Growth and Inhibition of Microorganisms in the Presence of Sorbic Acid: A Review

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ABSTRACT

Sorbate (sorbic acid) generally is an effective inhibitor of most molds and yeasts and some bacteria. Environmental factors such as pH, water activity, temperature, atmosphere, microbial load, microbial flora and certain food components can influence the effectiveness of sorbate. Strains of microorganisms resistant to sorbate exist and therefore are common causes of food spoilage. Some molds and bacteria are able to degrade sorbate. This paper reviews the factors that affect the antimicrobial effectiveness of sorbate in foods.

Sorbic acid was first isolated from the pressed unripened berry of the rowan or mountain ash tree in 1859 by the German chemist A. W. Hoffmann (6). It was not until 1939, however, that E. Müller and C. M. Gooding, working independently in Germany and the United States, respectively, discovered the antimicrobial activity of sorbic acid (95). In 1945, Gooding was awarded a U.S. patent for use of the antifungal properties of sorbic acid (64), and since the mid-1950s it has been used to a growing extent throughout the world to protect a variety of foods against deterioration by microorganisms.

REGULATORY STATUS

Because sorbic acid was not used as a food preservative until a time when extensive toxicological investigations were required for new food additives, it is among the most thoroughly investigated of all preservatives. It was found harmless in numerous acute, subchronic and chronic toxicity tests (52, 61, 94). Sorbic acid is metabolized in the body like other fatty acids. In humans and animals, it is subject to β-oxidation typical of fatty acid degradation. The half-life in the body is 40-110 min, depending on the dosage, and under normal conditions it is completely oxidized to CO₂ and H₂O (53, 95).

Use of sorbic acid in foods is permitted in most countries which regulate their food supply (95). The maximum permissible level, other than in exceptional situations, is between 0.1 and 0.2%. In the U.S., sorbic acid is a GRAS substance and its use is permitted in any food product to which preservatives may be added. No upper limits are imposed for foods not covered by Federal Standards of Identity. Thus, in natural cheese, the maximum quantity may not exceed 0.3% by weight, calculated as sorbic acid, and the maximum is set at 0.2% in pasteurized blended cheese, pasteurized cheese spread, cold pack cheese and cheese food and spread (39).

APPLICATIONS

Sorbic acid is a straight chain α, β-unsaturated monocarboxylic acid and has the structure

CH₃CH=CHCH=CHCOOH (179). The carboxyl group reacts readily and forms salts and esters. The salts are important in food applications because of their high solubility in water. Potassium sorbate was specifically developed to prepare the aqueous stock solutions needed for dip, spray and metering applications (36, 65). Sorbic acid is only slightly soluble in water; however, it is more soluble in fats than potassium sorbate. Sorbic acid can be incorporated with dry materials by mixing with salt, flour or corn starch. It can be solubilized with sodium or potassium hydroxide, or it may be dissolved in propylene glycol or ethanol for use in dips or sprays (36). Since sorbic acid sublimes upon heating, it should be added to foods only after any prolonged boiling or heating that may be employed in food processing (6, 36).

Sorbic acid and its potassium salt are the most widely used forms of the compound and are collectively known as sorbates. Their most common use is preservation of food, animal feed, cosmetic and pharmaceutical products as well as technical preparations that come in contact with food or the human body. Methods of application include: direct addition into the product; dipping, spraying, or dusting of the product; or incorporation into the wrapper (6, 7, 158).

