Calcium salt:** Add 5 ml of ammonium oxalate T.S. A white precipitate is formed, soluble in dilute hydrochloric acid T.S. but insoluble in dilute acetic acid T.S.

(b) **Sodium salt:** Add uranyl zinc acetate T.S.; a yellow crystalline precipitate appears within a few minutes.

C. Take the residue from the filter in test B, add 30 ml of sodium hydroxide T.S., heat for 30 minutes on a steam bath and filter. Add 20 ml of dilute hydrochloric acid 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 54.5° and 69°.

D. Add dilute sulfuric acid T.S. and potassium permanganate T.S. to calcium or sodium stearoyl lactylate. The odour of acetaldehyde is produced on heating.

**Specification**

The article of commerce can be specified as to loss on drying, calcium or sodium contents, pH of saturated solution, ester value, acid value, lactic acid and fatty acid content. For more complete analysis, see (1).

**LIMITS OF IMPURITIES**

Acids: Acids moieties other than lactic, lacticric and fatty acids should not be present.

Acrylic Acid: not more than 100 mg/kg (Method needed)
Arsenic: not more than 3 mg/kg
Lead: not more than 3 mg/kg
Heavy metals: not more than 10 mg/kg

STEARYL CITRATE

Chemical Description

Predominantly citric acid ester of n-octadecanol and n-hexadecanol.

Structural Formula

\[
\begin{align*}
\text{COOR} \\
\text{ROOC-CH}_2-\text{C}-\text{CH}_2-\text{COOR} \\
\text{OH}
\end{align*}
\]

Where R = stearyl, up to 50% palmityl or hydrogen.

Definition

Stearyl citrate is formed by esterifying citric acid with commercial stearyl alcohol, which consists essentially of n-octadecanol and n-hexadecanol.

Description

Stearyl citrate occurs as a cream-coloured unctuous substance.

Uses

Emulsifier.

Identification

A. Solubility:  
- Water: Insoluble
- Ethanol: Insoluble cold; soluble hot

B. Hydrolize approximately 2 g of the ester by heating with 50 ml sodium hydroxide T.S. under reflux for 1 hour. Cool and extract the aqueous solution with petroleum ether,
evaporate the petroleum ether in an evaporating dish. The residue has a melting range of 43 to 58°.

C. To part of the aqueous solution obtained according to B, add 5 ml of a 10 % solution of sodium citrate, 1 ml of calcium chloride T. S. and 3 drops of bromothymol blue T. S., and slightly acidify with dilute hydrochloric acid T. S. Add sodium hydroxide T. S. until the colour changes to a clear blue, then boil the solution for 3 minutes, agitating it gently during the heating period: a white crystalline precipitate appears which is insoluble in sodium hydroxide T. S. but is soluble in acetic acid T. S.

D. To part of the aqueous solution obtained according to B, add 10 ml of a 10 % solution of sodium citrate, 1 ml of mercuric sulfate T. S. Heat the mixture to boiling, and add a few drops of potassium permanganate T. S.: a white precipitate of the acetone dicarboxylic acid salt of mercury is formed.

**Purity Tests**

Chloroform insoluble material: Not more than 0.5
Dissolve about 50.0 g of sample in 400 ml chloroform.
Filter the solution through a sintered glass filter of porosity 3 previously weighed to the nearest 0.001 g. Keep filter warm and wash the residue in the filter with chloroform, then dry at 100°.
Other acids and alcohols: Acids other than citric and alcohols other than those present in stearyl alcohol must not be present.
Arsenic: Not more than 1 mg/kg.
Lead: Not more than 2 mg/kg.

Additional criteria: The article of commerce can be further specified by saponification value, total content and composition of stearyl alcohol, iodine value, acid value and citric acid content.
SUCROSE ESTERS OF FATTY ACIDS

DEFINITION

Functional Use in Foods: Emulsifier

Synonyms: Sucroesters, sucrose ester surfactants

Chemical Definition and Preparation: Sucrose fatty acid esters are the mono-, di and triesters of sucrose with edible fatty acids. They may be prepared as such from sucrose and the methyl and ethyl esters of edible fatty acids usually in the presence of a solvent. Another procedure is to react edible fats or oils and sucrose to produce a mixture of sucrose esters of fatty acids and mono- and diglycerides called "sucroglycerides." Both are usually produced in the presence of a solvent.

DESCRIPTION

Sucrose fatty acid esters occur as stiff gels, soft solids or white to slightly greyish white powders and are odourless.

CHARACTERISTICS

Identification:

A. Solubility: Water: insoluble
   Ethanol: soluble

B. Add 1 ml of alcohol to 0.1 g of sucrose fatty acid ester, dissolve by warming, add 5 ml of diluted sulfuric acid, heat in a water bath for 30 min and cool. A yellowish white solid or oil is formed, which is soluble in 3 ml of ether.

C. Take 2 ml of the solution separated from the solid in Test B and add 1 ml of anthrone T.S. carefully down the inside of the test tube: the boundary surface of the two layers turns to blue or green.
Specification

The article of commerce may be specified as to total fatty acid, total sucrose, total glycerol, ash, chloride, soap and moisture.

LIMITS OF IMPURITIES

Dimethyl formamide: not more than 50 mg/kg
Propylene glycol: content to be determined as described in Assay
Arsenic: not more than 3 mg/kg
Heavy metals: not more than 20 mg/kg

EXAMINATION

Assay:

Sucrose Ester Content: Accurately weigh about 2 g of sucrose fatty acid ester or sucroglyceride, previously dried over sulfuric acid, in a vacuum desiccator for 4 hours, and dissolve by warming in 100 ml of chloroform. Extract the chloroform solution four times with 50 ml portions of aqueous 5% sodium sulfate solution. The combined aqueous extract may be concentrated to 5 ml and, if propylene glycol was used as the solvent, the content of free propylene may be estimated by gas-liquid chromatography. Evaporate the chloroform solution of the sucrose ester to incipient dryness, dissolved with warming in 80 ml of water, add 10 ml of 10 N sodium hydroxide solution. Allow to stand 24 hours at 35-40°C, add 10 ml of concentrated hydrochloric acid and extract three times with 50 ml, 30 ml and 30 ml of ether. Evaporate the aqueous layer to about 50 ml. Allow to cool, add 2-3 drops of phenolphthalein T.S., neutralize with sodium hydroxide T.S., add water to 100 ml and use this solution as test solution. Take 20 ml of this solution, add 20 ml of Bertrand A T.S. and 20 ml of Bertrand B T.S., boil gently for 3 min. and let stand to allow cuprous oxide to precipitate. The upper solution should remain deep-blue. Filter the upper solution with a glass filter, wash the precipitate with hot water until the washings are no longer alkaline and filter the washings
using a glass filter. The cuprous oxide precipitate must not come in contact with air. Dissolve the precipitate in the flask by adding 20 ml of Bertrand C T.S filter this solution with the glass filter used before, wash with water, combine the washing with filtrate, titrate with Bertrand D T.S and calculate quantity of copper from the result of the titration; obtain the quantity of invert sugar and calculate quantity of sucrose as follows:

% Sucrose = mg invert sugar from Bertrand Table (1) x 0.95 x 500 weight of sample (mg).

The content of sucrose esters is calculated as sucrose distearate as follows:

% Sucrose Esters % Sucrose
0.391

If propylene glycol was used as solvent for the preparation of the sucrose ester, another 20 ml portion of the neutralized aqueous layer may be concentrated to 5 ml, and the content of combined propylene glycol may be estimated by gas-liquid chromatography.

**HYDROXYLATED LECITHIN**

*(Tentative)*

**Synonyms**

Hydroxylated phosphatides, dihydroxy phospholipids.

**Chemical Names**

Commercial hydroxylated lecithin is a mixture of phosphatides including:

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<th>CH&lt;sub&gt;2&lt;/sub&gt;OCOR</th>
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<td>CH&lt;sub&gt;2&lt;/sub&gt;OPOCH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;N+ (CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;OPOCH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>OH</td>
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- phosphatidyl choline ("lecithin fraction")
- phosphatidyl ethanolamine ("cephalin fraction")

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<tr>
<th>CH&lt;sub&gt;2&lt;/sub&gt;OCOR</th>
<th>CH&lt;sub&gt;2&lt;/sub&gt;OCOR</th>
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<td>OH</td>
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- phosphatidic acid
- phosphatidyl inositol

where R = various saturated and unsaturated hydroxylated fatty acid groups.
Definition

Commercial hydroxylated lecithin is a mixture of phosphatides prepared from vegetable oils and seeds which is treated with hydrogen peroxide, benzoyl peroxide, lactic acid and sodium hydroxide or with hydrogen peroxide, acetic acid and sodium hydroxide, under controlled conditions, whereby the separated fatty acid fraction of the resultant product has an hydroxyl value of 30 - 38. The commercial product is specified by its acetone-insoluble fraction which is usually in the range of 55 to 60 %.

Description

Commercial hydroxylated lecithin is a very light amber free-flowing liquid with a bland odour.

Uses

As an emulsifier.

Identification

A. Solubility: Water: Insoluble but hydrates characteristically with swelling.
   Acetone: Insoluble
   Chloroform: Soluble
   Benzene: Soluble
   The "lecithin fraction" is soluble in ethanol, the "cephalin fraction" insoluble.

B. Ignite 1 g of hydroxylated lecithin 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 T.S. and heat to boiling. A yellow precipitate is obtained.

