A REVIEW
OF THE TECHNOLOGICAL EFFICACY
OF SOME ANTIMICROBIAL AGENTS
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OF SOME ANTIMICROBIAL AGENTS

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Expert Committee on Food Additives which
met in Geneva, 24 June–2 July 19701/

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
WORLD HEALTH ORGANIZATION
ROME, 1971

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INTRODUCTION

At its 6th, 8th, 9th and 10th Sessions, the Joint FAO/WHO Expert Committee on Food Additives had prepared specifications and made toxicological evaluation of some antimicrobial agents. The Committee at its 14th Session held in Geneva from 24 June to 2 July 1970, reviewed the technological efficacy of certain antimicrobial agents and prepared the monographs contained in this publication. While reviewing the technological efficacy, the Committee stated that these monographs were not to be regarded as recommendations for use, "tolerances", legal restrictions or clearances, but constituted a review of data available in literature. The use levels given in the monographs therefore do not necessarily correspond either with those specified by legislation or with the optimal concentration for technological purposes. Readers should therefore consider these monographs only in line with the views of the Expert Committee as contained in their Report of the above mentioned meeting; FAO Nutrition Meetings Report Series No. 48 WHO/Food Add/462.

While the methods of assay of the pure substances are given in the respective monographs on specifications referred to in the documents, the Committee felt that the monographs on technological efficacy should also contain methods of analysis for determining the additives in food. It was advised that the elaboration of such methods was already being undertaken by the Joint FAO/WHO Codex Alimentarius Commission.
GENERAL CONSIDERATIONS (7, 12, 32, 46)

1. Principles

Many different types of micro-organisms occur in fresh foods immediately after harvest, catch or slaughter. Only part of this initial contamination will eventually play an important role in the spoilage of foods as encountered in commerce. The chemical composition of the food and the ways in which the foods are stored and handled permit only a small selection of the initial microflora to develop into what is customarily called the spoilage association of the food. Spoilage association has a very specific character: proteinaceous foods such as refrigerated meats, fish and poultry first spoil as a result of superficial slime formation by the Pseudomonas/Acinetobacter group of bacteria; fruit juices tend to be fermented by yeasts; and cereal products may grow mouldy during storage.

Whilst moisture control or other physical methods may be effectively used for preserving foods during storage, it is not always possible, for technological or other reasons including food quality, to use these as the sole means of preservation. It thus frequently becomes necessary to use chemical preservatives.

When selecting chemical preservatives the character of spoilage associations must be taken into account. A preservative effectively suppressing one type of micro-organism may
have no inhibitory effect at all on a different type: no preservative effect can be expected from a bacteriostatic agent, if the spoilage association of the food in which the preservative is to be used is almost purely fungal and vice-versa.

The following requirements should also be met by antimicrobial agents suggested for use in food:¹/

(i) Qualitative requirements: the preservation of the food under review is technologically necessary; the preservative is effective under conditions prevailing in the food to which it is to be added; the preservative has a sufficiently wide spectrum of activity (of Table 1); the preservative is reasonably stable in the food which it should protect on storage; the preservative conveys no taste, odour or discolouration to the food.

(ii) Quantitative requirements: additives should always be used at the lowest possible concentration. This is defined as the concentration that is required to preserve the food during a reasonable period of time, but only when all care is taken to limit the initial contamination of the food with potential spoilage agents.

As in the physical preservation of foods, the efficiency of chemical preservation depends largely on the initial numbers of potential spoilage agents per gramme of food: the higher the initial load of potential spoilage agents, the higher the concentration of a preservative required to attain a desired keeping quality. Limiting the amount of preservative in the lowest possible level is hence desirable for toxicological as well as for microbiological reasons. If unnecessarily high concentrations of preservative were used in food, hygienic requirements in food preparation could be neglected. This could well lead to higher levels of pathogenic or toxigenic organisms in foods, an obviously far from attractive prospect.

2. Factors influencing the mode of action of chemical preservatives

The antimicrobial activity of most chemical preservatives is particularly influenced by certain properties of the foods to which the preservatives are added. These are considered below:

**Acidity**

The most important parameter of a food determining the activity of a given preservative is its pH value. Although there are many potential antimicrobial food additives, most of the preservatives used are acids. It has been demonstrated that the activity of such preservatives is virtually nil in the neutral pH area and increases with decreasing pH. The reason for this is apparently that only the undissociated acid molecules can penetrate into the protoplasm of the microbial cells which are to be inhibited; whereas, at higher pH, most of these acids will be dissociated and the ions so formed cannot reach the site where they would exert antimicrobial activity.

The pH value of a food also often determines the efficiency of non-acid preservatives.

**Chemical Composition**

In addition to the effect of pH there is the important influence exerted by some components of a food on the activity of certain preservatives. Fats may partly extract some preservatives from the aqueous phase of a food and hence reduce their antimicrobial activity since the micro-organisms are in the aqueous phase. Sulfurous acid enters into chemical combination with reducing sugars or may be oxidized by air dispersed in, or occurring over foods and thus be inactivated.

3. Technological objections to the use of preservatives

In several instances the use of an otherwise acceptable antimicrobial preservative is rendered impossible because it may adversely affect nutritive value, taste or colour, or the chemical keeping quality of the food to be preserved. A
classical example of this is Vitamin K₂ which is subject to rapid auto-oxidation in the presence of air and then imparts a violet colour which renders food unacceptable.

4. Evaluation of the efficacy of preservatives under practical conditions

The efficacy of antimicrobial food additives under practical conditions is determined by the intrinsic antimicrobial spectrum of the additive which is mostly described in terms of minimal inhibitory concentration (at given pH, water activity, nutrient concentration, etc.) for the broad taxonomic groups of food spoilage organisms presented in Table 1. The water activity (a_w) of a food is defined as the ratio between water vapour pressure over the food and vapour pressure of pure water at the same temperature (47) and is inversely proportional to the osmotic pressure of a food. Efficiency is also determined by the pertinent properties of the food and the conditions of its storage. These latter attributes include such factors as the pH, redox potential, structure and concentration of lipids, sugars and proteins and the temperature at which the food is normally kept.

In the evaluation of the efficacy of an antimicrobial food additive, therefore, certain methodological principles have to be followed to draw valid conclusions about the minimum effective use levels. Food samples to which an adequate range of concentrations of the preservative have been added are usually subjected to storage tests under the conditions encountered in practice. Inoculation of the samples with pertinent spoilage organisms is advisable in such tests. The efficacy of the preservative may be expressed quantitatively by plotting either the time of storage elapsed before spoilage becomes organoleptically detectable or the increase with time of the viable counts of pertinent organisms, against the concentrations of preservative added (cf Fig. 1).
is readily soluble in water but insoluble in fats and oils; cf Table 1 below.

Table 1
Solubility in g per 100 g solvent

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Benzoic Acid</th>
<th>Sodium Benzoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, 25°</td>
<td>0.33</td>
<td>60.0</td>
</tr>
<tr>
<td>&quot; 50°</td>
<td>1.0</td>
<td>60.4</td>
</tr>
<tr>
<td>&quot; 80°</td>
<td>2.5</td>
<td>70.5</td>
</tr>
<tr>
<td>5% v/v ethanol, 25°</td>
<td>0.29</td>
<td>56.6</td>
</tr>
<tr>
<td>20% v/v &quot;</td>
<td>1.5</td>
<td>50.0</td>
</tr>
<tr>
<td>50% v/v &quot;</td>
<td>10.0</td>
<td>40.0</td>
</tr>
<tr>
<td>10% w/v sucrose solution, 25°</td>
<td>0.30</td>
<td>58</td>
</tr>
<tr>
<td>Fats and oils, 25°</td>
<td>3 to 4</td>
<td>-</td>
</tr>
<tr>
<td>Glycerine, 25°</td>
<td>0.4</td>
<td>-</td>
</tr>
</tbody>
</table>

In the presence of sodium chloride the solubility of the undissociated acid decreases considerably.

3.2 Chemical reactions occurring in foods
None have been reported.

3.3 Storage and Handling
No special precautions are indicated, since benzoic acid and benzoates are stable compounds.

4. Antimicrobial action (4, 9, 40)
Benzoic acid has a somewhat wider spectrum of antimicrobial activity than sorbic acid, but like sorbic acid, its activity depends on pH; Table 2 shows the proportions of undissociated benzoic acid present in the pH range 2.0 to 5.0.
Table 1
Taxonomic groups of food spoilage organisms

- Gram negative, rod-shaped, non-fermentative bacteria;
- Gram negative, rod-shaped, fermentative bacteria;
- Gram positive, catalase positive cocci;
- Gram positive, sporing rod-shaped bacteria;
- Gram positive, non-sporing rod-shaped catalase positive bacteria;
- Gram positive, catalase negative cocci;
- Gram positive, catalase negative rod-shaped bacteria;
- Moulds
- Yeasts

Fig. 1. Time elapsing until spoilage of a food becomes detectable ($t_s$) as determined by (i) degree of initial contamination with the spoilage association ($N_0$); (ii) concentration of added antimicrobial preservative ($P$)
BENZOIC ACID AND BENZOATES

1. Names
1.1 Benzoic acid
1.2 Sodium Benzoate
1.3 Potassium Benzoate

2. References
2.1 References to monographs on specifications
2.2 References to monographs on toxicology
2.2.1 Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants. Sixth Report; FAO Nutrition Meetings Reports Series No. 31; WHO Techn. Rep. Ser. No. 228; 1962, pp. 27-30 (Benzoic acid, Sodium benzoate).
2.3 Reference to Methods of Analysis in Foods
3. Some physical and chemical properties
3.1 Solubility
Benzoic acid is only sparingly soluble in water, but more soluble in ethanol, glycerine and oils; sodium benzoate

1/ See paragraph 6.1 of the report
Table 2 (45)

<table>
<thead>
<tr>
<th>pH</th>
<th>undissociated portion of benzoic acid, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>98</td>
</tr>
<tr>
<td>3.0</td>
<td>95</td>
</tr>
<tr>
<td>4.0</td>
<td>60</td>
</tr>
<tr>
<td>4.5</td>
<td>30</td>
</tr>
<tr>
<td>5.0</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 3 gives the minimum inhibitory concentrations for the most important groups of micro-organisms at various pH values.