Typical use levels in foods range from 0.02% in wine and dried fruits to 0.3% in some cheeses (Table 1). Foods in which sorbate has commercially useful antimicrobial activity include baked goods (cakes and cake mixes, pies and pie fillings, doughnuts, baking mixes, fudges, icings), dairy products (natural and processed...
TABLE 1. Typical concentration (%) of sorbic acid used in various food products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheeses</td>
<td>0.2-0.30</td>
</tr>
<tr>
<td>Beverages</td>
<td>0.03-0.10</td>
</tr>
<tr>
<td>Cakes and pies</td>
<td>0.05-0.10</td>
</tr>
<tr>
<td>Dried fruits</td>
<td>0.02-0.05</td>
</tr>
<tr>
<td>Margarine (unsalted)</td>
<td>0.05-0.10</td>
</tr>
<tr>
<td>Mayonnaise</td>
<td>0.10</td>
</tr>
<tr>
<td>Fermented vegetables</td>
<td>0.05-0.20</td>
</tr>
<tr>
<td>Jams and jellies</td>
<td>0.05</td>
</tr>
<tr>
<td>Fish</td>
<td>0.03-0.15</td>
</tr>
<tr>
<td>Semi-moist pet food</td>
<td>0.1-0.3</td>
</tr>
<tr>
<td>Wine</td>
<td>0.02-0.04</td>
</tr>
<tr>
<td>Fruit juices</td>
<td>0.05-0.20</td>
</tr>
</tbody>
</table>

cheeses, cottage cheese, sour cream, yogurt), fruit and berry products (artificially sweetened confections, dried fruits, fruit drinks, jams, jellies, wine), vegetable products (olives, pickles, relishes, salads) and other miscellaneous food products (certain fish and meat products, mayonnaise, margarine, salad dressings) (6, 108).

ENVIRONMENTAL FACTORS AFFECTING EFFECTIVENESS OF SORBATE

Environmental factors such as pH, water activity (a_w), temperature, atmosphere, initial microbial load, type of microbial flora, and certain food components, singly or in combination, can influence the antimicrobial activity of sorbate. Together with preservatives such as sorbic acid, they often act to broaden antimicrobial action or increase it synergistically. Use of other preservatives in combination with sorbate can also broaden or intensify antimicrobial action. The length and temperature of storage are other important considerations. If growth of spoilage or pathogenic microorganisms is inhibited, but the microorganisms are not killed, growth will eventually resume under proper conditions. The length of inhibition will vary with storage temperature as well as with any of the other factors discussed.

Effects of pH

Organic acids such as sorbic acid dissociate in aqueous solutions and release hydrogen ions. Some food preservatives such as acetic acid act mainly by lowering the pH of the environment to the point where microorganisms can no longer grow. Although the undissociated form of acetic acid has antimicrobial action, it is primarily the hydrogen ion and the resulting decrease in pH that inhibits microorganisms (45, 91).

With sorbic acid and other organic acids, it is the undissociated molecule that provides antimicrobial activity (45, 95, 99). The amount of the molecule in the undissociated form is determined by pH. This, along with solubility properties, determines the foods in which organic acids may effectively be used (135). The antimicrobial effectiveness of sorbate increases as the pH value approaches its dissociation constant (pKa), which is 4.75. At this pH value, 50% of sorbic acid is in the effective undissociated form (161) (Table 2). Therefore, sorbate is more effective in foods with low rather than high pH values (6, 7, 11, 14, 95, 161). The upper pH limit for activity of sorbate is 6.0-6.5, while those for propionate and benzoate are 5.0-5.5 and 4.0-4.5, respectively. Many investigators have demonstrated the importance of using sorbate at proper pH values. For example, Park and Marth (119) showed that Salmonella typhimurium would grow in nutrient broth (pH 6.7) or skim milk (pH 6.4) fortified with 0.3% sorbic acid when the media were not acidified. However, when the pH was reduced to 5.0, growth did not occur in either medium and, in time, a major portion of the population was inactivated.

TABLE 2. Effect of pH on sorbic acid dissociation.

<table>
<thead>
<tr>
<th>pH</th>
<th>Undissociated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.00</td>
<td>0.6</td>
</tr>
<tr>
<td>6.00</td>
<td>6.0</td>
</tr>
<tr>
<td>5.80</td>
<td>7.0</td>
</tr>
<tr>
<td>5.00</td>
<td>37.0</td>
</tr>
<tr>
<td>4.75 (pKa)</td>
<td>50.0</td>
</tr>
<tr>
<td>4.40</td>
<td>70.0</td>
</tr>
<tr>
<td>4.00</td>
<td>86.0</td>
</tr>
<tr>
<td>3.70</td>
<td>93.0</td>
</tr>
<tr>
<td>3.00</td>
<td>98.0</td>
</tr>
</tbody>
</table>