C. Fuse about 0.5 g of hydroxylated lecithin with about 0.05 g of sodium in a soft glass tube, and heat to redness. Plunge while hot into about 10 ml of distilled water, heat to boiling and filter. Add a few crystals of ferrous sulphate to
the filtrate, boil and add dilute sulphuric acid until just acid. Allow to stand for 15 minutes, filter and wash. A blue precipitate is obtained.

D. Reflux 1 g of hydroxylated lecithin for 1 hour with 25 ml of 0.5 N ethanolic potassium hydroxide. When cooled to 0°, a precipitate of potassium soap is obtained.

E. Determine the hydroxyl value *, it should be 30 - 38.

Purity Tests

Assay: Not less than 6

The hydroxylated lecithin content may be estimated by determination of the quantity of acetone-insoluble material.

Weigh 2 000 g of well-mixed sample into a centrifuge tube which has been previously tared together with a stirring rod. Add 15 ml of saturated acetone a/ from a burette. Warm in a water bath until the lecithin melts, but avoid evaporation of the acetone. Stir until the material is completely disintegrated. Place in an ice-water bath and chill for 5 minutes. Remove the tube from the bath and add about one-half of the volume of chilled (0° - 5°) saturated acetone required to make up to the final volume of 45 ml. Stir well to complete dispersion of remaining particles. Make to volume of 45 ml with chilled (0° - 5°) saturated acetone, stir, and return tube and contents to ice bath at 0° - 5° for 15 minutes. Then stir again while in the bath, remove rod and centrifuge immediately at 1 900 ± 100 r.p.m. for 5 minutes. Decant the acetone-soluble material into a clean beaker. Break up the centrifuged solids with the previously tared stirring rod and refill the centrifuge tube to the 40 ml mark with chilled (0° - 5°) saturated acetone; stir well and repeat as directed above. Centrifuge, pour off, return the stirring rod to the tube and break up the

solids. Place the tube and contents in a horizontal position on a laboratory bench until the excess of acetone evaporates. Mix again and place in a forced-draft oven at $105^\circ \pm 2^\circ$ until constant weight is obtained, usually 30 to 45 minutes. Cool to room temperature in an efficient desiccator and weigh immediately.

**Limits of Impurities**

Ether insolubles: Not more than 1.0 %. Weigh 10 g of well-mixed sample into a 250 ml flask. Add 100 ml of ether and shake until dissolved. Filter through a tared filter funnel. Wash the flask with 25 ml portions of ether and pour the washings through the funnel. Allow all ether to evaporate. Place the funnel in a forced-draft oven and weigh for drying for 1 hour at $105^\circ \pm 2^\circ$.

Arsenic: Not more than 3 mg/kg.

Lead: Not more than 10 mg/kg.

Heavy metals: Not more than 40 mg/kg.

**Additional criteria:** The article of commerce can be further specified by the saponification value, acid value and loss on drying.

---

*a/* Saturated acetone: Add purified lecithin to acetone at $5^\circ$; about 5 g of lecithin are sufficient for 16 litres of acetone. Maintain at $5^\circ$ for two hours, shaking vigorously at 15 minute intervals. Decant through a rapid filter paper avoiding as far as possible transfer of any of the solids to the paper and conducting the filtration under refrigerated conditions ($0^\circ - 5^\circ$) so as to maintain the same conditions for saturation as described under procedure.
AMMONIUM SALTS OF PHOSPHATIDIC ACIDS
(Tentative)

DEFINITION

Functional Use in Foods: Emulsifier

Synonyms: Emulsifier YN

Structural Formula: $R_3P = O$ where $R$ = mono- or diglyceride moiety or $-OH$ or $M-ONH_4$

DESCRIPTION

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, two or three glyceride moieties may be attached to phosphorus as indicated in the structural formula above. 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.

CHARACTERISTICS

Identification

A. Solubility:
   Water: ( Information
   Ethanol: ( needed
   Acetone: (   
   Fats: (   

B. 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 T.S. and heat to boiling. A yellow precipitate is obtained.
C. Reflux 1 g of the product for 1 hour 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. When cooled to 0°C, a precipitate of potassium soap is obtained.

D. Test for glycerol as in Annex on Identification of Emulsifiers.

Specification

Assay: Phosphorus content: 3.0 to 3.4 by weight
Ammonium content: 1.2 to 1.5 calculated as N.

The article of commerce can be specified further as to water content, hexane insoluble matter, inorganic hexane insoluble matter, pH and triglyceride content.

LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg.
Heavy metals: not more than 10 mg/kg.

EXAMINATION

Determination of phosphorus

Reagents The reagents used shall be of recognized analytical reagent quality and water refers to distilled water.

a. 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.
b. Standard phosphate solution

Stock solution: Dissolve 3.8346 g of potassium dihydrogen phosphate, previously dried at 110°C, in water and dilute to 1 000 ml. 1 ml of this solution = 2.0 mg $P_2O_5$.

Working solution: Dilute 50 ml of the stock solution to 500 ml with water. 1 ml of this solution = 0.20 mg $P_2O_5$.

c. Sulphuric acid, sp. gr. 1.84

d. Nitric acid, sp. gr. 1.42

e. 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 concentrated sulphuric acid and 10 ml of concentrated 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).

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 dilute phosphorus standard (\(= 5.0 \text{ mg } P_2O_5\))

(b) 30.0 ml of the same solution (\(= 6.0 \text{ mg } P_2O_5\)), and

(c) a 25 ml aliquot of the test solution which will contain
the equivalent of between 5 and 6 mg \(P_2O_5\).

Into each of the flasks containing the phosphorus standards,
i.e. (a) and (b) transfer an aliquot of the blank digest solu­
tion 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°C, dilute to the mark with
water and re-mix.

After 10 minutes measure the optical densities of both the
6 mg \(P_2O_5\) 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 mu, or with
an Ilford 604 filter if using a photo-electric colorimeter.

Calculation

\[
\text{Phosphorus} = \left( \frac{\text{OD test}}{\text{OD 6 mg}} \right) \times 0.873 \frac{\text{W}}{\text{W}}
\]

Where

\(\text{OD test} = \) Optical density difference
between the 5 mg standard
and the test solution

\(\text{OD 6 mg} = \) Optical density difference
between the 6 mg and 5 mg
standards

\(\text{W} = \) Sample weight taken (g)
Determination of ammonium salt nitrogen in neutral \( YN \)

1. **Apparatus for steam distillation**

   The apparatus consists of a 2 litre 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 sulphuric acid, 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 litre round bottomed B34 necked flask. The distillation head should be such that the steam inlet tube reaches almost to the bottom of the 1 litre flask and the outlet should be fitted with two splash traps, one near the top of the 1 litre flask and the other near the top of a 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.

2. **Reagents**

   Reagents a, b, c should be prepared using chemicals of analytical reagent grade.

   (a) Boric acid, 2 % \( w.v. \) aqueous solution.

   (b) 40 % \( w.v. \) aqueous solution of sodium hydroxide.

   (c) 0.02N, Hydrochloric acid.
(d) Mixed indicator. Mix 5.0 ml of a 0.1 % w.v. alcoholic solution of bromocresol green and 2.0 ml of a 0.1 % w.v. alcoholic solution of methyl red and dilute the mixture to 30 ml with 95 % alcohol.

(e) Silicone fluid 200/50 MS.

3. Procedure

Assemble and thoroughly steam out the apparatus. Accurately weigh about 0.2 gm. of a representative sample of neutral YN into a small glass phial (approx. 3/4'' diam., 1/2'' deep). Add approximately 250 ml distilled water and the phial and weighed contents to the distillation flask. 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.

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

\[ \% \text{ Nitrogen} = (\text{sample titre} - \text{blank titre}) \times 28.02 \]

(sample wt. in mg)
HYDROXYPROPYL CELLULOSE

DEFINITION

**Functional Use in Foods:** Emulsifier, stabilizer, thickener and suspending agent.

**Chemical name:** Hydroxypropyl ether of cellulose; cellulose hydroxypropyl ether.

**Chemical formula:**

\[
\left(\text{C}_6\text{H}_7\text{O}_2\text{(OH)}_x \text{ (OCH}_2\text{ - CHOH-CH}_3\text{)}_y \text{ (OCH}_2\text{-CH-CH}_3\text{)}_z\right)_n
\]

where 
- \(x = \text{less than 3.0}\)
- \(y = \text{not greater than 3.0}\)
- \(z = \text{not greater than (4.6 - y)}\)

**Structural formula:**

![Structural formula of Hydroxypropyl Cellulose](image)
Possible structural formula for repeating unit of a hydroxypropyl cellulose with molar substitution of 3.0 and a degree of polymerization of n.

Molecular weight:

Unsubstituted structural unit: 162.14
Trisubstituted structural unit: 336.37
Macromolecules: from about 30 000 (n about 100) up to 1 000 000 (n about 2 500)

DESCRIPTION

Chemical Description: Hydroxypropyl cellulose is an ether of cellulose containing hydroxypropyl substitution. It is prepared from cellulose by treatment with alkali and propylene oxide.

Appearance: Hydroxypropyl cellulose is a white or a slightly yellowish, almost odourless and tasteless, granular or fibrous powder.