Table 3

Minimum inhibitory concentrations of micro-organisms at various pH levels. (45)

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>pH</th>
<th>complete inhibition of growth obtained at a concentration of mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>5.0</td>
<td>2600</td>
</tr>
<tr>
<td>Mucor racemosus</td>
<td>5.0</td>
<td>1200</td>
</tr>
<tr>
<td>Penicillium glaucum</td>
<td>2.6</td>
<td>500</td>
</tr>
<tr>
<td>Penicillium glaucum</td>
<td>4.0</td>
<td>650</td>
</tr>
<tr>
<td>Penicillium glaucum</td>
<td>4.5</td>
<td>600-800</td>
</tr>
<tr>
<td>Pichia membranaefaciens</td>
<td>4.0</td>
<td>500</td>
</tr>
<tr>
<td>Pichia membranaefaciens</td>
<td>5.0</td>
<td>800</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>2.6</td>
<td>160</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>4.0</td>
<td>500</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>5.0</td>
<td>1600</td>
</tr>
<tr>
<td>Saccharomyces ellipsoideus</td>
<td>3.3</td>
<td>125</td>
</tr>
<tr>
<td>Saccharomyces ellipsoideus</td>
<td>4.1</td>
<td>500</td>
</tr>
<tr>
<td>Willia anomala</td>
<td>2.6</td>
<td>100</td>
</tr>
<tr>
<td>Willia anomala</td>
<td>4.0</td>
<td>340</td>
</tr>
<tr>
<td>Willia anomala</td>
<td>5.0</td>
<td>800</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>6.0</td>
<td>100</td>
</tr>
<tr>
<td>Betabacterium buchneri</td>
<td>4.3</td>
<td>2500</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5.2</td>
<td>500-1000</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6.0</td>
<td>1800</td>
</tr>
<tr>
<td>Lactobacillus arabinosus</td>
<td>6.0</td>
<td>7000</td>
</tr>
</tbody>
</table>
Table 3 (cont'd)

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>complete inhibition of growth obtained at a concentration of mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus flavus</td>
<td>5.5</td>
<td>1000</td>
</tr>
<tr>
<td>Pseudomonas ovalis</td>
<td>6.0</td>
<td>4500</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5.6</td>
<td>1000</td>
</tr>
</tbody>
</table>

Benzoic acid is thus only useful in foods with a pH below 4.5, and therefore more limited in its application than sorbic acid. Like sorbic acid, its partition between the lipid and aqueous phases, depends upon the pH of the aqueous phase. Lipids in concentration over 5% exert an antagonistic effect for this reason. Except for calcium chloride (which has a marked antagonistic effect) inorganic salts, such as potassium and magnesium chlorides have no effect; sodium chloride has a slight synergistic effect. Isobutyric acid, gluconic acid and cystein hydrochloride also have synergistic effects.

5. **Foods in which used** (7, 31, 41, 50)

5.1 **Margarine**
Margarine and similar fat emulsions have been preserved by the addition of about 0.1% of benzoic acid to the aqueous phase, depending upon pH, salt content and concentration of milk solids present.

5.2 **Mayonnaise, salad dressings, sauces**
These foods have been preserved with 0.1% to 0.15% benzoic acid when the fat content is comparatively low.

5.3 **Pickles, horse-radish preparations**
These rather acid foods are easily protected from yeasts and moulds (16, 17) with about 0.07% to 0.1% of benzoic acid. For further protection, and particularly for colour retention, the use of an additional 50 to 100 mg/kg of sulfur dioxide has been suggested.

5.4 **Egg yolk**
Salted yolks may be protected against spoilage by 0.1% to 0.2% benzoic acid; unsalted egg yolk may need at least 1.2% benzoic acid (19).
5.5 Marinades, semi-preserved fish and crustaceae products

These foods often have a pH well above 4.5 and cannot be preserved by the addition of only one type of preservative. Depending on salt and sugar content, mixtures of 0.02% to 0.1% benzoic acid with 0.02% to 0.05% of a mixture of p-hydroxybenzoates and/or 0.03% to 0.05% of sorbic acid have been suggested, although the results have not been entirely satisfactory.

5.6 Fruit juices

Fruit juices can be preserved with 0.1%-0.15% benzoic acid, preferably in combination with 0.003%-0.008% sulfur dioxide. Processed fruit juices for immediate consumption may not require chemical preservation but for juices stored for further processing, addition of preservatives may help avoid undue heat treatment.

5.7 Fruitpulp, fruit paste, press cake

These products may also be preserved with combinations of benzoic acid, sorbic acid, and sulfur dioxide.

5.8 Soft Drinks

The concentrations needed for soft drinks have been found to be between 0.025%-0.035% benzoic acid, in combination with 0.002%-0.005% sulfur dioxide.

5.9 Jams, jellies, filling masses for sweets, marzipan

Depending upon pH and fat content, 0.1%-0.2% of benzoic acid has been used to protect these products against spoilage by yeasts and, especially, lipolytic moulds.

5.10 Miscellaneous

Soybean sauce has been preserved with 0.06%, caviar with 0.25% of benzoic acid.

6. Levels used in practice

Margarine, mayonnaise, fruit juices, pulps, jellies, jams preserves .................................................0.1%-0.15%
marinades (plus 0.03%-0.05% parahydroxybenzoates 0.08%-0.1%
and/or sorbic acid)

pickled vegetables (plus 0.03%-0.05% sulfur dioxide)0.07%-0.1%
beverages .......................................................0.05%-0.1%
NITRATES AND NITRITES

1. Names
1.1 Sodium nitrate, Chile salpetre, cubic or soda nitre.
1.2 Potassium nitrate, Salpetre, nitre.
1.3 Sodium nitrite, potassium nitrite.
1.4 Mixtures of nitrates and sodium chloride are known as "pickling" or "curing" salts.

2. References
2.1 Reference to monographs on specifications
Specifications for Identity and Purity and Toxicological Evaluation of some Antimicrobials and Antioxidants; FAO Nutrition Meetings Report Series No. 38 A; WHO Food Add./24.65 pp. 31 - 41.

2.2 Reference to monographs on toxicology

2.3 Reference to Methods of Analysis in Food

3. Some physical and chemical properties
3.1 Solubility in gramme per 100 grammes of solvent

<table>
<thead>
<tr>
<th></th>
<th>in water</th>
<th>in ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium nitrate</td>
<td>88.0 (20°)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>175.5 (100°)</td>
<td></td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>31.5 (20°)</td>
<td>insoluble</td>
</tr>
<tr>
<td></td>
<td>246.0 (100°)</td>
<td></td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>66.7 (20°)</td>
<td>sparingly</td>
</tr>
<tr>
<td>Potassium nitrite</td>
<td>very soluble (300)</td>
<td>sparingly</td>
</tr>
</tbody>
</table>

1/ See paragraph 6.1 of the Report.
3.2. Chemical reactions occurring in foods

In addition to the normal reaction with myoglobin described in paragraph 5.1, nitrites may also react with traces of secondary amines (if present) with the formation of nitrosamines. The extent to which this reaction occurs in food processing is likely to be small and is still under investigation (23).

3.3. Storage and Handling

Nitrates and nitrites, when mixed with organic matter may ignite or explode if subjected to heat or pressure. Due precautions should therefore be taken in their storage and handling.

Curing salt mixtures tend to separate on storage under humid conditions, but present no risk of ignition or explosion.

4. Antimicrobial action (6, 13, 19, 21, 24, 38, 48)

Nitrates and nitrites are mostly, although not exclusively, used in conjunction with sodium chloride for curing meat. In the concentrations used in foods, nitrates exert an antimicrobial action solely against obligately anaerobic bacteria. Sodium chloride on the other hand inhibits only the bacterial types which show a great sensitivity to reduced water activity, such as many of the proteolytic Gram-negative rod-shaped bacteria; Staphylococcus aureus and many micrococci, for example, thrive in pickling brines at normal temperatures.

Nitrites have stronger antimicrobial properties; however, at the concentrations obtaining in, and at the pH of, cured meat products, they do not fully inhibit the growth of bacteria which are otherwise capable of developing in such products. At the levels encountered in cooked cured meat products, nitrites are unable to prevent the germination of spores of Bacillaceae, unless they have been heated. Nitrites inhibit vegetative cell growth; the spores so prevented from further development die out. Low microbial loads in the raw materials used and cool storage of the final products are necessary for the prevention of microbial deterioration of cured meat products. Similarly, smoking, fermentation and partial dehydration increase stability.
5. Foods in which used (7, 20, 46)

5.1. Curing of meat

The use of nitrates and nitrites in meat curing aims, in addition to preservation, at developing the typical flavour and red colour. Nitrous oxide is the effective compound in colour fixation and it reacts with myoglobin to give the heat stable nitroso-myoglobin. Nitrous oxide itself results from the decomposition of nitrite, either added as such or produced by reduction of nitrate by the action of bacteria. There appears to be no suitable substitute for nitrite in the curing of meat. Depending upon size of the pieces of meat and the duration of curing, nitrate is applied in concentrations of from 0.05% to 0.1%. For development of a stable colour, the level of nitrite in the meat should reach 0.01%.

5.2. Fish

Nitrites have also been used for the short time preservation of fresh fish by storage in nitrite-ice.