*From Sofos and Busta (148).*

Water activity

Addition of substances to food that reduce its a_w have a beneficial effect on the action of antimicrobial preservatives (10). The most important substances to consider are salt and sugar (87). Both intensify the action of sorbate primarily by reducing a_w. They may also induce cellular swelling, which makes many microorganisms more susceptible to action of preservatives (95). Costilow et al. (42-44) noted that salt (NaCl) increased the effectiveness of sorbate in inhibiting spoilage yeasts in cucumber fermentations. Sheneman and Costilow (155) saw the same effect with sucrose in sweet cucumber pickles. Robach and Stateler (148) found that certain combinations of NaCl and sorbate resulted in a synergistic inhibition of Staphylococcus aureus. Acott et al. (1) showed that sucrose (a_w =0.85) and sorbate in combination inhibited growth of Aspergillus glaucus and Aspergillus niger and slowed the growth of Staphylococcus epidermidis. At an a_w of 0.88, all three organisms were able to grow, but at a slower rate than in the absence of sorbate. Gooding et al. (65) indicated that salt and glucose have a marked synergistic effect on fungistasis by sorbic acid. In high sugar systems, increased antimicrobial activity occurred even at pH values above 6.5. In contrast, Beuchat (16) reported that the presence of sucrose or NaCl can, in some instances, reduce the synergistic effect of heat and sorbate on inactivation of molds.

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Temperature

Sorbate in combination with low temperature increased the storage life of grape juice (121). The combination of sorbate and a mild heat treatment greatly extended the shelf-life of fruit products (20, 150). Beuchat (16-19, 21) has shown that sorbate synergistically enhances cellular injury during heating of several yeast and mold strains. Shibasaki and Tsuchido (156) found that sorbate increased sensitivity of A. niger to heat but had no effect on Penicillium thomii. Beuchat (16, 17) demonstrated that NaCl and sucrose can impart greater resistance to heat, probably because cells are dehydrated, but regardless of solute concentration sorbate treatment increased the sensitivity of microorganisms to heat. Sorbate also retards or prevents repair of thermal injury in bacteria and yeasts (19, 156). Tsuchido et al. (175) found that sorbate did not inhibit recovery of nucleic acid synthesis in thermally injured cells of Candida utilis, but it did inhibit recovery of protein synthesis and respiratory activity. Generally, temperatures outside the optimal growth range for the microorganism in question will increase the effectiveness of sorbate in inhibiting growth.

Atmosphere

Several investigators have shown that CO\(_2\) and sorbate in combination will effectively inhibit microorganisms. Elliott et al. (55, 56) showed that CO\(_2\) and sorbate acting synergistically inhibited Salmonella enteriditis and S. aureus. Carbon dioxide and sorbate will inhibit mold spoilage of grains (48). Sorbate will delay the growth of psychrotrophic bacteria in vacuum-packaged pork loins (111).

Microbial flora

The numbers and variety of microorganisms present in a food to be preserved will affect the ability of sorbate to prevent microbial growth and spoilage. Experiments have shown that the lower the initial microbial load, the longer sorbate will afford protection by preventing growth of the microbes (6, 65). A low initial microbial count allows microorganisms to be inhibited in the initial lag phase of growth and not in the exponential (log) phase where the microorganisms will multiply exponentially. Therefore, in fat/water emulsions a relatively high proportion of sorbate remains in the microbiologically susceptible water phase. This property makes sorbate useful for preservation of margarine, mayonnaise and salad dressings. However, an increase in fat content of a product will lower the amount of sorbate in the aqueous phase where it is needed for microbial control (116). Soluble components such as salts and sugars will also decrease the amount of sorbate in the aqueous phase (65). This effect may be offset, however, by the synergistic action of sorbate and salt or sugar to give an increased antimicrobial effect (1, 16, 42-44, 63, 148, 155). Some acids decrease the solubility of sorbate in water (65), but acids also enhance antimicrobial activity by increasing the proportion of sorbate in the active undissociated form. Citric and lactic acids potentiate the antimicrobial action of sorbate to a greater extent than do inorganic acids (140, 141, 159). Acetic acid, which is often used in conjunction with sorbate in mayonnaise and salad dressings, decreases the fat-to-water ratio of sorbate partition (65). Food ingredients that have antimicrobial activity by themselves, such as spices, can increase the spectrum or intensity of activity of sorbate.