CHARACTERISTICS

Identification

A. Solubility: Water: The product swells in water and produces a clear to opalescent, viscous colloidal solution.
   Ethanol: Soluble
   Ether: Insoluble

Specification

Assay: The dry product contains not more than 4.6 hydroxypropyl groups per anhydroglucose unit. The article of commerce can be specified further by viscosity and loss on drying.
LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg
Heavy metals: not more than 10 mg/kg
Sulphated ash content: not more than 0.2

EXAMINATION

Assay:

Hydroxypropyl Content

Principle: The sample is oxidized with chromic acid; the acetic acid from the hydroxypropyl groups plus some chromic acid are distilled over. The acids are titrated with base and a correction for the chromic acid is obtained by iodometric titration which is subtracted from the total acid titre to give the hydroxypropyl value.

Fig. 7—Apparatus for Hydroxypropoxyl Group Determination
Apparatus: (See Fig. 1)

The boiling flask A is fitted with a distilling head B. The distilling head is fitted with a gas delivery tube, D for the introduction of nitrogen, a 50-ml graduated dropping funnel C for the introduction of water, and a condenser E. The boiling flask is immersed in an oil bath equipped with a thermoregulator so that the oil can be maintained at a temperature of 155° and the distillate is collected in a 50-ml graduate.

Procedure:

Transfer about 50 mg of hydroxypropyl methylcellulose previously dried at 105° for 3 h and accurately weighed into a flask and add 10 ml of chromium trioxide solution (430 g/1). Immerse the flask in the oil bath to two thirds of its height, assemble the apparatus and pass nitrogen gas through it at the rate of ninety bubbles per minute. Raise the temperature until the end of the determination. Distillation begins between 135° and 140° and as successive 5 ml volumes of distillate collect in the 50-ml graduate, add 5 ml portions of water to the flask from the 50-ml graduated dropping funnel. Continue this procedure until 50 ml of water has been added and 55 ml of distillate, which should be faintly yellow in colour, has been collected. Detach the condenser from the stillhead and wash both of its arms with distilled water, collecting the washing in a 250-ml flask.

Transfer the contents of the 50-ml graduate to the flask containing the washings. Add a few drops of phenolphthalein and titrate the combined solution with 0.02 N sodium hydroxide until the end point just begins to fade. Heat the solution nearly to the boiling point to remove carbon dioxide, cool to room temperature and continue to titrate until the pink colour remains stable for 10 sec. Record the volume (x) of the 0.02 N sodium hydroxide used, and then add 500 mg of sodium hydrogen carbonate and 10 ml of dilute sulfuric acid T.S. After evolution of carbon dioxide has ceased, add 1 g of potassium iodide, stopper the flask, shake the mixture and allow the solution to stand in the dark for 5 min.
Titrate the liberated iodine with 0.02 N sodium thiosulfate, adding a few drops of starch T.S. as the end point is approached and record the volume (y) required. Correct the volume (x) of 0.02 N sodium hydroxide required for the initial titration in order to obtain the equivalent of acetic acid, by the formula $x - Ky$, in which $K$ represents the ratio (ml 0.02 N sodium hydroxide from the blank)/ (ml 0.02 N sodium thiosulfate from the blank), obtained by performing a blank determination with the same quantities of the same reagents and in the same manner. Each ml of the corrected volume of 0.02 N sodium hydroxide is equivalent to 1.5 mg of hydroxypropoxyl groups (-OCH$_2$CHOHCH$_3$).
PECTIN

CHEMICAL DESCRIPTION

Pectin consists of the partial methyl-esters of polygalacturonic acid and their salts.

DEFINITION

Function Use in Foods: Jelling, thickening and stabilizing agent. Pectin is a purified carbohydrate product generally obtained from the dilute acid extract of the inner portion of the rind of citrus fruits or from apple pomace.

DESCRIPTION

Pectin is a white, yellowish, light greyish or light brownish powder.

The commercial product, pectin, is usually diluted with 15-35% sugars and mixed with up to 40% buffer salts, calculated on the final product; buffer salts normally used are:

- Calcium citrate
- Calcium monophosphate
- Potassium tartrate
- Sodium citrate
- Sodium hexametaphosphate
- Sodium pyrophosphate

Buffer salts are required for pH utilization and for obtaining different setting temperatures.

CHARACTERISTICS

Identification

A. Solubility: Water: soluble, forming a colloidal, opalescent solution
   Ethanol: insoluble
B. Heat 1 g of pectin with 9 ml of water on a steam bath until a solution is formed, replacing water lost by evaporation: it yields a gel upon cooling.

C. To a solution of pectin (1 in 100) add an equal volume of alcohol: a translucent, gelatinous precipitate is formed.

D. To 10 ml of a solution of pectin (1 in 100) add 1 ml of thorium nitrate T.S., stir, and allow to stand for 2 minutes: a stable precipitate or gel forms.

E. To 5 ml of a solution of pectin (1 in 100) add 1 ml of a solution of potassium hydroxide (1 in 50) and allow to stand at room temperature for 15 minutes: a translucent gel or semi-gel forms.

F. Acidify the gel from the preceding test with diluted hydrochloric acid and shake well: a voluminous, colourless, gelatinous precipitate forms, which upon boiling becomes white and flocculent (pectic acid).

Specifications

Moisture: not more than 12

Ash: Low Methoxyl Pectins: not more than 12%
Medium and High Methoxyl Pectins: not more than 6%

Acetyl groups: not more than 1%

Galacturonic acid: not less than 70% ± 5% calculated on the ash and moisture free basis.

Methoxyl content: Low Methoxyl Pectins: from 0 to 7% ± 2%
Medium and High Methoxyl Pectins: not less than 7% ± 2%

The article of commerce may be further specified as to grade strength and pH.
LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg

Lead: not more than 10 mg/kg

Copper: not more than 60 mg/kg

Sulphur dioxide: not more than 50 mg/kg

Alcohol (methyl, ethyl or isopropyl): not more than 10 mg/kg

EXAMINATION

**Determination of moisture content:** drying at 105° for 2 h.

**Determination of ash content:** 3 h at 600°.

**Determination of acetyl groups:** The pectin is saponified for 3 h in nitrogen current by 5 % H₂SO₄ at 100°, is distilled, and the distillate is titrated after elimination of CO₂ by heating again with 0.1 N NaOH.

**Determination of alcohol contents:** gas chromatography

**Determination of methoxyl and galacturonic acid content:** Transfer exactly 5 g of pectin to a suitable beaker and stir for 10 min with a mixture of 5 ml of hydrochloric acid and 100 ml of 60 % alcohol.

Transfer to a fritted glass filter tube (30 to 60 ml) and wash with six 15 ml portions of the hydrochloric acid - 60 % alcohol mixture, followed by 60 % alcohol until the filtrate is free of chlorides. Finally wash with 20 ml of alcohol and dry for 1 h in an oven at 100°, cool and weigh.

Transfer exactly one-tenth of the total net weight of the dried sample (representing 0.5 g of the original unwashed sample) to a 250 ml Erlenmeyer flask and moisten the sample with 2 ml of alcohol.

Add 100 ml of recently boiled and cooled distilled water, stopper and swirl occasionally until the pectin is completely dissolved.
Add 5 drops of phenolphthalein T.S., titrate with 0.5 N sodium hydroxide and record the results as the initial titre. Add exactly 20 ml of 0.5 N sodium hydroxide, stopper, shake vigorously and let stand for 15 minutes.
Add exactly 20 ml of 0.5 N hydrochloric acid and shake until the pink colour disappears.
After adding 5 drops of phenolphthalein T.S., titrate with 0.5 N sodium hydroxide to a faint pink colour which persists after vigorous shaking: record this value as the saponification titre.
Each ml of 0.5 N sodium hydroxide used in the saponification titre is equivalent to 0.0155 g of OCH₃.

Assay for galacturonic acid: Each ml of 0.5 N sodium hydroxide used in the total titration (the initial titre added to the saponification titre) is equivalent to 0.09707 g of C₅H₉O₅COOH.
PROPYLENE GLYCOL ALGINATE

Chemical Name

1, 2 propane-diol ester of alginic acid.

Definition

Propylene glycol alginate consists of esters of alginic acid in which the carboxyl groups are partially esterified with propylene glycol and partly neutralized with alkalis approved in the Ninth Report.

Description

Propylene glycol alginate is a white to yellowish fibrous or granular powder. It is almost odourless and tasteless.

Uses

As a stabilizer, thickener and emulsifier.

Identification

A. Solubility: Water: Soluble to give viscous colloidal solution.
   Ethanol: Soluble in up to 60% aqueous ethanol depending upon degree of esterification.

B. To 10 ml of a 1% solution of the sample add 1 ml of sodium hydroxide T.S. Heat in a boiling water bath for about 5 minutes, cool and add 1 ml of dilute sulphuric acid T.S. A gelatinous precipitate is formed.

C. To 5 ml of a 1% solution add 1 ml lead acetate T.S. A gelatinous precipitate is formed.

D. Identify propylene glycol as indicated in Annex I to "Specifications for the Identity and Purity of Food Additives: Some Emulsifiers and Stabilizers and certain other substances."
Purity Tests

Ash: Not more than 10 %.
Insoluble matter: Not more than 0.2 %.
Arsenic: Not more than 3 mg/kg.
Heavy metals: Not more than 40 mg/kg.
Lead: Not more than 10 mg/kg.

Additional criteria: The article of commerce can be further specified by viscosity, acid value, loss on drying, free esterified and neutralized carboxyl content and percentage carbon dioxide produced on acid decarboxylation.

Note: Assay of propylene glycol in this product is required.
ANNEX

Identification Tests for Emulsifiers

The tests are specific for the component mentioned in the heading.