5.3. Cheese

The addition of up to 0.2% of sodium or potassium nitrate to the milk is used as a measure against spoilage mainly by butyric acid forming clostridia in the manufacture of certain types of cheeses. Where nitrates are added to milk, traces are likely to occur in the whey.

6. Levels used in practice

In some countries the level of use is limited by prescribing admixture of nitrates and nitrites with common salt.

Approximate levels in cured meat products:

a) Canned chopped meat,
   Canned corned beef,
   Canned pork shoulder,
   Canned ham,
   Luncheon meat – nitrate, potassium or sodium as sodium nitrate
   nitrite, potassium or sodium as sodium nitrite

b) Milk for cheese making (certain cheeses) sodium as sodium nitrate
Note: The degradation of nitrates in cheese is reported to proceed from the initial level of about 80 mg/kg to a range between 30 and 2 mg/kg after storage for 2 to 10 weeks. The rate of degradation depends upon the type of cheese, its pH, and temperature of storage (51, 52). Small amounts of nitrite (100 μg/100 g) were found in cheese after 11 days, but none later (53).
ESTERS OF P-HYDROXYBENZOIC ACID

1. Names
   1.1 Methyl p-hydroxybenzoate; methylester of p-hydroxybenzoic acid; methyl paraben.
   1.2 Ethyl p-hydroxybenzoate; ethyl ester of p-hydroxybenzoic acid; ethyl paraben.
   1.3 Propyl p-hydroxybenzoate; n-propyl ester of p-hydroxybenzoic acid; propyl paraben.
   1.4 Butyl p-hydroxybenzoate; n-butyl ester of p-hydroxybenzoic acid; butyl paraben.

2. References
   2.1 References to monographs on specifications
      2.1.2 Specifications for butyl p-hydroxybenzoate - not published. See 2.2.2

2.2 References to monographs on toxicology
      Butyl p-hydroxybenzoate - evaluation postponed - p. 11

2.3 Reference to Methods of Analysis in Foods

3. Some physical and chemical properties

3.1 Solubility

The esters are only slightly soluble in water, but readily soluble in other solvents and alkali; cf Table 1

Table 1 (7)

Solubility of p-hydroxybenzoic acid esters in g per 100 g solvent:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>methyl</th>
<th>ethyl</th>
<th>propyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water 15°</td>
<td>0.16</td>
<td>0.08</td>
<td>0.023</td>
</tr>
<tr>
<td>25°</td>
<td>0.3</td>
<td>0.17</td>
<td>0.05</td>
</tr>
<tr>
<td>50°</td>
<td>1.4</td>
<td>0.23</td>
<td>0.10</td>
</tr>
<tr>
<td>80°</td>
<td>3.2</td>
<td>0.86</td>
<td>0.45</td>
</tr>
<tr>
<td>Ethanol 20%, v/v 25°</td>
<td>2.0</td>
<td>0.61</td>
<td>0.30</td>
</tr>
<tr>
<td>50%, v/v 25°</td>
<td>32.0</td>
<td>23.0</td>
<td>21.0</td>
</tr>
<tr>
<td>96%, v/v 25°</td>
<td>53.0</td>
<td>61.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Sodium chloride solution</td>
<td>0.24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5%, 25°</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose solution 10%, 25°</td>
<td>0.5</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>0.5</td>
<td>1.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

3.2 Chemical reactions occurring in foods

None of any significance have been reported.

3.3 Storage and handling

No special precautions are necessary

4. Antimicrobial activity

(1)

Esters of p-hydroxybenzoic acid are effective antimicrobial compounds, their activity being independent of pH up to pH 9.5. In this respect they have a decisive advantage over such preservatives as benzoic, sorbic, propionic or sulphurous acid. Table 2 gives a summary of the antifungal efficacy of benzoic, propionic and sorbic acid and of the methyl and propyl esters of p-hydroxybenzoic acid (7).

1/ See paragraph 6.1 of the report.
Table 2
Minimum Inhibitory Concentrations (%) of p-hydroxybenzoate esters and certain other preservatives against four types of moulds, as affected by pH

<table>
<thead>
<tr>
<th>At pH - 3.0</th>
<th>Alternaria</th>
<th>Aspergillus</th>
<th>Chaetomium</th>
<th>Penicillium</th>
<th>Globosum</th>
<th>Citrinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>0.10</td>
<td>0.04</td>
<td>0.08</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl p-hydroxy-</td>
<td>0.06</td>
<td>0.08</td>
<td>0.06</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>benzoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propyl p-hydroxy-</td>
<td>0.015</td>
<td>0.02</td>
<td>0.008</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>benzoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionic acid</td>
<td>0.04</td>
<td>0.08</td>
<td>0.04</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>0.005</td>
<td>0.04</td>
<td>0.01</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>At pH - 5.0</th>
<th>Alternaria</th>
<th>Aspergillus</th>
<th>Chaetomium</th>
<th>Penicillium</th>
<th>Globosum</th>
<th>Citrinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>0.15</td>
<td>0.20</td>
<td>0.10</td>
<td>0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl p-hydroxy-</td>
<td>0.08</td>
<td>0.10</td>
<td>0.06</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>benzoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propyl p-hydroxy-</td>
<td>0.02</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>benzoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionic acid</td>
<td>0.06</td>
<td>0.08</td>
<td>0.04</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>0.02</td>
<td>0.08</td>
<td>0.06</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>At pH - 7.0</th>
<th>Alternaria</th>
<th>Aspergillus</th>
<th>Chaetomium</th>
<th>Penicillium</th>
<th>Globosum</th>
<th>Citrinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl p-hydroxy-</td>
<td>0.10</td>
<td>0.15</td>
<td>0.10</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>benzoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propyl p-hydroxy-</td>
<td>0.05</td>
<td>0.05</td>
<td>0.04</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>benzoate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 (cont.d)

<table>
<thead>
<tr>
<th>Alternaria solani</th>
<th>Aspergillus niger</th>
<th>Chaetomium globosum</th>
<th>Penicillium citrinum</th>
</tr>
</thead>
</table>

At pH = 9.0

<table>
<thead>
<tr>
<th>Compound</th>
<th>Methyl p-Hydroxybenzoate</th>
<th>Ethyl p-Hydroxybenzoate</th>
<th>Propyl p-Hydroxybenzoate</th>
<th>Butyl p-Hydroxybenzoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl p-Hydroxybenzoate</td>
<td>0.10</td>
<td>0.15</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>Propyl p-Hydroxybenzoate</td>
<td>0.05</td>
<td>0.05</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>Propionic acid</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Minimum inhibitory concentration not attained.

The intrinsic efficacy increases from methyl to butyl esters; while the solubility decreases in the same direction.

While the esters are most active against moulds and yeasts, they are also sufficiently active against bacteria to be of use for the preservation of certain foods with neutral pH values. Table 3 shows the effect of the esters on various yeasts, moulds and bacteria.

Table 3 (45)

Inhibitory effect of esters of p-hydroxybenzoic acid at pH 5-6
(concentrations in mg/kg)

<table>
<thead>
<tr>
<th>Methyl-Ester</th>
<th>Ethyl-Ester</th>
<th>Propyl-Ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>400</td>
<td>300</td>
</tr>
<tr>
<td>Aspergillus oryzae</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>Mucor racemosus</td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>Penicillium digitatum</td>
<td>200</td>
<td>300</td>
</tr>
<tr>
<td>Penicillium glaucum</td>
<td>600</td>
<td>400</td>
</tr>
<tr>
<td>Pichia membranaefaciens</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>800</td>
<td>400</td>
</tr>
<tr>
<td>Torula lipolytica</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Willia anomala</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Bac. Subtilis</td>
<td>1200</td>
<td>600</td>
</tr>
<tr>
<td>Betabact. buchneri</td>
<td>4000</td>
<td>4000</td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

19
Table 3 (cont.d)

<table>
<thead>
<tr>
<th></th>
<th>Methyl-Ester</th>
<th>Ethyl-Ester</th>
<th>Propyl-Ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>2000</td>
<td>800</td>
<td>500</td>
</tr>
<tr>
<td>Lactobacillus arabinosus</td>
<td>2500</td>
<td>1500</td>
<td>500</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>2000</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4000</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>1200</td>
<td>1500</td>
<td>200</td>
</tr>
</tbody>
</table>

The efficacy of the preservative in foods with a considerable fat content depends upon its distribution between the lipid and aqueous phases, since only the part dissolved in the aqueous phase is effective. The lipid/water distribution coefficient increases from the methyl to the propyl ester as shown in Table 4.

Table 4

<table>
<thead>
<tr>
<th></th>
<th>methyl-ester</th>
<th>ethyl-ester</th>
<th>propyl-ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>in arachis oil, 20°</td>
<td>5.8</td>
<td>26</td>
<td>87</td>
</tr>
<tr>
<td>in arachis oil, 46°</td>
<td>-</td>
<td>19</td>
<td>58</td>
</tr>
</tbody>
</table>

Various salts and acids exert little influence on the efficacy of the esters, with the exception of calcium chloride and acetic acid, which have antagonistic effects. The antagonistic effect of lipids exceeds that for sorbic or benzoic acid, because of the different distribution coefficient (14). Because of their relatively low solubility, it is beneficial to use mixtures of the esters. The esters have a characteristic taste, and they may with advantage be partially replaced by sorbic, benzoic or propionic acid when used in foods with a pH value below 5.

Foods in which used (7, 31)

5.1 Marinades, semi-preserved fish and crustaceae products

Since these types of food often have a pH well above 4.5 they cannot be preserved by the addition of one type of preservative alone. Depending on salt and sugar content,
mixtures of 0.02% to 0.1% benzoic acid with 0.02% to 0.05% of a mixture of p-hydroxybenzoates and/or 0.03% to 0.05% of sorbic acid have been suggested; but the results have not been entirely satisfactory.