Preservative interactions

Mixtures of preservatives may reinforce or reduce their separate growth-inhibitory activities. They may work synergistically, additively or antagonistically with one another, as illustrated in Fig. 1. As an example, if two compounds are combined in this preservative system and they react synergistically, only 40% of the minimum inhibitory concentration (MIC) of each compound is required to give 100% inhibition of the test microorganism. Conversely, in an antagonistic relationship, 50% of the MIC of one compound and 85% of the MIC of the other compound are required to achieve 100% inhibition of growth. The type of relationship between two preservatives can vary with the proportion of each used, and with

Food components

Effectiveness of sorbate is influenced by food components which can affect distribution of sorbate within the microenvironment. In comparison with other preservatives, sorbate has a favorable fat-to-water ratio of partition. Therefore, in fat/water emulsions a relatively high proportion of sorbate remains in the microbiologically susceptible water phase. This property makes sorbate useful for preservation of margarine, mayonnaise and salad dressings. However, an increase in fat content of a product will lower the amount of sorbate in the aqueous phase where it is needed for microbial control (116). Soluble food components such as salts and sugars will also decrease the amount of sorbate in the aqueous phase (65). This effect may be offset, however, by the synergistic action of sorbate and salt or sugar to give an increased antimicrobial effect (1, 16, 42-44, 63, 148, 155). Some acids decrease the solubility of sorbate in water (65), but acids also enhance antimicrobial activity by increasing the proportion of sorbate in the active undissociated form. Citric and lactic acids potentiate the antimicrobial action of sorbate to a greater extent than do inorganic acids (140, 141, 159). Acetic acid, which is often used in conjunction with sorbate in mayonnaise and salad dressings, decreases the fat-to-water ratio of sorbate partition (65). Food ingredients that have antimicrobial activity by themselves, such as spices, can increase the spectrum or intensity of activity of sorbate.

Figure 1. Minimum inhibitory concentration (MIC) when two preservatives are combined at various concentrations. ADD = additive, ANT = antagonistic and SYN = synergistic. Adapted from Rehm (136).
the microorganism to be inhibited. For example, combinations of benzoic acid and boric acid react antagonistically against *Escherichia coli* but are synergistic in inhibiting *Aspergillus* spp. (10, 136). A combination of sorbic acid and formic acid reacts antagonistically against *Saccharomyces cerevisiae*, but is synergistic in inhibiting *A. niger* (95, 138). Sorbic acid in combination with sulfur dioxide, formic acid, benzoic acid or p-hydroxybenzoic acid acts additively in inhibiting *E. coli* (137). A mixture of sorbic acid and sulfur dioxide will act synergistically to inhibit *A. niger* (138). The combination of sorbic acid and benzoic acid has an additive effect in inhibiting sensitive microorganisms (10, 95, 137, 138). An additive effect does not enable a reduction in the total amount of chemicals used, but often the spectrum of antimicrobial activity is increased.

The antimicrobial effectiveness of antioxidants in combination with sorbate has been investigated. Several researchers have shown that butylated hydroxyanisole (BHA) or tertiary butylhydroquinone (TBHQ) inhibit *S. aureus* and *S. typhimurium* more effectively when combined with sorbate in trypticase soy broth (49, 84, 88, 148). Morad et al. (109) demonstrated that BHA in combination with sorbate acted synergistically to inhibit *S. typhimurium* and the natural flora in cooked turkey. Ahmad and Branen (2) found that BHA combined with sorbate totally inhibited growth of *Aspergillus flavus* in a liquid growth medium.

Many studies have shown that sorbate and nitrite act synergistically to retard botulinal toxin production and extend the time necessary for toxin to be produced under abuse conditions (78, 100, 112, 113, 125, 154, 160, 161). For this reason, sorbate has been suggested as a partial replacement for nitrite in cured meat products. It has been postulated that nitrite and sorbate react and form compounds that are more inhibitory than the parent substances. However, these compounds have been found only in non-food systems at very low pH values and with very high nitrite and sorbate concentrations (112, 161).