1. Fatty acids

Reflux 1 g of sample with 15 ml of 0.5 N ethanolic potassium hydroxide for 1 h. Add 15 ml of water, acidify with dilute hydrochloric acid TS (about 6 ml). Oily drops or a white to yellowish-white solid is produced which is soluble in 5 ml of hexane.

Remove the hexane layer, extract again with 5 ml of hexane and remove again the hexane layer. The fatty acids thus extracted may be identified by gas-liquid chromatography.

The aqueous layer is used for tests 2 to 9.

2. Acetic acid

Transfer about 5 ml of the aqueous layer resulting from test 1 into a dish, add excess calcium carbonate and evaporate until dry. Transfer the major part of the residue into a glass tube. Place a filter paper, moistened with Reagent for acetone, on top of the tube. Heat as indicated in the figure.

![Figure 1](image-url)
The yellow colour of the paper changes into greenish blue by reaction with acetone, formed from calcium acetate.

Reagent for acetone: a saturated solution of c-nitrobenzaldehyde in sodium hydroxide TS, freshly prepared.

3. **Succinic acid**

Transfer one drop of the aqueous layer resulting from test 1 and a drop of a 0.5 % solution of ammonium chloride and several mg of zinc powder into a micro test tube. The mouth of the tube is covered with a disk of filter paper moistened with a solution in benzene of 5 % p-dimethylaminobenzaldehyde and 20 % trichloroacetic acid. The bottom of the test tube is heated vigorously with a micro flame (Fig. 1) for about 1 minute. Depending on the amount of succinic acid or succinimide, a red-violet or pink stain appears on the paper.

4. **Fumaric acid**

Transfer 1 ml of the aqueous layer resulting from test 1 with 1 ml of 2 N sodium carbonate into a test tube. Add 2 or 3 drops of 0.1 N potassium permanganate. The solution is promptly discoloured.
5. **Tartaric acid**

Evaporate about 5 ml of the aqueous layer resulting from test 1 in a porcelain dish until dry. Add 2 ml of concentrated sulfuric acid containing 0.5% of pyrogallol and heat on a steam bath. An intense violet colour is produced.

6. **Citric acid**

6.1 To 3 ml of the aqueous layer resulting from test 1 add a few drops of 1% potassium permanganate and warm until the colour has disappeared. Then add an excess of bromine TS. A white precipitate (pentabromoacetone) is formed immediately on cooling.

6.2 Evaporate 1 ml of the aqueous layer resulting from test 1 in a porcelain dish, add 1 ml of a mixture of 1 vol. acetic anhydride and 5 vol. of pyridine into the warm dish. A red colour is produced. (Tartaric acid produces a green colour).

7. **Lactic acid**

Transfer 0.2 ml of the aqueous layer resulting from test 1 and 2 ml of conc. sulfuric acid into a test tube and place for 2 min in boiling water. Cool and add 1 or 2 drops of a 5% guajacol solution in ethanol. A red colour is immediately produced.

If tartaric acid is present according to test 5, it must be removed as follows: transfer 3 ml of the aqueous layer resulting from test 1 and an excess of calcium hydroxide as a powder into a test tube, place in boiling water for 5 min, shaking several times, cool and filter.

8. **Calcium**

Dissolve a grain (about 1 mg) of solid murexide in one drop of 2 N sodium hydroxide and 2 drops of water on a porcelain spot-plate. Add a drop of the solution resulting from test 1. The colour changes from violet into red.
9. **Glycerol**

Transfer 5 ml of the aqueous layer resulting from test 1 into a test tube. Add excess calcium hydroxide as a powder, place in boiling water for 5 min. shaking several times, cool and filter.

Transfer one drop of the filtrate into a tube as indicated in the figure in Test 1 and add about 50 mg of potassium hydrogen sulfate. Place a filter paper, moistened with Reagent for acrolein, on the top of the tube. Heat as indicated in the figure. A blue colour of the filter paper indicates the presence of glycerol. The colour changes into light red after addition of sodium hydroxide TS.

The test cannot be employed in the presence of ethylene glycol or lactic acid, since they decompose under the prescribed conditions yielding acetaldehyde which reacts with the reagents in the same manner as acrolein.

**Reagent for acrolein:** Prepare a 5% solution of disodium pentacyanonitrosylferrate in water and a 20% piperidine solution in water. Mix solutions 1:1 immediately before use.

10. **Polyols**

**Principle:** The product is hydrolyzed. Fatty acids are removed by ion-exchange in combination with hexane extraction. The filtrate is separated by thin layer chromatography.

**Procedure:** Reflux 1 g of sample with 15 ml of 0.5 N ethanolic potassium hydroxide for 1 h. Add 25 ml of Amberlite Resin IR-120(H), 50 ml of hexane and 25 ml of water. Stir the mixture for about 1 h. Filter and separate the layers of the filtrate and use the aqueous layer.

Prepare a sililic acid G (Merck) plate and allow it to dry at room temperature in the air. Place 2 to 5 µl of the aqueous layer on the plate and also 2µl of 5% solutions of glycerol, ethylene glycol and 1,2-propylene glycol.
Use a mixture of chloroform, acetone and 5 N ammonia (10:80:10) as a solvent. After chromatographing dry in a stream of air until water and ammonia have disappeared. Spray with 0.1% aqueous sodium periodate solution. When the plate is half dry spray with a solution of 2.8 g benzidine in 80 ml of 96% ethanol, to which 70 ml of water, 30 ml of acetone and 1.5 ml of N hydrochloric acid are added.

1.2-Diols show a yellow or white spot on blue background with following Rf-values:

**According to Stahl**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycerol</td>
<td>0.35</td>
</tr>
<tr>
<td>ethylene glycol</td>
<td>0.70</td>
</tr>
<tr>
<td>1.2-propyleneglycol</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Tartaric acid produces a white spot with Rf 0.
SOME ANTICAKING AGENTS
FERROCYANIDES OF CALCIUM*, POTASSIUM AND SODIUM

DEFINITION

Functional Use in Foods: Anticaking agent in the manufacture of salt

Synonyms: Yellow prussiate of lime, soda or potash, hexacyanoferrate of calcium, sodium or potassium

Formulae: Ca$_2$Fe(CN)$_6$$\cdot$12H$_2$O;
K$_4$Fe(CN)$_6$$\cdot$3H$_2$O;
Na$_4$Fe(CN)$_6$$\cdot$10H$_2$O.

Molecular Weights:
- Calcium salt 508.3
- Potassium salt 422.4
- Sodium salt 484.1

DESCRIPTION

Yellow crystals or crystalline powder.

CHARACTERISTICS

Identification

A. Solubility: Water: soluble (Na, K salt); insoluble (Ca salt)
Ethanol: insoluble

B. Test for Ferrocyanide: To 10 ml of a 1% solution of the ferrocyanide add 1 ml of ferric chloride T.S. A dark blue precipitate is formed. This test should be supplemented by a negative test for cyanide.

* The specification for calcium ferrocyanide is incomplete due to lack of a test for calcium.
C.1. Test for Calcium*: to be developed

C.2. Test for Potassium: Potassium compounds impart a violet colour to a non-luminous flame if not masked by the presence of small quantities of sodium. In neutral, concentrated or moderately concentrated solutions of potassium salts, sodium hydrogen tartrate T.S. slowly produces a white, crystalline precipitate which is soluble in ammonia T.S. and in solutions of alkali hydroxides or carbonates. The precipitation may be accelerated by stirring or rubbing the inside of the test tube with a glass rod or by the addition of a small amount of glacial acetic acid or ethanol.

C.3. Test for Sodium: Sodium compounds, after conversion to chloride or nitrate, yield with cobalt-uranyl acetate T.S. a golden-yellow precipitate, which forms after several minutes agitation. Sodium compounds impart an intense yellow colour to a non-luminous flame.

**Specification**

**Assay:** Not less than 99 percent of the respective ferrocyanide.

The article of commerce can be specified further by limits on chloride, free moisture, insoluble matter and sulphate.

**EXAMINATION**

**Assay:**

Transfer about 3 grams, accurately weighed, into a 400-ml beaker, dissolve in 225 ml of water, and add cautiously about 25 ml of sulphuric acid T.S. Add, with stirring 1 drop of orthophenanthroline T.S., and titrate with 0.1 N ceric sulphate until the colour changes sharply from orange to pure yellow. Each ml of 0.1 N ceric sulphate is equivalent to 101.66 mg of \( \text{Ca}_2\text{Fe(CN)}_6\cdot12\text{H}_2\text{O} \); 84.48 mg of \( \text{K}_4\text{Fe(CN)}_6\cdot3\text{H}_2\text{O} \) or 96.81 mg of \( \text{Na}_4\text{Fe(CN)}_6\cdot10\text{H}_2\text{O} \).

---

* The specification for calcium ferrocyanide is incomplete due to lack of a test for calcium.
CALCIUM PHOSPHATE, TRIBASIC

DEFINITION

Functional Use in Foods: Anticaking Agent, Buffer

Synonyms: Tricalcium phosphate. Precipitated Calcium Phosphate

DESCRIPTION

Tribasic calcium phosphate consists of a variable mixture of calcium phosphates having the approximate composition of $10\text{CaO} \cdot 3\text{P}_2\text{O}_5 \cdot \text{H}_2\text{O}$. It occurs as a white, odourless, tasteless powder which is stable in air.