5.2 Mayonnaise, salad dressings, and sauces

Such products can be preserved with 400-500 mg/kg ethyl plus propyl esters in the ratio 2:1; depending upon the pH, 0.02% sorbic acid may also be added.

5.3 Baked goods

Combinations of about 0.03%-0.06% of methyl and propyl esters in the proportion 3:1 have been suggested for cakes, pie crusts and other unleavened goods, including filling masses and creams.

5.4 Beverages

0.03%-0.05% of a 2:1 mixture of methyl and propyl esters has been used for improving the keeping quality of soft drinks.

5.5 Cheese

The occurrence of mould on hard cheese has been controlled by dipping in a 3%-5% alcoholic solution of the methyl or ethyl ester.

5.6 Jams, jellies, and preserves

Spoilage of these products has been prevented with about 70 mg/kg of a 2:1 mixture of methyl and propyl esters.

5.7 Olives and pickles

The esters can usefully be added, singly or in combination, and also combined with benzoic acid, at a total concentration of 100 mg/kg.

6. Levels used in practice

- marinaades, preserves in oil, mayonnaise, sauces, creams, salad dressings
  - jams, jellies, preserves

0.02%-0.04% of esters singly or in combination, if required and possibly with an extm
0.05% benzoic or sorbic acid

0.07%
PROPIONIC ACID AND PROPIONATES

1. Names
1.1 Propionic acid
1.2 Calcium propionate
1.3 Potassium propionate
1.4 Sodium propionate

2. References
2.1 Reference to monographs on specifications

2.2 References to monographs on toxicology

2.3 Reference to Methods of Analysis in Food 1/

1/ see paragraph 6.1 of the report.

22
3. Some Physical and Chemical Properties

3.1. Solubility
Propionic acid is completely miscible with water, ethanol and ether; its salts are soluble to the extent of 20-30% in water, and virtually insoluble in lipids.

3.2. Chemical reactions occurring in foods
Interactions between propionic acid or propionates and food components have not been observed so far.

3.3. Storage and handling
Propionic acid is rarely used as such in the food industry. It is corrosive and should be handled with care. The sodium calcium and potassium salts need no special care other than protection from moisture. At high temperatures the salts decompose and emit acid fumes.

4. Antimicrobial activity (33, 34, 35)
As in the case of other acidic preservatives, only the undissociated propionic acid shows antimicrobial activity; the action of propionates is thus dependent upon the pH of the food to which they are added, although somewhat less so than in the case of sorbic acid. However, this is counter-balanced by the higher intrinsic antimicrobial activity of sorbic acid. Propionates have a rather limited antimicrobial spectrum; they have little effect on bacteria other than Bacillus mesentericus, which causes "ropiness" in bread. They do not strongly inhibit yeasts but are effective against moulds.

5. Foods in which used (7, 22, 37)

5.1 Bread and baked goods
The main field of application of propionic acid and its salts is in the prevention of mould spoilage and ropiness. High levels of propionates may inhibit the activity of yeast; this can be overcome by longer proofing times, by the addition of sugar or to a certain extent by the use of the less soluble calcium propionate. Propionates may confer a cheese-like flavour to foods, so that in practice only 0.1% of propionic acid (based on flour) or 0.3 - 0.5% calcium propionate or 0.5% sodium propionate are used. The use of
a bacterial culture producing 1.3% to 2.5% propionic acid has also been found effective against ropiness. Propionates are also used against mould spoilage and ropiness in other baked goods, such as rolls and cakes.

5.2 Dairy products

Propionic acid is a natural constituent of certain types of cheese. It is reported to reach a concentration of 1% in some types of Swiss cheese (7). 0.2 - 0.3% propionate has been found useful for the protection of processed cheese against mould spoilage (7).

6. Levels used in practice

<table>
<thead>
<tr>
<th>Product</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread and baked goods</td>
<td>0.12% - 0.5% (based on flour)</td>
</tr>
<tr>
<td>White bread</td>
<td>0.32%</td>
</tr>
<tr>
<td>Whole wheat bread</td>
<td>0.38%</td>
</tr>
<tr>
<td>Processed cheese</td>
<td>0.2% - 0.3%</td>
</tr>
<tr>
<td>Malt extract and syrups</td>
<td>0.2% - 0.4%</td>
</tr>
</tbody>
</table>
SODIUM DIACETATE

1. **Name**
   Sodium diacetate

2. **References**

2.1 **Reference to monographs on specifications**

2.2 **Reference to monographs on toxicology**

2.3 **Reference to Methods of Analysis in Foods**

3. **Some physical and chemical properties**

3.1 **Solubility**
   Sodium diacetate is readily soluble in water and soluble in vegetable oil, for instance, in sunflower oil to the extent of about 3%.

3.2 **Chemical reactions occurring in foods**
   Sodium diacetate will hydrolyse to its equilibrium pH in water and foods of relatively high water activity.

3.3 **Storage and handling**
   Sodium diacetate is comparatively stable if protected against moisture.

4. **Antimicrobial activity**
   The antimicrobial effect of sodium diacetate is similar to that of acetic acid, depending partly on the lowering of the pH and partly on the intrinsic effect of the acetic acid molecule. *Bacillus mesenterious* (which causes "ropiness" in bread) and *Staph. aureus* are controlled (7) at a level of 0.02 to 0.03% at pH 5.0-5.2; and killed by concentrations of 0.04% at pH 4.9. A lethal effect is exerted on

1/ See paragraph 6.1 of the report.
Saccharomyces cerevisiae and Aspergillus niger only at a concentration of 0.6% at pH 3.9. Since it lowers the pH of foods, sodium diacetate increases the activity of sorbic and benzoic acids.

5. Foods in which used

5.1 Bread and baked goods

Sodium diacetate is used at a level of 0.14% (based on flour) for rope and mould control in yeast-leavened and sour-dough bread and baked goods; it does not impair the activity of yeast at the amounts normally employed (37). Use in white bread and cakes is limited by its taste; in dark bread, levels of 0.2 - 0.4% have been used.

5.2 Cheese

Sodium diacetate has been used to protect cheese spread against mould growth; concentrations of 0.1 - 2.0% were found to be effective.

5.3 Salads, salad dressings, pickles

Sodium diacetate is also used for lowering the pH and imparting an acetic taste; levels range from 1 - 5% (2).

5.4 Other uses

Sodium diacetate is used as a dry form of vinegar in dry spice mixtures, herb specialties, soup mixtures, vegetables, sauces, fruit powders, meat and fish dishes. Quantities used range from 0.5 - 10%.

6. Levels used in practice

In bread, levels vary from 0.15 - 1.0% on flour.
SORBIC ACID AND SORBATES

1. **Names**
   Sorbic acid; 2,4- Hexadienoic acid.

2. **References**

2.1 **Reference to monographs on specifications**

2.2 **References to monographs on toxicology**

2.3 **Reference to Methods of Analysis in Food**

3. **Some physical and chemical properties.**
3.1 **Solubility (26)**

---

\[1/\] See paragraph 6.1 of the report.
### Table 1

**g per 100 g solvent**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Sorbic acid</th>
<th>Potassium sorbate</th>
</tr>
</thead>
<tbody>
<tr>
<td>water at 20°</td>
<td>0.16</td>
<td>138</td>
</tr>
<tr>
<td>water at 50°</td>
<td>0.6</td>
<td>150</td>
</tr>
<tr>
<td>water at 100°</td>
<td>3.9</td>
<td>175</td>
</tr>
<tr>
<td>ethanol 5% v/v</td>
<td>0.16</td>
<td>130</td>
</tr>
<tr>
<td>ethanol 20% v/v</td>
<td>0.3</td>
<td>120</td>
</tr>
<tr>
<td>ethanol 50% v/v</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>ethanol absolute</td>
<td>14.5</td>
<td>2</td>
</tr>
<tr>
<td>sodium chloride solution 5%</td>
<td>0.1</td>
<td>90</td>
</tr>
<tr>
<td>sodium chloride solution 10%</td>
<td>0.07</td>
<td>45</td>
</tr>
<tr>
<td>sucrose-solution 10%</td>
<td>0.14</td>
<td>132</td>
</tr>
<tr>
<td>sucrose-solution 50%</td>
<td>0.1</td>
<td>55</td>
</tr>
<tr>
<td>fats and oils</td>
<td>about 0.6-0.8</td>
<td>about 0.01</td>
</tr>
</tbody>
</table>

Calcium sorbate is practically insoluble in water, organic solvents, fats and oils.

#### 3.2 Chemical reactions occurring in foods

Interactions between sorbic acid and food components have not been observed so far. Studies on the stability of aqueous solutions of sorbic acid for periods up to one year showed some photoactivated decomposition (27, 28, 44).

#### 3.3 Storage and handling

Sorbic acid and sorbates must be protected from light and should be stored at temperatures below 40°. Containers should be moisture proof.

#### 4. Antimicrobial action (26)

Sorbic acid is almost exclusively effective in the form of the undissociated free acid. Hence, the antimicrobial effect depends largely on the pH of the environment. The percentage of undissociated acid varies from 98% at pH 3 to 9.6% at pH 7. Contrary to most other preservatives whose action also depends on the pH, sorbic acid is still reasonably effective in the pH range from 5 to 6. In this range, sorbic
acid is more effective than benzoic acid and propionic acids as is shown in table 2 of the section on esters of p-hydroxybenzoic acid. The antimicrobial effect of sorbic acid disappears below a certain minimum level, depending upon the type of micro-organism. Sub-inhibitory concentrations are metabolised by mould cultures and similar microbiological decomposition of sorbic acid has also been observed in wine. Some organisms, like Lactobacillaceae, clostridia and to a lesser extent, Staph. aureus are virtually insensitive. In emulsion foods, the undissociated portion of sorbic acid and its salts can partly be dissolved in both phases, according to the distribution coefficient. Temperature has no marked influence on the distribution coefficient because both solubilities are influenced similarly, nor have various types of lipids, so long as the fat-phase retains its original consistency. The addition of salt reduces the solubility of sorbic acid in the aqueous phase, changing the distribution coefficient considerably; sugars do not have this effect. Since the spoilage to be controlled by sorbic acid is located in the aqueous phase, the amount of undissociated preservative in the aqueous phase is of special interest. The antimicrobial effect of sorbic acid is increased by the addition of sodium chloride or sugar, although salt reduces the solubility of the undissociated acid. Certain amino acids, especially cystine and cysteine, as well as short chain fatty acids, exert a synergistic effect on the action of sorbic acid. Higher concentrations of malic, malonic and tartaric acids have a slight antagonistic effect. Condensed phosphates reinforce the antimicrobial effect of sorbic acid. Calcium and magnesium chlorides have a slight antagonistic influence. Fats and fatty acids above 5% also exert a slight antagonistic effect.