Amano et al. (4) found a tylosin-sorbate combination effective in preserving fish sausage held at elevated temperatures (30 and 37°C). Boyd and Tarr (26) saw that the combination of smoke and sorbate inhibited mold spoilage in fish. Perry et al. (123) reported that the shelf-life of eviscerated cut-up poultry was extended by the synergistic effect of acidic hydration followed by sorbate application.

**EFFECTS OF SORBATE ON GROWTH OF MOOLS**

One of the most common uses of sorbate is as an inhibitor of mold growth on cheese and cheese products (6, 36), and its effectiveness has been demonstrated by several workers (52, 53, 102-106, 157, 158). The permitted use level for cheeses under the Federal Standard of Identity is 0.20-0.3% (39). This concentration will inhibit growth of most molds that contaminate cheeses. However, Bullerman (29) found that about 70% of the molds isolated from moldy cheese were able to grow in the presence of 0.3% potassium sorbate in a solid laboratory growth medium.

In a study of moldy Cheddar cheese, Bullerman and Olivigni (34) found that 82% of the isolates were of the genus *Penicillium*, 7% *Aspergillus* and 1% *Fusarium*. Seven percent of all molds were able to produce mycotoxins. In another study of Swiss cheese, 87% of the isolates were *Penicillium* species and less than 1% were *Aspergillus* species. A total of 5.5% were able to produce mycotoxins (28). Toxic *Penicillium* and *Fusarium* species are generally more capable of growth at low temperatures than are toxic species of aspergilli (114). Mold growth on cheese is a problem during ripening as well as refrigerated storage (28, 29, 31, 171, 172).

In 1966, Marth et al. (101) demonstrated that some molds in the genus *Penicillium* could grow in the presence of up to 0.71% (7100 ppm) potassium sorbate (Table 3). The molds were isolated from natural and processed Cheddar cheese previously treated with sorbate. Some of the penicillia degraded sorbate and produced 1, 3-pentadiene, a volatile compound with an extremely strong kerosene-like odor. Later Finol et al. (59) reported that penicillia, also isolated from cheese, could grow in the presence of up to 1.2% (12,000 ppm) potassium sorbate (Table 4). Liewen and Marth (93) examined several strains of penicillia and aspergilli for their abilities to grow in the presence of sorbate. They found that only molds isolated from sorbate-treated cheeses were able to grow in the presence of 0.3% (3000 ppm) or more sorbate and none of the aspergilli they tested were able to grow in the presence of more than 0.1% (1000 ppm) sorbate (Tables 5, 6). The aspergilli generally are more sensitive to sorbate than are the penicillia.

**TABLE 3. Amount of sorbate that permitted growth of penicillia isolated from sorbate-treated cheese.**

<table>
<thead>
<tr>
<th>Species of Penicillium</th>
<th>Maximum amount of sorbate permitting growth (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>roqueforti</td>
<td>5,400</td>
</tr>
<tr>
<td>roqueforti (variant)</td>
<td>7,100</td>
</tr>
<tr>
<td>notatum</td>
<td>2,300</td>
</tr>
<tr>
<td>frequentans</td>
<td>2,800</td>
</tr>
<tr>
<td>cyano-fulvum</td>
<td>1,800</td>
</tr>
</tbody>
</table>

*From: Marth et al. (101).*

**TABLE 4. Amount of sorbate that permitted growth of penicillia isolated from sorbate-treated cheese.**

<table>
<thead>
<tr>
<th>Species of Penicillium</th>
<th>Maximum amount of sorbate permitting growth (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>roqueforti</td>
<td>10,000</td>
</tr>
<tr>
<td>cyclopium</td>
<td>3,000</td>
</tr>
<tr>
<td>cyclopium (variant)</td>
<td>12,000</td>
</tr>
<tr>
<td>viridicatum</td>
<td>6,000</td>
</tr>
<tr>
<td>puberulum</td>
<td>12,000</td>
</tr>
<tr>
<td>crustosum</td>
<td>7,000</td>
</tr>
<tr>
<td>lanoso-viride</td>
<td>3,000</td>
</tr>
</tbody>
</table>