CHARACTERISTICS

Identification

A. Solubility: Water: almost insoluble
   Ethanol: insoluble
   Dilute hydrochloric acid: soluble
   Dilute nitric acid: soluble

B. To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate T.S. A yellow precipitate forms.

C. Dissolve about 100 mg by warming with 5 ml of diluted hydrochloric acid T.S. and 5 ml of water, add 1 ml of ammonia T.S., dropwise, with shaking, and then add 5 ml of ammonium oxalate T.S. A white precipitate forms.

Specifications

Assay: not less than the equivalent of 90 percent of $\text{Ca}_3(\text{PO}_4)_2$, calculated on the ignited basis.

The article of commerce may be specified further as to titration value and loss on ignition.
LIMITS OF IMPURITIES

Arsenic (as As): not more than 3 mg/kg
Fluoride (F): not more than 50 mg/kg
Heavy metals (as Pb): not more than 30 mg/kg
Lead: not more than 5 mg/kg

EXAMINATION

Arsenic: A solution of 1 gram in 25 ml of diluted hydrochloric acid T.S. meets the requirements of the Arsenic Test.

Fluoride: Weigh accurately 1.0 gram, and proceed as directed in the Fluoride Limit Test.

Heavy Metals: Warm 1.33 grams with 7 ml of diluted hydrochloric acid T.S. until no more dissolves, dilute to 50 ml with water, and filter. A 25-ml portion of the filtrate meets the requirements of the Heavy Metals Test, using 20 mcg of lead ion (Pb) in the control (Solution A).

Lead: A solution of 250 mg in 5 ml of diluted hydrochloric acid T.S. meets the requirements of the Lead Limit Test, using 1.25 mcg of lead ion (Pb) in the control.

Assay:

Weigh accurately about 200 mg, and dissolve it in a mixture of 25 ml of water and 10 ml of diluted nitric acid T.S. Filter, if necessary, wash any precipitate, add sufficient ammonia T.S. to the filtrate to produce a slight precipitate, then dissolve the precipitate by the addition of 1 ml of diluted nitric acid T.S. Adjust the temperature to about 50°C, add 75 ml of ammonium molybdate T.S., and maintain the temperature at about 50°C for 30 minutes, stirring occasionally. Wash the precipitate once or twice with water by decantation, using from 30 to 40 ml each time. Transfer the precipitate to a filter, and wash with potassium nitrate solution (1 in 100) until the last washing is not acid to litmus paper. Transfer the precipitate and filter to the precipitation vessel, add 40.0 ml of 1 N sodium hydroxide, agitate until the precipitate is dissolved, add 3 drops of phenolphthalein T.S., and then titrate the excess alkali with 1 N sulphuric acid. Each ml of 1 N sodium hydroxide corresponds to 6.743 mg of Ca₃(PO₄)₂.
MAGNESIUM PHOSPHATE, TRIBASIC

DEFINITION

Functional Use in Foods: Anticaking Agent

Synonyms: Trimagnesium phosphate

Chemical Formula: $\text{Mg}_3(\text{PO}_4)_2$ (various hydrates)

Molecular Weight: 262.9

DESCRIPTION

Tribasic magnesium phosphate may contain 4, 5, or 8 molecules of water of hydration. It occurs as a white, odourless, tasteless crystalline powder.

CHARACTERISTICS

Identification

A. Solubility: Water: almost insoluble
   Ethanol: insoluble
   Dilute mineral acids: soluble

B. To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate T.S. A yellow precipitate of ammonium phosphomolybdate forms which is soluble in ammonia T.S.

C. Dissolve about 100 mg in 0.7 ml of diluted acetic acid T.S. and 20 ml of water. Add 1 ml of ferric chloride T.S., let stand for 5 minutes, and filter. The filtrate gives a positive test for Magnesium.

Specifications

Assay: not less than 98 percent and not more than the equivalent of 101.5 percent of $\text{Mg}_3(\text{PO}_4)_2$ after ignition.
The article of commerce may be specified further as to titration value and loss on ignition.

**LIMITS OF IMPURITIES**

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic (as As)</td>
<td>not more than 3 mg/kg</td>
</tr>
<tr>
<td>Fluoride (F)</td>
<td>not more than 10 mg/kg</td>
</tr>
<tr>
<td>Heavy metals (as Pb)</td>
<td>not more than 30 mg/kg</td>
</tr>
<tr>
<td>Lead</td>
<td>not more than 5 mg/kg</td>
</tr>
</tbody>
</table>

**EXAMINATION**

**Assay:**

Weigh accurately about 200 mg, previously ignited at about 425°C to constant weight, and dissolve it in a mixture of 25 ml of water and 10 ml of diluted nitric acid T.S. Filter, if necessary, wash any precipitate, then dissolve the precipitate by the addition of 1 ml of diluted nitric acid T.S. Adjust the temperature to about 50°C, add 75 ml of ammonium molybdate T.S., and maintain the temperature at about 50°C for 30 minutes, stirring occasionally. Wash the precipitate once or twice with water by decantation, using from 30 to 40 ml each time. Transfer the precipitate to a filter, and wash with potassium nitrate (1 in 100) until the last washing is not acid to litmus paper. Transfer the precipitate and filter to the precipitation vessel, add 40.0 ml of 1 N sodium hydroxide, agitate until the precipitate is dissolved, add 3 drops of phenolphthalein T.S., and then titrate the excess alkali with 1 N sulphuric acid. Each ml of 1 N sodium hydroxide corresponds to 5.715 mg of Mg₃(PO₄)₂.

**Arsenic:** A solution of 1 gram in 10 ml of diluted hydrochloric acid T.S. meets the requirements of the Arsenic Test.

**Fluoride:** Proceed as directed in the Fluoride Limit Test.
**Heavy metals:** Suspend 1.33 grams in 20 ml of water, and add hydrochloric acid, dropwise, until the sample just dissolves. Adjust the pH to between 3 and 4, filter, and dilute the filtrate to 40 ml with water. For the control (Solution A), add 20 mcg of lead ion (Pb) to 10 ml of the filtrate, and dilute to 40 ml. For the sample (Solution B), dilute the remaining 30 ml of the filtrate to 40 ml. Add 10 ml of hydrogen sulphide T.S. to each solution, and allow to stand for 5 minutes. Solution B is no darker than Solution A.

**Lead:** Dissolve 1 gram in 20 ml of diluted hydrochloric acid T.S., evaporate the solution to a volume of about 10 ml on a steam bath, dilute to about 20 ml with water, and cool. This solution meets the requirements of the Lead Limit Test, using 5 mcg of lead ion (Pb) in the control.
SALTS of MYRISTIC, PALMITIC AND STEARIC ACID
with BASES ACCEPTED FOR FOOD USE

DEFINITION

Scope: The sodium, potassium, ammonium, calcium, magnesium and aluminium salts of commercial myristic, palmitic and stearic acids produced from edible fats and oils.

Functional Use in Foods: Anticaking agents.

DESCRIPTION

The products occur as hard, white or faintly yellowish somewhat glossy and crystalline solids or as white or yellowish white powder.

CHARACTERISTICS

Identification

A. Solubility: Sodium, Potassium and ammonium salts:
   Water: soluble
   Ethanol: soluble

B. Heat 1 g with a mixture of 25 ml water and 5 ml hydrochloric acid. Fatty acids are liberated, floating as a solid or oil layer on the surface which is soluble in hexane. After cooling, the aqueous layer is decanted, evaporated to dryness and the residue gives positive tests for the appropriate cation.

Ammonium: Add sodium hydroxide T.S.; the odour of ammonia is observed.

Sodium: Add uranyl zinc acetate T.S.; a yellow crystalline precipitate appears within a few minutes.
Potassium: Add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

Calcium: Add ammonium oxalate T.S. The white precipitate formed is soluble in hydrochloric acid, but insoluble in acetic acid.

Magnesium: Add ammonium chloride T.S. and ammonium carbonate T.S. A white crystalline precipitate is formed which is insoluble in ammonia T.S.

Aluminium: Add ammonia T.S. A white gelatinous precipitate is formed which is insoluble in excess ammonia but dissolves in sodium hydroxide T.S.

Specification:

The article of commerce can be specified by gas or thin layer chromatography of the fatty acids obtained from the salts, saponification value, free fatty acids, solidification point for the acids obtained from the salts, iodine value, residue on ignition including assay of the cation, unsaponifiable matter and moisture content.

LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg
Heavy metals (as Pb): not more than 10 mg/kg

EXAMINATION

Arsenic: Prepare test solution as directed for organic compounds.

Heavy metals: Prepare and test a 2 g sample as directed under Method II in Heavy Metals Test, using 20 mcg lead ion in the control (Solution A).
SILICON DIOXIDE, AMORPHOUS

DEFINITION

**Functional Use in Foods:** Anticaking agent

**Scope:** The products included under this specification are: Silica Aerogel (precipitated silicon dioxide); Hydrated Silica; "Silicic Acid"; Dehydrated silica gel.

**Chemical name:** Silicon dioxide

**Empirical Formula:** \((\text{SiO}_2)_x\)

DESCRIPTION

Silica aerogel is a microcellular silica occurring as a fluffy powder or granules. Hydrated silica is a precipitated, hydrated silicon dioxide occurring as a fine, white, amorphous powder, or as beads or granules.