5. Foods in which used (7, 18, 26)

5.1 Cheese

Rindless Swiss cheese, matured in foil does not develop mould during the maturing process if the surface is treated with a 15 to 20% potassium sorbate solution. Cottage cheese
has been protected against mould spoilage with 0.05% to 0.075% sorbic acid depending upon pH, without inhibiting the bacteria necessary for maturation; to achieve this, 0.01 to 0.02% sorbate may be added to the milk. Sorbic acid is effective for preserving processed cheese under various storage conditions. Other milk products such as yoghurt may also be protected against unwanted moulds and yeasts by sorbic acid, without hindering the yoghurt fermentation.

5.2 Margarine

Sorbic acid has a comparatively favourable distribution coefficient for water/oil emulsions. For salt free margarine with pH values up to 5.7 levels of 0.03% to 0.1% have been suggested. If used in margarine containing milk potassium sorbate may be added to the extent of 0.04% to 0.07%.

5.3 Fish Products (31)

Sorbic acid has been used for the protection of dried or smoked fish against mould and yeast spoilage. Non-heat processed marinades have been preserved to a certain extent by the addition of 0.1% to 0.2% sorbic acid, although they are better preserved by mixtures of sorbic acid with benzoic acid, or p-hydroxybenzoic esters (see paras 5.5 and 5.1 under benzoic acid, and p-hydroxybenzoic acid respectively).

5.4 Wine (3, 39)

Sorbic acid in concentrations of 0.01% to 0.02% has been found suitable for stabilizing wines with residual sweetness. Because it does not inhibit oxidation nor the development of acetic acid and lactic acid bacteria, additional measures such as the use of sulphur dioxide or ascorbic acid are required. If the alcohol content is below 10 vol. % v/v the amounts of sorbic acid have to be increased. The level at which the flavour of sorbic acid can be detected depends upon the type of wine and varies from 0.015 to 0.04%.

5.5 Fruit and Vegetable Products

Dried prunes with a moisture content of about 35% have been protected against mould by dipping in a 5% solution of potassium sorbate, to give a final content of about 0.03% sorbic acid. In citrus juices the taste level for sorbic acid is of the order of 0.1%. Concentrations even below
this level may suffice for preservation, but do not prevent browning; for the latter purpose, and for protection against oxidation, sulphur dioxide addition is required (Lafuente Perriola 1963). In apple juice concentrate of 45° Brix, the addition of 0.04% sorbic acid delays the growth of osmophilic yeasts while the taste level was found to be 0.1% in single strength juice. Tomato juice has been preserved with 0.03% to 0.05% sorbic acid (Kalsevci 1960).

5.6 Sour Preserves - fermented fruit and vegetable products

The addition of sorbic acid at levels not exceeding 0.07% has been suggested for controlling the fermentation of sauerkraut and cucumbers. The addition of 0.03% of sorbic acid prevents surface mould of the brine in case of pickled olives.

5.7 Baked goods

Cakes have been protected against mould spoilage by 0.1% sorbic acid; lowering the pH improves the keeping quality but exerts an unfavourable effect on taste and structure. The antimicrobial effect of sorbic acid is 2 to 3 times higher than that of propionic acid. For yeast-raised goods the use of sorbic acid is limited by its effect on yeasts. This may be overcome by extended proofing periods. The inhibitory effect on yeast during proofing may be considerably decreased by using sorbyl palmitate, a mixed anhydride of sorbic and palmitic acids; during the baking process this compound is split into its components. The addition of 0.07% of sorbic acid, calculated on the flour content, protects wrapped sliced bread against mould for 2 weeks; higher concentrations give prolonged protection. (26)

5.8 Miscellaneous

Salted egg yolk (6% salt) has been preserved with 0.4% to 0.6% sorbic acid (41). Mayonnaise and delicatessen containing mayonnaise have also been treated against yeast and mould spoilage with sorbic acid. Where protection against lactic acid bacteria is required combinations of sorbic and benzoic acids are to be used.
5.9 Packing Material

In order to protect packing materials against mould, a treatment with a 5% to 10% solution of potassium sorbate has been used. For wrapping cheese, 2 to 4 g of sorbic acid per square meter of the material has been proposed; extensive work has been done on the migration of the preservative into cheese and margarine. When treated wrapping material is used, the concentration in the outer layers reaches 0.05% to 0.1%. For margarine and butter, 0.1 g/m² of sorbic acid in the wrappers suffices to prevent mould marks.

6. Levels used in practice

<table>
<thead>
<tr>
<th>up to 0.03%</th>
<th>up to 0.1%</th>
<th>up to 0.2%</th>
<th>&gt; 0.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>wine</td>
<td>cottage cheese</td>
<td>hard cheeses</td>
<td>liquid egg products</td>
</tr>
<tr>
<td>margarine</td>
<td>fruit pulps</td>
<td>fruit juices</td>
<td></td>
</tr>
<tr>
<td>dried fruits</td>
<td>fruit juices</td>
<td>bread</td>
<td></td>
</tr>
<tr>
<td>processed cheese</td>
<td>bakery products</td>
<td>confectionery</td>
<td></td>
</tr>
<tr>
<td>fruit juices</td>
<td></td>
<td>marinated fish</td>
<td></td>
</tr>
<tr>
<td>tomato juice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sauerkraut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>soft drinks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>marmalade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dried fish</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SULFUR DIOXIDE AND RELATED SUBSTANCES

1. Names
1.1 Sulfur dioxide, sulfurous acid anhydride
1.2 Sodium sulfite
1.3 Sodium hydrogen sulfite, sodium bisulfite, sodium acid sulfite
1.4 Sodium metabisulfite, sodium pyrosulfite
1.5 Potassium metabisulfite, potassium pyrosulfite

2. References
2.1 Reference to monographs and specifications

2.2 Reference to monographs on toxicology

2.3 References to methods of analysis in foods

1/ See paragraph 6.1 of the Report.
3. Physical and chemical properties

3.1 Solubility

Table 1 (12)
Solubility in water

<table>
<thead>
<tr>
<th>Substance</th>
<th>Solubility in g in 100 g solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur dioxide at 0°C</td>
<td>80 vol. in 1 vol</td>
</tr>
<tr>
<td></td>
<td>20°C 40 vol.</td>
</tr>
<tr>
<td></td>
<td>Sulfurous acid exists only in the aqueous solution.</td>
</tr>
<tr>
<td>Sodium sulfite anhydrous</td>
<td>25</td>
</tr>
<tr>
<td>Sodium sulfite heptahydrate</td>
<td>50</td>
</tr>
<tr>
<td>Sodium bisulfite</td>
<td>40</td>
</tr>
<tr>
<td>Sodium metabisulfite</td>
<td>50</td>
</tr>
<tr>
<td>Potassium sulfite</td>
<td>28.5</td>
</tr>
<tr>
<td>Potassium metabisulfite</td>
<td>40</td>
</tr>
</tbody>
</table>

3.2 Chemical Reactions occurring in foods (5, 11, 12, 49)

Sulfur dioxide reacts with aldehyde and ketone groups and is then no longer active against micro-organisms; on the other hand, by blocking these groups, SO₂ inhibits enzymic as well as non-enzymic browning. Owing to its reducing power, SO₂ protects ascorbic acid and other compounds against oxidation. Thiamine is decomposed by SO₂ but this effect is negligible at low pH values and low SO₂ concentrations.

3.3 Storage and handling

Sulfur dioxide is usually kept as liquified gas under pressure and dispensed with special equipment. The gas is highly irritating and poisonous, and appropriate care must be taken in its use. Sulfite salts are not very stable and should be kept at low temperature and protected from moisture. Elemental sulfur is sometimes used as a source of sulfur dioxide, particularly for burning in wine casks for the purpose of disinfecting them; care must be taken not to enter such casks before the SO₂ has completely disappeared.

4. Antimicrobial activity (9, 10, 40, 42, 43)

The undissociated forms of sulfurous acid have the maximum antimicrobial effect; active compounds are essentially the undissociated acid H₂SO₃ rather than the HSO₃⁻ ion. However,
the unstable dissociation equilibria make it difficult to determine the antimicrobial action of individual compounds. Sulfurous acid is more active against bacteria than against moulds or yeasts. Different strains react differently to sulfites; minimum inhibitory concentrations for various species have been compiled by Rehm and are given in Table 3. The antimicrobial action is stronger at low pH's, and also depends on the composition of the food. In addition sulfites display other properties useful in food processing such as inhibition of discoloration, oxidation and enzymatic and non-enzymatic browning.