*From: Finol et al. (59).*
The shelf-life of cottage cheese can be extended by adding sorbate (5, 7, 40). Bradley et al. (27) found that 0.1% potassium sorbate inhibited growth of Penicillium frequentans, as well as other spoilage microorganisms. Other workers have also noted that sorbate inhibits spoilage organisms in cottage cheese (41, 72, 122). Mold growth was also inhibited in unsalted butter as a result of sorbate treatment (80, 81, 117).

Mold growth in grains can be inhibited by sorbate treatment (128, 131, 164). Danzinger et al. (48) found that 0.05% potassium sorbate increased the time required to obtain yeast and mold counts of $1.3 \times 10^5$ g of dry weight (termed Safe Storage Time, or SST) of wet corn slightly over the control. The SST was increased by almost 2 d with 0.10% potassium sorbate, while 0.20% increased the SST by 3 d. The primary organisms inhibited were yeasts and molds. Sauer and Burroughs (151) reported that with 0.5 and 1.0% potassium sorbate, the mold-free storage time for corn containing 18% moisture was 2 and 3 weeks, respectively, compared to less than 1 week for the control. At the same levels of sorbic acid, the mold-free storage time was increased to 7 and 12 weeks, respectively.

Sorbate also inhibits mold growth in hams (9,82,83) and sausage. Wallhäuser and Lück (177) reported that 0.02 to 0.04% potassium sorbate inhibited growth of Aspergillus versicolor in culture media and fermented sausage at pH 5.7-5.9.

Bandelin (11) determined the MIC of sorbic acid required to inhibit several mold species. At pH 5.0, the MICs were 0.02% for Alternaria solani, 0.08% for Penicillium citrinum and 0.08% for A. niger. Bullerman (31) found that potassium sorbate at 0.05, 0.10 and 0.15% delayed or prevented spore germination and initiation of growth, and slowed mycelial growth by strains of Aspergillus parasiticus and A. flavus. Similar results were obtained with Penicillium patulum and P. roqueforti (33). Liewen and Marth (92) noted that sorbate had detrimental effects on viability of conidia of P. roqueforti. Viability decreased as the sorbate concentration increased (Fig. 2). Przbylski and Bullerman (130) noted a similar trend for the conidia of A. parasiticus. Lukas (96) reported that 0.1% potassium sorbate hindered germination of conidia of A. niger and 0.15% inhibited mycelial growth.

A small number of molds found growing on foods produce mycotoxins (30, 107, 153). Tong (170) demonstrated that potassium sorbate inhibited growth and ochratoxin A production by Aspergillus sulphureus and Penicillium viridicatum. Bullerman (31) found that potassium sorbate greatly reduced or prevented production of aflatoxin B1 by A. parasiticus and A. flavus. The same investigator (32, 33) showed that potassium sorbate had a similar effect on patulin production by P. patulum. However, while potassium sorbate reduced growth by P. roqueforti, sublethal doses actually stimulated patulin production by this mold (33). Yousef and Marth (186) reported in 1981 that sublethal amounts of sorbate sometimes resulted in greater production of aflatoxin by A. parasiticus than

### TABLE 5. Maximum concentration of potassium sorbate (ppm) which permitted growth of various penicillia at 25 and 4°C.