CHARACTERISTICS

**Identification**

A. Solubility:
   - Water: insoluble
   - Ethanol: insoluble
   - HF: soluble (CAUTION*)
   - Alkali \((80^\circ\text{C}100^\circ)\): soluble

B. Test for silica - Volatility of SiF4 - see Assay of silicon dioxide.

**Specification**

**Assay:** On the ignited basis: Silica aerogel: not less than 90 % of SiO\(_2\). Hydrated silica: not less than 89 % of SiO\(_2\).

* Toxic, corrosive, must not contact skin.
The article of commerce may be further specified as to loss on drying, loss on ignition and soluble ionizable salts.

LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg
Heavy metals (as Pb): not more than 30 mg/kg
Lead: not more than 10 mg/kg

EXAMINATION

Assay

Transfer about 2 grams, accurately weighed, into a tared platinum crucible, ignite at 1000° for 1 hour, cool in a dessicator, and weigh. Moisten the residue with 7 or 8 drops of ethanol, add 3 drops of concentrated sulphuric acid, and, with CAUTION*, add enough hydrofluoric acid to cover the wetted sample. Evaporate to dryness on a hot plate, using medium heat (95-105°), then add 5 ml of hydrofluoric acid, swirl the dish carefully to wash down the sides, and again evaporate to dryness. Ignite the dried residue to a red heat over a Meker burner, cool in a dessicator, and weigh. The difference between the total weight loss and the weight loss after ignition at 1000° represents the weight of SiO₂ in the sample taken.

Sample Solution for the Determination of Arsenic, Heavy Metals and Lead: Transfer 10.0 grams of the sample 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 minutes, 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.

* Toxic, corrosive, must not contact skin.
**Arsenic:** A 10-ml portion of the Sample Solution meets the requirements of the Arsenic Test.

**Heavy Metals:** Dilute 20.0 ml of the Sample Solution to 30.0 ml with water. A 10-ml portion of the dilution meets the requirements of the Heavy Metals Test, using 20 mcg of lead ion (Pb) in the control (Solution A).

**Lead:** A 10-ml portion of the Sample Solution meets the requirements of the Lead Limit Test, using 10 mcg of lead ion (Pb) in the control.
ALUMINIUM SILICATE
(KAOLIN)

DEFINITION

Functional Use in Foods: Anticaking agent

Synonyms: Kaolin, light or heavy

DESCRIPTION

Kaolin is a native hydrated aluminium silicate, freed from most of its impurities by elutriation and dried. It is a soft, whitish powder free from gritty particles, odourless and tasteless.

CHARACTERISTICS

Identification

A. Solubility: Water: insoluble
   Ethanol: insoluble
   Mineral acids: insoluble

B. Mix 0.2 g of Kaolin with 1.5 g of a mixture of anhydrous sodium carbonate and anhydrous potassium carbonate (1:1), and heat in a platinum or nickel crucible until the mixture melts completely. Cool, add 5 ml of water, and allow to stand for 3 minutes. Heat the bottom of the crucible gently to detach the melt easily, and transfer the melt into a beaker with water. Add gradually hydrochloric acid until no effervescence is observed, and add another 10 ml of the acid. Evaporate the mixture to dryness on a water bath. Add 200 ml of water, boil and filter. Reserve the filtrate for test C. Transfer the gelatinous residue into a platinum dish, and add, with CAUTION* 5 ml of hydrofluoric acid. The precipitate dissolves and on heating it volatilizes almost completely.

* Toxic, corrosive, must not contact skin.
C. Add ammonia TS to the filtrate from B above. A white gelatinous precipitate is formed which is insoluble in excess ammonia but dissolves in sodium hydroxide TS.

D. To 8 g of aluminium silicate (Kaolin) add 5 ml water and mix well. The mixture is plastic.

**Specification**

Water soluble substances not more than 0.3 %

Hydrochloric acid soluble substances: not more than 2 %

The commercial product may be further specified as to chloride, foreign substances, particle size, loss on drying, loss on ignition and pH.

**LIMITS OF IMPURITIES**

Arsenic: not more than 3 mg/kg

Heavy Metals: not more than 10 mg/kg

Asbestos: absent - microscopic test needed

**EXAMINATION**

*Water soluble substances*: To 10 g of Kaolin, add 100 ml of water, and boil for 30 minutes, supplementing water occasionally. Cool, add water to 100 ml, and filter through a glass filter. Evaporate a 50 ml portion of the filtrate to dryness, and dry the residue at 105° for 1 hour. The weight of the residue does not exceed 15 mg.

*Hydrochloric acid soluble substances*: Boil 2.0 g with 100 ml of 0.2N hydrochloric acid under a reflux condenser for five minutes, cool, and filter; evaporate 50 ml of the filtrate to dryness; the residue, after gentle ignition to constant weight, weighs not more than 10 mg.

*Arsenic*: To 0.5 g of Kaolin, add 5 ml of diluted hydrochloric acid, and heat at 70° for 15 minutes, shaking well. Cool immediately, and filter. Wash the residue with 5 ml of diluted hydrochloric acid, and subsequently with 10 ml of
water. Combine the washings to the filtrate, and add water to 20 ml. A 10 ml portion of the solution meets the requirements of the test for Arsenic, as directed in General Tests.

**Heavy Metals:** To 10 g of Kaolin, add 70 ml of water, 10 ml of hydrochloric acid and 5 ml of nitric acid, and heat on a water bath for 15 minutes with shaking. Cool, add water to 100 ml, and filter. Evaporate a 50 ml portion of the filtrate to dryness on a water bath. Add 2 ml of diluted acetic acid and 20 ml of water to dissolve and filter if necessary. Proceed as directed under Heavy Metals Test in General Tests.
CALCIUM SILICATE

DEFINITION

Functional Use in Foods: Anticaking agent
Synonyms and Varieties: Calcium silicates and poly-silicates

DESCRIPTION

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

It is a very fine, white or off white powder with low bulk density and high physical water absorption.

CHARACTERISTICS

Identification

A. Solubility: Water: insoluble
   Ethanol: insoluble

B. Mix 500 mg with about 200 mg of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate, and heat the mixture in a platinum crucible until fusion is complete. Cool, and transfer the fused mixture to a dish or beaker with the aid of about 50 ml of hot water. Add hydrochloric acid to the liquid until effervescence ceases, 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: an insoluble residue of silica remains. Transfer the gelatinous residue into a platinum dish, and add, with CAUTION*, 5 ml of hydrofluoric acid. The precipitate dissolves and on heating it volatilizes almost completely.

* Toxic, corrosive, must not contact skin.
C. Neutralize the filtrate from B above with ammonia T. S. using 2 drops methyl red T. S. as indicator, then add diluted hydrochloric acid T. S. dropwise until the solution is acid. Upon the addition of ammonium oxalate T. S. a white granular precipitate of calcium oxalate forms. This precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

**Specification**

The commercial product may be specified as to calcium and silicon dioxide contents, loss on drying, loss on ignition, pH of 10% water slurry, bulk density, moisture, sulphate and chloride.

**LIMITS OF IMPURITIES**

- **Arsenic:** not more than 3 mg/kg
- **Lead:** not more than 10 mg/kg
- **Heavy metals (as Pb):** not more than 40 mg/kg
- **Fluorine:** not more than 50 mg/kg
- **Asbestos:** absent, microscopic test needed.
MAGNESIUM SILICATE
(Talc and Magnesium trisilicate)

DEFINITION

Functional Use in Foods: Anticaking agent

Synonyms and Varieties: Talc; Magnesium trisilicate

DESCRIPTION

Sources: Talc is a native, hydrous magnesium silicate, sometimes containing a small portion of aluminium silicate. Magnesium trisilicate also coming under this specification may be a synthetic product.

Appearance: Very fine, white or greyish white, odourless crystalline powder. Is unctuous, adheres readily to the skin, and is free from grittiness.

CHARACTERISTICS

Identification:

A. Solubility: Water: insoluble
   Ethanol: insoluble

B. Mix 500 mg with about 200 mg of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate, and heat the mixture in a platinum crucible until fusion is complete. Cool, and transfer the fused mixture to a dish or beaker with the aid of about 50 ml of hot water. Add hydrochloric acid to the liquid until effervescence ceases, 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; an insoluble residue of silica remains. Transfer the gelatinous residue into a platinum dish, and add, with CAUTION*, 5 ml of hydrofluoric acid. The precipitate dissolves and on heating it volatilizes almost completely.

* Toxic, corrosive, must not contact skin.
C. In the filtrate from B above, dissolve about 2 g of ammonium chloride, and add 5 ml of ammonia T.S. Filter if necessary, and add sodium phosphate T.S. to the filtrate: a white, crystalline precipitate of magnesium ammonium phosphate separates.

**Specification**

- **pH**: Neutral to litmus
- **Water soluble substances**: not more than 0.2
- **Hydrochloric acid-soluble substances**: not more than 2
- The article of commerce can be specified further by magnesium oxide and silicon dioxide contents, loss on ignition, chloride, sulphate and iron.

**LIMITS OF IMPURITIES**

- **Arsenic**: not more than 3 mg/kg
- **Heavy metals**: not more than 40 mg/kg
- **Lead**: not more than 10 mg/kg
- **Fluoride**: not more than 20 mg/kg
- **Asbestos**: absent, microscopic test needed

**EXAMINATION**

- **Hydrochloric Acid-soluble substances**: Digest 1.00 g with 20 ml of diluted hydrochloric acid at $50^\circ$ for 15 minutes, add water to restore the original volume, mix, and filter. To 10 ml of the filtrate add 1 ml of diluted sulfuric acid, evaporate to dryness, and ignite to constant weight.