5. Foods in which used (7, 12)

5.1 Wine

SO₂ obtained by burning sulfur has been used in wine making since antiquity for disinfecting the vessels, for preserving the freshly pressed juice during settling and clarification, and for controlling the fermentation process by protecting the yeasts against undesirable bacterial actions. The levels used vary considerably. Full preservation of pressed grapes or grape juice requires levels above 1000 mg/kg. Since sulfur dioxide disappears slowly as a result of evaporation and oxidation, the level has to be maintained by successive additions. More sulfur dioxide, 50 to 400 mg/kg, is later added to the finished wine in order to enhance its keeping quality, particularly for sweet wines containing residual sugar and lacking acidity and tannin. Much lower doses, 25 to 50 mg/kg, suffice to control undesirable bacteria during the alcoholic fermentation; most of the sulfur dioxide disappears eventually. Combined use of sorbic acid and sulfur dioxide may permit a substantially lower level of the latter; this possibility deserves more attention, if only on the grounds of palatability. Pasteurization has also been used with satisfactory results. For inhibition of non-enzymic browning reactions and oxidation degradation, doses between 15–25 mg/kg have been found sufficient.
5.2 Fruit pulps and purées, straight and concentrated fruit juices

Complete preservation requires high doses (above 1000 mg/kg) of sulfur dioxide, which render the food inedible as such; it is therefore only used for bulk preservation of raw materials to be subsequently processed into jams and jellies, fruit based beverages, etc. Straight fruit juices to be consumed as such are seldom preserved with sulfur dioxide; grape juice, on account of the long settling period which is required for the deposition of potassium bitartrate, is an exception; when so handled, it is "de-sulfited" before final packing for distribution. Complete elimination of residual sulfite from fruit pulps and juices is practically impossible; however the residual amounts are useful in the prevention of non-enzymic browning, although they may be objectionable from the points of view of flavour or canning: $SO_2$ accelerates corrosion and is reduced to $H_2S$ particularly in concentrates which are very prone to this type of deterioration.

Physical methods of preservation (pasteurization, cold storage, freezing, storage under carbon dioxide pressure) make the use of sulfur dioxide unnecessary. Combined use of sorbic or benzoic acid and $p$-hydroxybenzoic acid with sulfur dioxide is also a means of reducing the levels of the latter.

5.3 Cider, beer, vinegar

Sulfite in limited amounts (50-100 mg/kg) is sometimes used to protect these products from bacterial spoilage.

5.4 Sugars

Sulfites are often used at various steps in sugar manufacturing operations; residual sulfite may reach 50-75 mg/kg. Such sugars cannot be used in canned products.

5.5 Minced meat and fresh sausages (14, 25)

In some countries sodium sulfate equivalent to 300 to 450 mg/kg of sulfur dioxide is added to fresh minced meat and sausages in order to increase storage life under refrigeration. At lower doses, however, sulfites may not inhibit the proliferation of clostridia while maintaining the red colour; this practice can thus be misleading as to apparent freshness (6th Report, para 2.2.1 p. 96).
5.6 **Dried and dehydrated fruit and vegetables**

Fruits for drying have traditionally been treated with fumes of burning sulfur; vegetables are sprayed with sulfite solutions. The treatment aims both at protection against microorganisms and insects and at preserving a light colour. The high levels (1000 mg/kg and above) applied diminish during storage and transportation; as a consequence the products reaching the consumer usually contain 300–400 mg/kg.

5.7 **Pectin solutions**

Pectin solutions distributed in small containers for household use are sometimes preserved with high doses of sulfites. Preservations by heat treatment or other antimicrobial agents is possible. Dry pectin is now replacing pectin solutions.

5.8 **Other products**

Sulfur dioxide and related compounds are widely used both as antimicrobial agents and to prevent discoloration and oxidation, in spite of the fact that taste is sometimes adversely affected. Small amounts may therefore be encountered in many products, e.g. gelatin, potato products, glucose syrup, candied and glazed fruits, starch, pickles, etc. Strict attention to hygiene and use in conjunction with other preservatives and antioxidants, would allow the average sulfite load of foodstuffs to be reduced. In many cases, e.g. in fruit pulps and juices, jams and jellies, sulfites could be dispensed with entirely. Nevertheless sulfur dioxide is an efficient, versatile and very cheap preservative.

6. **Levels used in practice**

<table>
<thead>
<tr>
<th></th>
<th>mg/kg calculated as S\textsubscript{2}O\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit pulps and fruit juices for manufacturing purposes</td>
<td>1000–2000</td>
</tr>
<tr>
<td>Dried fruits and vegetables</td>
<td>500–1000</td>
</tr>
<tr>
<td>Pectin solutions</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 (cont'd).

<table>
<thead>
<tr>
<th>Concentrated fruit juices</th>
<th>Glucose syrup</th>
<th>Wine, beer, cider, vinegar</th>
<th>Gelatin</th>
<th>Potato products</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg calculated as SO₂</td>
<td>100-500</td>
<td>up to 100</td>
<td>up to 100</td>
<td>up to 100</td>
</tr>
</tbody>
</table>

Table 3

Minimal inhibitory concentration of sodium sulfate at pH 2.5-7 (43)

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>pH</th>
<th>Minimum inhibitory concentration mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>6.3</td>
<td>50</td>
</tr>
<tr>
<td>B. cereus</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>B. cohaerens</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>B. megatherium</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>B. plicatus</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>B. ubicuitarius</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>Corynebacterium nicotinovorans</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>6.0</td>
<td>200</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5.2</td>
<td>15</td>
</tr>
<tr>
<td>E. coli</td>
<td>5.9</td>
<td>80</td>
</tr>
<tr>
<td>E. coli</td>
<td>6.0</td>
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<td>100</td>
</tr>
<tr>
<td>E. coli</td>
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<td>200</td>
</tr>
<tr>
<td>E. coli</td>
<td>6.0</td>
<td>105</td>
</tr>
<tr>
<td>Lactobacillus arabinosus</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>L. casei</td>
<td>6.0</td>
<td>100</td>
</tr>
<tr>
<td>Micro-organism</td>
<td>pH</td>
<td>Minimum inhibitory concentration mg/kg</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>Ps. effusa</td>
<td>6.0</td>
<td>50</td>
</tr>
<tr>
<td>Ps. ovalis</td>
<td>6.0</td>
<td>100</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>6.0</td>
<td>100</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida krusei</td>
<td>5.0</td>
<td>160</td>
</tr>
<tr>
<td>C. pseudotropicalis</td>
<td>5.0</td>
<td>240</td>
</tr>
<tr>
<td>Hansenula anomala</td>
<td>5.0</td>
<td>240</td>
</tr>
<tr>
<td>Pichia membranaefaciens</td>
<td>6.0</td>
<td>2500</td>
</tr>
<tr>
<td>S. anamensis</td>
<td>5.0</td>
<td>240</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>4.0</td>
<td>125</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>4.0</td>
<td>125</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>5.0</td>
<td>80</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>6.0</td>
<td>1750</td>
</tr>
<tr>
<td>S. ellipsoideus</td>
<td>2.5</td>
<td>20</td>
</tr>
<tr>
<td>S. ellipsoideus</td>
<td>3.5</td>
<td>80</td>
</tr>
<tr>
<td>S. ellipsoideus</td>
<td>5.0</td>
<td>1400</td>
</tr>
<tr>
<td>S. ellipsoideus</td>
<td>7.0</td>
<td>&gt;500</td>
</tr>
<tr>
<td>S. spec (Johannesberg)</td>
<td>5.0</td>
<td>160</td>
</tr>
<tr>
<td>S. spec (Karlsberg)</td>
<td>5.0</td>
<td>160</td>
</tr>
<tr>
<td>S. spec (Hautes Sauternes)</td>
<td>5.0</td>
<td>160</td>
</tr>
<tr>
<td>S. spec (Tokayer)</td>
<td>4.2</td>
<td>203</td>
</tr>
<tr>
<td>Torula lipolytica</td>
<td>5.0</td>
<td>80</td>
</tr>
<tr>
<td>Willia anomala</td>
<td>6.0</td>
<td>2500</td>
</tr>
<tr>
<td>Zygosaccharomyces nussbaumii</td>
<td>4.0</td>
<td>200</td>
</tr>
<tr>
<td>Z. nussbaumii</td>
<td>4.0</td>
<td>200</td>
</tr>
<tr>
<td><strong>Moulds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>4.5</td>
<td>217</td>
</tr>
<tr>
<td>A. niger</td>
<td>5.0</td>
<td>350</td>
</tr>
<tr>
<td>A. niger</td>
<td>6.0</td>
<td>1250</td>
</tr>
<tr>
<td>Trichoderma lignorum</td>
<td>5.0</td>
<td>200</td>
</tr>
<tr>
<td>Fusarium spec</td>
<td>5.0</td>
<td>160</td>
</tr>
</tbody>
</table>
Table 3 (cont.d)

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>pH</th>
<th>Minimum inhibitory concentration mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oliocladium roseum</td>
<td>5.0</td>
<td>&gt;400</td>
</tr>
<tr>
<td>Mucor spec.</td>
<td>2.5</td>
<td>30</td>
</tr>
<tr>
<td>M. spec.</td>
<td>3.5</td>
<td>60</td>
</tr>
<tr>
<td>M. spec.</td>
<td>7.0</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Penicillium glaucum</td>
<td>4.5</td>
<td>280</td>
</tr>
<tr>
<td>P. glaucum</td>
<td>6.0</td>
<td>1250</td>
</tr>
<tr>
<td>P. spec.</td>
<td>2.5</td>
<td>20</td>
</tr>
<tr>
<td>P. spec.</td>
<td>3.5</td>
<td>60</td>
</tr>
<tr>
<td>P. spec.</td>
<td>5.0</td>
<td>160</td>
</tr>
<tr>
<td>P. spec.</td>
<td>7.0</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>
1. **Names**

Diethylpyrocarbonate, diethyldicarbonate, pyrocarbonic acid diethylester.