<table>
<thead>
<tr>
<th>Culture</th>
<th>25°C</th>
<th>4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. cyclopium-8</td>
<td>3000</td>
<td>2000</td>
</tr>
<tr>
<td>P. roqueforti-22a</td>
<td>9000</td>
<td>3000</td>
</tr>
<tr>
<td>P. cyclopium (atypical)-25a</td>
<td>9000</td>
<td>9000</td>
</tr>
<tr>
<td>P. ruberulum-33a</td>
<td>9000</td>
<td>9000</td>
</tr>
<tr>
<td>P. viridicatum-34a</td>
<td>9000</td>
<td>2000</td>
</tr>
<tr>
<td>P. ruberulum-37a</td>
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<td>6000</td>
</tr>
<tr>
<td>P. cyclopium (atypical)-40a</td>
<td>9000</td>
<td>9000</td>
</tr>
<tr>
<td>P. crustosum-42a</td>
<td>6000</td>
<td>2000</td>
</tr>
<tr>
<td>P. lanoso-viride-44a</td>
<td>3000</td>
<td>2000</td>
</tr>
<tr>
<td>P. roqueforti-KVa</td>
<td>9000</td>
<td>9000</td>
</tr>
<tr>
<td>P. camemberti-877</td>
<td>500</td>
<td>NGb</td>
</tr>
<tr>
<td>P. chrysogenum</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>P. digitatum</td>
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<tr>
<td>P. frequentans</td>
<td>500</td>
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<tr>
<td>P. notatum</td>
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<tr>
<td>P. expansum</td>
<td>1000</td>
<td>NG</td>
</tr>
<tr>
<td>P. roqueforti-849</td>
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<td>2000</td>
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<tr>
<td>P. patulum-M59</td>
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<td>NG</td>
</tr>
<tr>
<td>P. sp.-K2a,c</td>
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<tr>
<td>P. sp.-S1a,c</td>
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</tr>
<tr>
<td>P. sp.-S2a,c</td>
<td>9000</td>
<td>9000</td>
</tr>
<tr>
<td>P. sp.-S3a,c</td>
<td>2000</td>
<td>1000</td>
</tr>
<tr>
<td>P. sp.-B1a,c</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>P. sp.-B2a,c</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>P. sp.-B7a,c</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

*a*Isolated from sorbate-treated cheese.

*b*Isolated from cheese not treated with sorbate.

*c*Mold of genus Penicillium, species not identified.

*d*From Liewen and Marth (93).

### TABLE 6. Maximum concentration of potassium sorbate (ppm) which permitted growth of various aspergilli at 25 and 4°C.

<table>
<thead>
<tr>
<th>Culture</th>
<th>25°C</th>
<th>4°C</th>
</tr>
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<tr>
<td>A. ochraceus 3174</td>
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*a*NG = no growth.

*b*From Liewen and Marth (93).
SORBIC ACID INHIBITS MICROBIAL GROWTH

been reported by Bauer et al. (174) and Tsai et al. (174). Similar results have been reported by Yousef and Marth (187) when media were free of sorbate. Similar results have been reported by Yousef and Marth (187) when media were free of sorbate. Similar results have been reported by Yousef and Marth (187) when media were free of sorbate. Similar results have been reported by Yousef and Marth (187) when media were free of sorbate. Similar results have been reported by Yousef and Marth (187) when media were free of sorbate. When media were free of sorbate. Similar results have been reported by Bauer et al. (12) and Tsai et al. (174). Yousef and Marth (187) demonstrated that sorbate inhibited aquisition of aflatoxin in resting cultures of A. parasiticus and concluded that sorbate inhibited aflatoxin biosynthesis by inhibiting transfer of substances from the growth medium into the cell.

EFFECTS OF SORBATE ON GROWTH OF YEASTS

Sorbates are generally very effective in controlling spoilage yeasts in foods, although they cannot be used directly in yeast-leavened products because of inhibition of baker’s yeast (36). In broth culture at pH 4.5, Bell et al. (14) found 0.1% sorbic acid to be inhibitory to 32 species of yeasts.

Since yeasts do not compete effectively with most bacteria in neutral to slightly acidic food products with high aw (0.98-0.995), this group of microorganisms has been of minor importance in the spoilage of most food products. However, under certain storage conditions (low pH, intermediate aw, temperature slightly below room temperature) a product may be susceptible to spoilage by yeast (141).

Salad dressings and related products are sometimes spoiled by yeasts (159). Saccharomyces baltii, because of its tolerance of acidic conditions, a high osmotic pressure and preservatives such as sorbate, is a universally recognized problem in the salad dressing industry (126, 127, 159). Other products subject to spoilage by osmotolerant yeasts include carbonated beverages, cordials, tomato sauce, honey, maple syrup, molasses, some candy, jams, jellies and chocolate syrup (141). Under certain conditions sorbates are effective in controlling spoilage yeasts.