- **pH and water-soluble substances**: Boil 10 g with 50 ml of water for 30 minutes, adding water from time to time to maintain approximately the original volume, and filter. The filtrate is neutral to litmus paper. Evaporate one-half of the filtrate to dryness, and dry at $105^\circ$ for 1 hour.
Arsenic: Mix 0.5 g of Talc in 5 ml of diluted hydrochloric acid, and boil while stirring. Cool the mixture, and filter. Wash the residue with 5 ml of diluted hydrochloric acid, and subsequently with 10 ml of water. A mixture of the filtrate and the washings meets the requirements of the test for Arsenic.

Heavy metals: Mix 2 g of Talc with 8 ml of diluted hydrochloric acid and 10 ml of water, shake well, and boil gently. Cool, add water to produce a 50 ml solution, and filter. To 25 ml of the filtrate, add ammonia T.S. to adjust the pH to 5.2, and add 2 ml of diluted acetic acid: it meets the heavy metals limit, as specified above using the General Test procedure.
SODIUM ALUMINO SILICATE

DEFINITION

Functional Use in Foods: Anticaking agent

Synonyms: Sodium silicoaluminate

DESCRIPTION

Sodium silicoaluminate is the name for a series of hydrated sodium aluminum silicates. It occurs as a fine, white, amorphous powder, or as beads. It is odourless and tasteless.

CHARACTERISTICS

Identification

A. Solubility:
   Water: insoluble
   Ethanol: insoluble
   Strong acids: partially soluble
   Alkali Hydroxides: partially soluble

B and C. Mix 0.2 g of material with 1.5 g of a mixture of anhydrous sodium carbonate and anhydrous potassium carbonate (1:1), and heat in a platinum or nickel crucible until the mixture melts completely. Cool, add 5 ml of water, and allow to stand for 3 minutes. Heat the bottom of the crucible gently to detach the melt easily, and transfer the melt into a beaker with water. Add gradually hydrochloric acid until no effervescence is observed, and add another 10 ml of the acid. Evaporate the mixture to dryness on a water bath. Add 200 ml of water, boil and filter. Reserve the filtrate for test C. Transfer the gelatinous residue into a platinum dish, and add, with CAUTION*, 5 ml of hydrofluoric acid. The precipitate dissolves and on heating it volatilizes almost completely.

* Toxic, corrosive, must not contact skin.
C. Add ammonia TS to a part of the filtrate from B above. A white gelatinous precipitate is formed which is insoluble in excess ammonia but dissolves in sodium hydroxide TS.

D. Add uranyl zinc acetate TS to a part of the filtrate from B above; a yellow crystalline precipitate appears within a few minutes.

Specifications

The commercial product may be specified as to silicon dioxide, aluminum oxide, and sodium oxide content, loss on drying, loss on ignition and pH of the slurry with water.

LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg
Heavy metals (as Pb): not more than 10 mg/kg

EXAMINATION

Sample Solution for the Determination of Arsenic and Heavy Metals: Transfer 10.0 grams of the sample 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 minutes, 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 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.

Arsenic: A 10-ml portion of the Sample Solution meets the requirements of the Arsenic Test.

Heavy metals: A 20-ml portion of the Sample Solution meets the requirements of the Heavy Metals Test, using 20 µg of lead ion (Pb) in the control (Solution A).
CERTAIN OTHER FOOD ADDITIVES
MONOSODIUM L-GLUTAMATE

(Tentative)

DEFINITION

Functional Use in Foods: Flavour enhancer

Synonyms: Monosodium Glutamate; Sodium Glutamate; MSG

Empirical Formula: \( \text{C}_5\text{H}_8\text{NNaO}_4\cdot\text{H}_2\text{O} \)

Structural Formula:

\[
\text{HOOC} - \text{CH} - \text{CH}_2\text{CH}_2\text{C00Na} \cdot \text{H}_2\text{O} \\
\text{NH}_2
\]

Molecular Weight: 187.1

DESCRIPTION

White, practically odourless, free-flowing crystals or crystalline powder, with either a slightly sweet or a slightly salty taste.

CHARACTERISTICS

Identification

A. Solubility: Water: soluble
   Ethanol: insoluble

B. To 1 ml of a 1 in 30 solution add 1 ml of ninhydrin T.S. and 100 mg of sodium acetate, and heat in a boiling water bath for 10 minutes. An intense violet-blue colour is formed.

C. To 10 ml of a 1 in 10 solution add 5.6 ml of 1 N hydrochloric acid. A white crystalline precipitate of glutamic acid forms on standing. When 6 ml of 1 N hydrochloric acid is added to the turbid solution, the glutamic acid dissolves on stirring.
D. To 1 ml of a 1 in 100 solution add uranyl zinc acetate T. S; a yellow crystalline precipitate appears within a few minutes.

Specifications

Assay: Not less than 99 percent of C₅H₈NNaO₄·H₂O.

Specific rotation: \[ [\alpha]^{25}_D + 24.2^0 \text{ to } +25.5^0 \]

Loss on drying: not more than 0.2 % on drying at 98 ± 1°C for 5 hours.

The article of commerce can be further specified by nitrogen content, pH and chloride content.

LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg
Heavy metals (as Pb): not more than 20 mg/kg
Pb: not more than 10 mg/kg

EXAMINATION

Assay:

Dissolve about 250 mg, accurately weighed, in 100 ml of glacial acetic acid. A few drops of water may be added prior to the addition of the acetic acid to effect faster dissolution of the sample. Titrate with 0.1 N perchloric acid in glacial acetic acid, determining the end-point potentiometrically. Each ml of 0.1 N perchloric acid is equivalent to 9.356 mg of C₅H₈NNaO₄·H₂O.

Specific Rotation

\[ [\alpha]^{25}_D \] Determine in a 4 dm tube on a solution containing 400 mg in each 10 ml of 2.3 N hydrochloric acid.
CALCIUM SULPHATE

DEFINITION

Functional Use in Foods: Firming Agent

Chemical Formula: CaSO$_4$.2H$_2$O and CaSO$_4$ (anhydrous)

Molecular Weight: 172.2; 136.1 (anhydrous)

DESCRIPTION

Calcium sulphate is a fine, white to slightly yellow-white odourless powder.

CHARACTERISTICS

Identification

A. Solubility: Water: slightly soluble
   Ethanol: insoluble

B. Warm 0.2 g calcium sulphate with 4 ml dilute hydrochloric acid T.S. and 16 ml water. (Solution A). To 10 ml of Solution A add 5 ml ammonium oxalate T.S. A white precipitate forms.

C. To the remaining 10 ml of Solution A add barium chloride T.S. A white precipitate forms which is insoluble in hydrochloric and nitric acids.

Specification

Assay: not less than 99% CaSO$_4$ after drying at 250$^\circ$ to constant weight.
LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg
Fluoride: not more than 30 mg/kg
Heavy metals (as Pb): not more than 10 mg/kg
Selenium: not more than 30 mg/kg

EXAMINATION

Arsenic: Mix 1 g with 10 ml of water, add 12 ml of diluted hydrochloric acid T. S., 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 Heavy Metals Test, using 20 mcg of lead ion (Pb) in the control (Solution A).

Selenium: A solution of 2 grams in 40 ml of dilute hydrochloric acid (1 in 2) meets the requirements of the Selenium Limit Test.

Assay: Dissolve about 350 mg of the dried sample accurately weighed, in 100 ml of water and 4 ml of diluted hydrochloric acid T. S. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 N disodium ethylenediaminetetraacetate from a 50-ml buret, then add 15 ml of sodium hydroxide T. S. and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 N disodium ethylenediaminetetraacetate is equivalent to 6.807 mg of CaSO₄.
POTASSIUM CHLORATE

(Identification only)

Chemical Formula: KClO₃

DESCRIPTION

Colourless crystals, white granules or powder.

CHARACTERISTICS

Caution

In admixture with organic or readily oxidizable substances, it is liable to explode if heated or subjected to percussion or trituration.

Identification

A. Solubility: Water: soluble
   Ethanol: insoluble

B. To a 1% aqueous solution of potassium chlorate add silver nitrate T.S. No precipitate is formed. Reduce chlorate in this solution to chloride with urea. Cool and add 10 ml of water and silver nitrate T.S. A flocculent white precipitate is formed.

C. To an aqueous solution of potassium chlorate add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.
ASCORBYL STEARATE

DEFINITION

Synonyms: Vitamin C stearate

Structural Formula:

\[
\begin{align*}
\text{O} & \equiv \text{C} \\
\text{HO} & \equiv \text{C} \\
\text{HO} & \equiv \text{C} \\
\text{H} & \equiv \text{C} \\
\text{HO} & \equiv \text{C} - \overset{\text{R}}{\text{H}} \\
\text{CH}_2\text{CO} & \equiv \text{R}
\end{align*}
\]

Molecular Weight: 442.6

Where R is the aliphatic chain of stearic acid.

Ascorbyl stearate contains not less than 93% of the product and conforms to the following specifications.

DESCRIPTION

Ascorbyl stearate is a white or yellowish-white solid with a citrus-like odour.

CHARACTERISTICS

Identification

A. Solubility: Water: insoluble 
   Ethanol: soluble

B. Melting range 115-118°

C. A solution of ascorbyl stearate in ethanol will decolourize a solution of 2,6-dichlorophenol-indophenol T.S.
PURITY TESTS

Sulphated ash

Not more than 0.1%.