2. **References**

2.1.1 **References to Monographs on specifications**


2.2 **References to monographs on toxicology**


2.3 **Reference to Methods of Analysis in Foods**

3. **Some physical and chemical properties** (15, 17, 36)

3.1 **Solubility**

Diethylpyrocarbonate is but sparingly soluble in water; special devices (metering pumps, pulverizers) are necessary for mixing it with aqueous products.

3.2 **Chemical reactions occurring in foods**

Diethylpyrocarbonate is very rapidly hydrolyzed in water to ethanol and carbon dioxide; in ethanol, alcoholysis leads to carbon dioxide and diethylcarbonate, which then reacts with various compounds which may be present e.g. amines, amino acids, sulphydryl groups, phenolic compounds, and alcohols yielding the corresponding carbethoxy compounds. As much as

\[1/\] See paragraph 6.1 of the Report.
35% DEPC can react in this way, e.g. in beer. Although the carboxy compounds also undergo hydrolysis, they are much more stable than DEPC.

3.3 Storage and handling

Diethylpyrocarbonate is extremely irritating to the eyes, lungs, mucous membranes and skin; great care should be taken in using it. It keeps unchanged in unopened containers, but exposure to moisture or contact with other substances leads to decomposition with the evolution of carbon dioxide. A violent exothermic reaction may result on exposure to aluminium or iron dust, amines and ammonia.

4. Antimicrobial Action

Because DEPC decomposes so quickly in aqueous products, it acts as a chemical sterilisant rather than as a microbial inhibitor. The effect depends on microbial species and pH; the preservative should only be used in foods of a pH below 4.0. The initial microbial load should not be higher than 500 to 1000/ml. Very good conditions of hygiene are therefore necessary where DEPC is used.

5. Foods in which used (7, 15)

5.1 Wine

Diethylpyrocarbonate at doses of 50 to 150 mg/kg is used to stabilize wines which have a tendency to secondary fermentation particularly those with a lower alcohol content and containing residual sugars. The use of DEPC may help to reduce the amount of sulphur dioxide, traditionally used as a preservative for wine, to the levels necessary for preventing oxidation.

5.2 Beer

Diethylpyrocarbonate has been used for preserving beer. It is however not effective against sarcina and lactic acid bacteria; moreover, its efficacy depends upon the pH and protein content of the beer.

5.3 Soft drinks

Both carbonated and non-carbonated beverages have been sterilized with DEPC using 50-80 mg/kg for the former and
180–200 mg/kg for the latter. However, previous flash pasteurization or filtration have been recommended so as to insure a low initial microbial load; carbonation may by itself be sufficient to insure the keeping quality of carbonated beverages.

5.4 Fruit juices
Diethylpyrocarbonate may be used for sterilizing fruit juices; doses range from 100 to 250 mg/kg (30); a low initial microbial count is imperative and flash pasteurization alone may be sufficient. DEFC has been reported to induce malo-lactic fermentation in fruit juice. Small amounts (20–30 mg/kg) of sulfur dioxide or ascorbic acid enhance the action of DEFC.

5.5 Other uses
Diethylpyrocarbonate has been used, or suggested, for the preservation of fruit pulp and juice intended for fermentation; for the control of fermentation of sake; for freeing enzyme preparations from viable organisms; and as a 1% alcoholic solution for dipping strawberries and wiener sausages to increase their keeping quality.

6. Levels used in practice

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Level (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wine</td>
<td>50–150</td>
</tr>
<tr>
<td>Carbonated soft drinks</td>
<td>50–80</td>
</tr>
<tr>
<td>Non-carbonated soft drinks</td>
<td>100–200</td>
</tr>
<tr>
<td>Fruit juices</td>
<td>100–250</td>
</tr>
</tbody>
</table>
ANNEX I

POTASSIUM BENZOATE

CHEMICAL NAME
Potassium benzoate; potassium salt of benzene-carboxylic acid.

EMPirical FORMULA
C_7H_5O_2K. 3H_2O

STRUCTURAL FORMULA

\[
\begin{align*}
\text{COOK} \\
\text{\( \cdot 3H_2O \)}
\end{align*}
\]

MOLECULAR WEIGHT
214.27

DEFINITION
Potassium benzoate contains not less than 99% C_7H_5O_2K after drying at 105\(^\circ\)\text{C}, and conforms to the following specifications.

DESCRIPTION
White crystalline powder

USES
as an antimicrobial agent.

IDENTIFICATION TESTS

A. Solubility
Water: freely soluble.
Ethanol: soluble.

B. A 10% solution of potassium benzoate in water yields a buff-coloured precipitate with ferric chloride T.S.

C. Melting range: Acidify a 2% solution of potassium benzoate with dilute hydrochloric acid T.S. Collect precipitate on a filter paper. Wash free of chloride with water and dry at 105\(^\circ\)\text{C} for 1 h. Its melting range is 121.50-123.50.

D. Acidify a 10% solution of potassium benzoate with dilute hydrochloric acid T.S. and filter. Make the filtrate neutral with sodium hydroxide T.S. and add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.
Acidity and alkalinity: 2 g of potassium benzoate dissolved in 20 ml of water, require for neutralization not more than 0.5 ml of either 0.1 N sodium hydroxide or 0.1 N hydrochloric acid, using phenolphthalein T.S. as indicator.

Polycyclic acids: Dissolve 10 g of potassium benzoate in water, neutralize if necessary, using phenolphthalein T.S. and adjust the volume to 100 ml. Add 4 ml of 0.1 N hydrochloric acid, dissolve the precipitate by heating and allow to stand for 12 h. Collect the precipitate on a filter paper, wash free of chloride with water and dry at 105° for 1 h. The melting range of the dried precipitate is 121.5°-123.5°.

Chlorinated organic compounds: Dissolve 0.25 g of potassium benzoate in 10 ml of water. Acidify with nitric acid and filter off the precipitate. Mix the precipitate with 0.5 g of calcium carbonate, dry the mixture and then ignite. Dissolve the ignition residue in 20 ml of dilute nitric acid T.S. and filter. Mix the solution with 0.5 ml of 0.1 N silver nitrate. The turbidity should be not more than that obtained in a similar volume of water by addition of 0.5 ml of 0.1 N silver nitrate and 0.5 ml of 0.01 N hydrochloric acid.

Arsenic: Not more than 3 mg/kg

Heavy metals: Not more than 20 mg/kg.
ASSAY

Weigh accurately 2.5 to 3 g of potassium benzoate previously dried at 105° and dissolve in 50 ml of water. Neutralize the solution, if necessary, with 0.1 N hydrochloric acid, using phenolphthalein T.S. as indicator. Add 50 ml of ether and a few drops of bromophenol blue T.S. and titrate with 0.5 N hydrochloric acid, shaking constantly, until the colour of the indicator begins to change. Separate the lower layer, wash the ethereal layer with 10 ml of water, and to the separated aqueous layer add the washings and an additional 20 ml of ether. Complete the titration with the 0.5 N hydrochloric acid, shaking constantly. Each ml of 0.5 N hydrochloric acid is equivalent to 80.11 mg of C₇H₅O₂K.
ANNEX II

PROPIONIC ACID

CHEMICAL NAMES
Propionic acid; Propanoic acid.

EMPIRICAL FORMULA
C₃H₆O₂

STRUCTURAL FORMULA
CH₃CH₂COOH

MOLECULAR WEIGHT
74.08

DEFINITION
Propionic acid contains not less than 99.5% C₃H₆O₂ and conforms to the following specifications.

DESCRIPTION
Propionic acid is a liquid with a slightly pungent odour.

USES
As mould inhibitor.

IDENTIFICATION TESTS

A. Solubility
Water: miscible.
Ethanol: miscible.

B. Melting point: -22°C
C. Boiling point: 141°C

PURITY TESTS

Distillation range: Between 138.5°C and 142.5°C
Non-volatile residue: Not more than 0.01% when dried at 140°C to constant weight.

Readily oxidizable substances:
Dilute 2 ml propionic acid in a glass-stoppered container with 10 ml of water and add 0.1 ml of 0.1 N potassium permanganate. The pink colour is not changed to brown within 30 min.
Arsenic: Not more than 3 mg/kg
Heavy metals: Not more than 10 mg/kg
Aldehydes: Not more than 0.1%, calculated as formaldehyde.

ASSAY

Mix about 3 g propionic acid accurately weighed, with water in a 250-ml Erlenmeyer flask, add phenolphthalein T.S., and titrate with 1 N sodium hydroxide to the first appearance of a faint pink endpoint which persists for at least 30 seconds. Each ml of 1 N sodium hydroxide is equivalent to 74.08 mg of C₃H₆O₂.
ANNEX III

CALCIUM SORBATE

CHEMICAL NAME Calcium sorbate, calcium salt of trans-trans-2-4-hexadianoic acid.

EMPIRICAL FORMULA C₁₂H₁₄CaO₄

STRUCTURAL FORMULA

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

MOLECULAR WEIGHT 262.32

DEFINITION Calcium sorbate contains not less than 90% and not more than the equivalent of 10.2% of C₁₂H₁₄CaO₄ after drying for 4 hours in a dessicator over sulfuric acid and conforms to the following specifications.

DESCRIPTION Fine white crystalline powder not showing any change in colour after 90 minutes heating at 105°.

USES As an antimicrobial preservative and fungistatic agent.

IDENTIFICATION TESTS

A. Solubility

Water: Soluble.
Ethanol: Almost insoluble.

B. Melting range of sorbic acid: Acidify a calcium sorbate solution with dilute hydrochloric acid T.S. Collect the precipitate on a filter paper, wash it free of chloride with water and dry it under vacuum over concentrated sulfuric acid. Its melting range is 130° - 135°.
C. To a saturated solution of calcium sorbate add ammonium oxalate T.S. The white precipitate formed is soluble in hydrochloric acid, but insoluble in acetic acid.

D. To 2 ml of a 1 in 10 solution add a few drops of bromine T.S. The colour of the bromine disappears.

**PURITY TESTS**

**Loss on drying:** Not more than 3% (by drying in a dessicator over sulfuric acid for 4 h.)

**Colour stability:** No change in colour when heated for 90 min. at 105°.

**Aldehydes:** Prepare a 0.3% solution of calcium sorbate. In a test tube add 5 ml of this solution to 2.5 ml of Schiff's reagent T.S. and allow to stand for 10 - 15 min. Compare the colour with that produced by 5 ml of formaldehyde solution (containing 15 µg) with the same amount of Schiff's reagent under the same conditions. The colour of the test solution should not be more intense than that of the formaldehyde solution, corresponding to not more than 0.1% of aldehydes. (Calculated as formaldehyde).

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

**Heavy metals:** Not more than 10 mg/kg.

**ASSAY**

Dissolve about 250 mg calcium sorbate, previously dried for 4 h. in a dessicator over sulfuric acid and accurately weighed, in 36 ml of glacial acetic acid and 4 ml of acetic anhydride in 250 ml glass-stoppered flask, warming to effect solution. Cool to room temperature, add 2 drops of crystal violet T.S. and titrate with 0.1 N perchloric acid in glacial
acetic acid to a blue-green end point which persists for at least 30 sec. Perform a blank determination and make any necessary correction. Each ml of 0.1 M perchloric acid is equivalent to 26.23 mg of $\text{C}_{12}\text{H}_{14}\text{O}_4$.
ANNEX IV

POTASSIUM SORBATE

CHEMICAL NAMES
Potassium sorbate; Potassium salt of trans, trans-2,4 hexadienoic acid.

EMPIRICAL FORMULA
C₆H₇O₂K

STRUCTURAL FORMULA

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

MOLECULAR WEIGHT
150.22

DEFINITION
Potassium sorbate contains not less than 90% and not more than the equivalent of 102% of C₆H₇O₂K and conforms to the following specifications.

DESCRIPTION
Potassium sorbate occurs as white or yellowish-white crystals or crystalline powder.

USES
As an antimicrobial agent.

IDENTIFICATION TESTS

A. Solubility
Water: freely soluble (1 g in 1 ml)
Ethanol: soluble

B. Melting range of sorbic acid:
Acidify a potassium sorbate solution with diluted hydrochloric acid T.S. Collect the precipitate on a filter paper, wash it free of chloride with water and dry it under vacuum over sulfuric acid. The melting range is 130° - 135°.

C. To a 1% solution of potassium sorbate add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is
formed.

D. To 2 ml of a 1 in 10 solution add a few drops of bromine T.S. The colour of the bromine is discharged.

**PURITY TESTS**

**Alddehydes:** Prepare a 0.3% solution of potassium sorbate. In a test tube, add 5 ml of this solution to 2.5 ml of Schiff's reagent T.S. and allow to stand for 10 – 15 min. Compare the colour with that produced by 5 ml of formaldehyde solution (containing 15 μg) with the same amount of Schiff's reagent under the same conditions. The colour of the test solution should not be more than that of the formaldehyde solution, corresponding to not more than 0.1% of aldehydes (calculated as formaldehyde).

**Loss on drying:** Not more than 1% (by drying in a dessicator over sulfuric acid for 4 h.)

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

**Heavy metals:** Not more than 10 mg/kg

**ASSAY**

Dissolve about 250 mg accurately weighed in 36 ml of glacial acetic acid and 4 ml acetic anhydride in 250 ml glass-stoppered conical flask, warming to effect solution. Cool to room temperature, add 2 drops of crystal violet T.S. and titrate with 0.1 N perchloric acid in glacial acetic acid to a bluegreen end point which persists for at least 30 seconds. Perform a blank determination and make any necessary correction. Each ml of 0.1 N perchloric acid is equivalent to 15.02 mg of C₆H₇NO₂.
ANNEX V

POTASSIUM METABISULFITE

CHEMICAL NAMES
Potassium metabisulfite; potassium pyrosulfite; potassium disulfite.

EMPirical FORMULA
$K_2S_2O_5$

MOLECULAR WEIGHT
222.33

DEFINITION
Potassium metabisulfite contains not less than 90% of $K_2S_2O_5$ and conforms to the following specifications:

DESCRIPTION
Potassium metabisulfite occurs as colourless free-flowing crystals, crystalline powder, or granules, usually having an odour of sulfur dioxide.

USES
As an antimicrobial and antibrowning agent.

IDENTIFICATION TESTS

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

B. To 1 volume of 1% solution of potassium metabisulfite add 1 volume of saturated sodium hydrogen tartrate solution and 1 volume of ethanol and shake. A white crystalline precipitate is formed.

C. To potassium metabisulfite add dilute hydrochloric acid T.S. A strong odour of sulfur dioxide is detected.

PURITY TESTS

Selenium: Not more than 30 mg/kg on the basis of sulfur dioxide content. (see under "Examination").

Arsenic: Not more than 3 mg/kg.

Heavy metals: Not more than 10 mg/kg.
ASSAY

Weigh accurately about 250 mg of potassium metabisulfite add it to 50 ml (measured accurately) of 0.1 N iodine solution contained in a glass-stoppered flask and stopper the flask. Allow to stand for 5 min., add 5 ml of dilute hydrochloric acid T.S. and titrate the excess iodine with 0.1 N sodium thiosulfate, using starch T.S. as the indicator. Each ml of 0.1 N iodine is equivalent to 5.558 mg of K₂S₂O₅.

EXAMINATION

Selenium
Selenium Stock Solution: Transfer 120.0 mg of metallic selenium (Se) into a 1000 ml volumetric flask, add 100 ml of dilute nitric acid (1 in 2), warm gently on a steam bath to effect solution and dilute to volume with water. Transfer 5.0 ml of this solution into a 200 ml volumetric flask, dilute to volume with water and mix. Each ml of this solution contains 3 μg of selenium.

Standard solution: On the day of use, transfer 20.0 ml of Selenium Stock Solution (60 μg/Se) into a 200 x 25 mm test tube and add 20 ml of hydrochloric acid.

Sample Solution: Transfer 3.5 g of the sample into a 250 ml conical flask and cautiously add 10 ml of 30% hydrogen peroxide. After the initial reaction has subsided, add 6 ml of 70% perchloric acid, and heat slowly until white fumes of perchloric acid are copiously evolved and continue heating gently for a few minutes to ensure decomposition of excess peroxide. If the solution is brownish in colour due to undecomposed organic matter, add a small portion of the peroxide solution and heat again to white perchloric acid fumes, repeating if necessary until the decomposition of organic matter is complete and a colourless solution is obtained. Cool, add 10 ml of water and filter with hot water until the filtrate measures 20 ml and add 20 ml of hydrochloric acid and mix.
Procedure: Place the test tubes containing the Standard Solution and the Sample Solution in a water bath and heat until the temperature of the solutions reaches 40°. To each tube add 400 mg of ascorbic acid, stir until dissolved and maintain both at 40° for 30 min. Cool the solutions, dilute with water to 50 ml and mix. Any pink colour produced should not exceed that produced by the standard.
CHEMICAL NAME: Diethyl pyrocarbonate.

EMPIRICAL FORMULA: C₆H₁₀O₅

STRUCTURAL FORMULA: 

![Structural formula diagram]

MOLECULAR WEIGHT: 162.14

DEFINITION: Diethyl pyrocarbonate contains not less than 99.0% C₆H₁₀O₅ and conforms to the following specifications.

DESCRIPTION: Diethyl pyrocarbonate is a colourless liquid with a slightly fruity ester-like odour.

CAUTION: Avoid inhalation of vapours and exposure to eyes, skin and mucous membranes.

USES: As an antimicrobial preservative.

IDENTIFICATION TESTS

A. Solubility
   Water: Slightly soluble, with decomposition.
   Ethanol: Miscible.

B. To a solution of 1 ml ethylenediamine in 3 ml acetone add slowly 2 ml diethylpyrocarbonate and shake gently. The mixture becomes warm and a viscous oil is deposited. The warm upper layer is filtered and yields, after cooling, crystals of diethyl ethylenedicarbamate, mp 110°. After addition of a few ml acetone to the remaining oil, crystals of (2-aminoethyl) carbonic acid form, mp 165° in a closed capillary tube; sublimes at about 160° in an open tube.
PURITY TESTS

Relative density 20°/20°: 1.12

Boiling point: 155° with decomposition

Refractive index n_25^D: 1.395 to 1.398

Non volatile residue: Not more than 0.05% when heated in a bath at 160°-180°.

Arsenic: Not more than 3 mg/kg

Heavy metals: Not more than 10 mg/kg

ASSAY

Solutions: Morpholine Solution: Dilute 44 ml of redistilled morpholine to 1000 ml with methanol.

Indicator Solution: Dissolve 1.0 g of methyl yellow (4-dimethylaminoazobenzene) and 100 mg of methylene blue (3,7-bis(dimethylamino phenazathonium chloride) in 125 ml of methanol.

0.5N Methanolic Hydrochloric Acid: Dilute 84 ml of 6 N hydrochloric acid to 1000 ml with methanol. Standardize daily against 0.5 N sodium hydroxide, using phenolphthalein T.S. as indicator.

Procedure: Transfer about 1 g (approx. 1 ml) of the sample, accurately weighed, into a 125-ml glass-stoppered Erlenmeyer flask. Carefully pipet 25-ml of the morpholine solution into the flask, shake carefully and let stand for 5 min. Add 4 or 5 drops of the Indicator Solution and titrate with 0.5 N methanolic hydrochloric acid to the disappearance of the green colour. Perform a blank determination and make any necessary corrections. Each ml of 0.5 N methanolic hydrochloric acid is equivalent to 81.07 mg of C_6H_{10}O_5.
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