Heavy metals: Add 1 ml of hydrochloric acid and 0.2 ml of nitric acid to the residue obtained at the test for residue on ignition, and evaporate to dryness on a water bath. Dissolve the residue in 1 ml of diluted hydrochloric acid and 15 ml of water by heating. Cool, add 1 drop of phenolphthalein T.S., add ammonia T.S. dropwise until a slightly pink colour appears, and add 2 ml of diluted acetic acid. Proceed as directed under Heavy Metals Test of Section 10 in General Tests using the solution. The heavy metals limit for L-Ascorbyl Stearate is not more than 0.001 percent.

Arsenic: Place 2 g of L-Ascorbyl Stearate in a digestion flask, add 20 ml of nitric acid, and liquefy by heating gently. Cool, add 5 ml of sulphuric acid, and heat until white fume evolves. If the solution is still brown, add 5 ml of nitric acid after cooling, and heat. Repeat this process, until the colour of the solution turns into colourless or pale yellow. Cool, add 15 ml of saturated ammonium oxalate solution, and heat until white fumes evolve. Cool, and add water to 25 ml. A 5 ml portion of the solution meets the requirements of the test for Arsenic, as directed under Arsenic Test of Section 19 in General Tests. For the control, place 2 ml of arsenic standard solution in the digestion flask, add 20 ml of nitric acid, and proceed in the same manner as the sample.

Loss on drying: not more than 2% after drying over sulphuric acid in a vacuum dessicator for 4 hours.
Arsenic: not more than 3 mg/kg
Heavy metals (as Pb): not more than 10 mg/kg

Assay

Titrimetric method: Add 0.800 g of ascorbyl stearate to a mixture of 50 ml of carbon dioxide-free water, 50 ml of chloroform and 25 ml of dilute sulphuric acid T.S. Titrate the mixture at once with 0.1 N iodine making sure that the mixture is well shaken. Add a few drops of starch T.S. as indicator, as the end point is reached. Each ml 0.1 N iodine is equivalent to 0.0214 g of ascorbyl stearate.
DIMETHYLPOLYSILOXANE

DEFINITION

**Functional Use in Foods:** Deoaming agent

**Synonyms:** Polydimethylsiloxane, Silicone, Silicone fluid, oil

**Structural Formula:**

![Structural Formula](image)

The average value for \( n \) is 200 to 300.

DESCRIPTION

Dimethylpolysiloxane frequently is used in commerce as such, as a liquid containing silica gel and as an aqueous emulsion formulation containing in addition to silica gel, emulsifying agents and preservatives. The pure substance treated here can be isolated by centrifuging from the silica gel containing liquid. Dimethylpolysiloxane is a clear, colourless, viscous liquid.

CHARACTERISTICS

**Identification**

A. **Solubility:**
   - Water: insoluble
   - Ethanol: insoluble
   - Carbon tetrachloride: soluble
B. Ash 0.1 to 0.2 gram of sample in a platinum crucible with \( \text{H}_2\text{SO}_4 \) and \( \text{HNO}_3 \). After ashing is complete, transfer a portion of the silica residue to a nickel crucible, add about 1 gram of NaOH pellets and heat the mixture until fusion is completed. Cool and dissolve in about 50 ml water and filter out any residue. Place a drop of the filtrate on a sheet of filter paper, followed by a drop of ammonium molybdate T. S. * and a drop of benzidine T. S. ** Let the test paper come into contact with ammonia fumes. If silicon is present, a blue spot will be developed.

* Ammonium Molybdate T. S. 5 g ammonium molybdate dissolved in 100 ml \( \text{H}_2\text{O} \), and this solution added to 35 ml of 25 % \( \text{HNO}_3 \).

** Benzidine T. S. 0.05 g benzidine hydrochloride is dissolved in 100 cc of 10 % acetic acid.

C. Infrared Spectrum: Characteristic for dimethylpolysiloxane, and shall not indicate presence of phenyl compounds.

**Specification**

Specific gravity \( 25^\circ/25^\circ \): 0.972 ± 0.004
Refractive index at \( 25^\circ \): 1.400 to 1.404
Viscosity: 300 to 600 centistokes at \( 25^\circ \)
Loss on heating for 4 h at \( 200^\circ \): not more than 18%

Dimethylpolysiloxane in the article of commerce used as an antifoaming agent can be specified further as to total silicon.

**LIMITS OF IMPURITIES**

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>3 mg/kg</td>
</tr>
<tr>
<td>Lead</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>40 mg/kg</td>
</tr>
</tbody>
</table>
OXYSTEARIN

DEFINITION

**Functional Use in Foods:**

Crystallization inhibitor in vegetable oils.

DESCRIPTION

"Oxystearin" is a mixture of glycerides of partially oxidized stearic and other fatty acids. The product is obtained by heating hydrogenated cottonseed or soybean oil under controlled conditions in the presence of air and a suitable catalyst (not present in the final product). It occurs as a tan to light brown fatty or wax-like substance having a bland taste. *(Information needed from manufacturer on nature of the oxidation products, ketonic and polymeric acids).*

CHARACTERISTICS

**Identification**

A. **Solubility:**
   - Water: insoluble
   - Ethanol: soluble

B. Identify fatty acids as indicated in Annex to FA3/IV.

C. Oxystearin can be acetylated with acetic anhydride and pyridine (see hydroxyl number under Assay, below).

**Specification**

**Assay:** Hydroxyl number between 30 and 45.

The article of commerce can be specified as to acid number, iodine number, saponification number, unsaponifiable material, refractive index.
LIMITS OF IMPURITIES

Arsenic: not more than 3 mg/kg
Heavy metals (as Pb): not more than 10 mg/kg
Epoxides: not more than 50 mg/kg

EXAMINATION

Hydroxyl Number: see 6th Report of the Joint FAO/WHO Expert Committee on Food Additives, p. 182
Arsenic and heavy metals: as for fatty acids.
ISOAMYL GALLATE

(Tentative)

DEFINITION

Functional Use in Foods: Antioxidant

Chemical Name: iso-Amyl ester of 3, 4, 5 trihydroxy benzoic acid

Empirical Formula: $\text{C}_{12}\text{H}_{16}\text{O}_5$

Structural Formula:

\[
\begin{array}{c}
\text{HO} \\
\text{CO(CH}_2)_2\text{CH(CH}_3)_2 \\
\text{HO} \\
\text{OH}
\end{array}
\]

Molecular Weight: 240.3

DESCRIPTION

Isoamyl gallate is a white to pale brownish yellow crystalline solid, odourless, with a slightly bitter taste.

CHARACTERISTICS

Identification

A. Solubility: Water: insoluble
   Ethanol: soluble
B. Melting range: 140-145°

C. Dissolve 0.5 g of isoamyl gallate in 10 ml of sodium hydroxide T.S., distil, and take 4 ml of the distilled portion. This solution is separated in two layers, emitting a strong isoamyl alcohol odour.

D. Dissolve 0.1 g of isoamyl gallate in 5 ml of ethanol, and add 1 drop of diluted ferric chloride T.S.; a violet colour is produced.

E. Add 1 ml of ammonium hydroxide to 5 ml of a 1% ethanolic solution of isoamyl gallate. A pink to red colour appears.

**Specification**

**Assay:** Method needed

The commercial product may be further specified as to limits for chloride, sulphate, colour of solution, loss on drying, residue on ignition.

**LIMITS OF IMPURITIES**

<table>
<thead>
<tr>
<th>Impurity</th>
<th>Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy metals (as Pb)</td>
<td>not more than 40 mg/kg</td>
</tr>
<tr>
<td>Arsenic</td>
<td>not more than 3 mg/kg</td>
</tr>
<tr>
<td>Lead</td>
<td>not more than 10 mg/kg</td>
</tr>
</tbody>
</table>
ETHYL PROTOCATECHUATE

(Identification only)

DEFINITION

Chemical Name: Ethyl ester of 3, 4-dihydroxy-benzoic acid

Empirical Formula: C₉H₁₀O₄

Structural Formula:

\[
\begin{array}{c}
\text{HO} \\
\text{HO--} \\
\text{COOC}_2\text{H}_5 \\
\text{HO}
\end{array}
\]

Molecular Weight: 182.2

DESCRIPTION

Ethyl protocatechuate is a white or pale brownish yellow, crystalline powder. It is odourless or has a faint phenol-like odour with a slight bitter taste.

CHARACTERISTICS

Identification

A. Solubility: Water: insoluble
   Ethanol: soluble

B. Melting range: 132-135°C

C. Dissolve 0.5 g of ethyl protocatechuate in 10 ml of sodium hydroxide T.S., distil, and take 4 ml of the initial distillate. This is clear. To this initial distillate add an
equal volume of sodium hydroxide T.S. and 2 or 3 drops of iodine T.S., and heat the solution: iodoform odour evolves.

D. Dissolve 0.1 g of ethyl protocatechuate in 5 ml of ethanol, and add 1 drop of diluted ferric chloride T.S.: a green colour develops.

E. Acidify the residue from the distillation in C, to precipitate an acid which on recrystallization has a melting range of 190-194° with decomposition.
ANNEX

General References

REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES


* These documents can be obtained on request from: Food Additives, World Health Organization, Avenue Appia, 1211 Geneva. Switzerland or: Food Science Branch, Food and Agriculture Organization of the United Nations, 00100 Rome, Italy.


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