Restaino et al. (141) studied the effects of acids combined with sorbate on growth of four osmophilic yeasts. At 0.05% potassium sorbate (pH 5.0), growth of all four strains was slowed, although Saccharomyces rouxii and Saccharomyces bisporus were more resistant than Saccharomyces acidifaciens and S. baltii to the antimycotic agent independent of the acid used to acidify the growth medium. At 0.1% potassium sorbate, growth of all four yeast strains was completely inhibited. Effects of potassium sorbate at pH 5.0 on growth of S. rouxii are shown in Fig. 3. While growth was rapid in the absence of preservative, at 0.05% potassium sorbate growth was slowed and at 0.1% cell death occurred. Some strains of S. rouxii can acquire resistance to sorbate. Bills et al. (22) showed that a strain of S. rouxii preconditioned in 0.1% sorbate showed an increased resistance to sorbate when inoculated into a growth medium or chocolate syrup. Beech and Carr (13) found strains of S. cerevisiae, S. rouxii, Saccharomyces carlsbergensis, (Saccharomyces uvarum), Torulopsis colliculosa, Brettanomyces bruxellensis, C. utilis and Triganopsis varabilis that were resistant to 0.1% sorbic acid in a wort medium at pH 5.4.

Sorbates inhibit growth of yeasts in cucumber fermentations (3, 24, 42, 44, 124, 135), on the surface of hams (9), on high-moisture dried fruits (23, 115), in cottage cheese (27, 73), on smoked fish (26, 62) and in fruit juices (15, 58, 121).

EFFECTS OF SORBATE ON GROWTH OF BACTERIA

Information on the effect of sorbate against bacteria is not nearly as comprehensive as it is for yeasts and molds. Some reports suggest that sorbate exerts a selective inhibition against all catalase-positive microorganisms and can be used as a selective agent for catalase-negative lactic acid bacteria and clostridia (57, 124, 176, 181, 182). However, these conclusions may have been incomplete. In some instances, pH of the medium was not controlled and hence the active undissociative form of sorbic acid may not have been present in amounts sufficiently large for effectiveness. The effectiveness of sorbate in these studies was dependent upon factors such as concentration of the compound, laboratory medium used, pH of the medium and type and strain of microorganism examined (161, 162). Hansen and Appleman (69) re-

Figure 2. Viability of conidia of Penicillium roqueforti when exposed to various sorbic acid solutions at pH 4.75. From Lieven and Marth (92).

Figure 3. The effects of potassium sorbate on the growth of S. rouxii, in supplemented potato dextrose broth, pH 5.0. O = control. □ = 500 ppm sorbate. △ = 1000 ppm sorbate. Adapted from Restaino et al. (129).
ported that sorbic acid neither inhibited nor stimulated growth of clostridia in laboratory media at pH 6.7. Hammond et al. (68) found that catalase-negative yeast starter bacteria were inhibited by 0.1% sorbic acid. Costilow et al. (43) found two strains of catalase-positive *Pediococcus cerevisiae* which were as tolerant to sorbate as were catalase-negative bacteria. Doell (54) found that 0.75% sorbic acid at pH 5.0 inhibited salmonellae and *E. coli* in 48 h at 37°C. Concentrations as low as 0.075% were bacteriostatic. Park and Marth (779) were among the first in the U.S. to demonstrate the effectiveness of the undisassociated sorbic acid against bacteria. They found that 0.3% sorbic acid at pH 5.0 inactivated *S. typhimurium* in 12 h in nutrient broth at 37°C. Complete inactivation in evaporated milk (pH 5.0) at 7°C required 55 d. Reducing sorbic acid concentration or raising the pH of the substrate increased the time needed for inactivation. Growth occurred in nutrient broth and skim milk with 0.2 and 0.3% sorbic acid when the pH was not adjusted. Inactivation of *S. typhimurium* was more rapid in cold-pack cheese food that contained sorbic acid rather than sodium propionate or no antimycotic agent (120). Growth of two strains of *Pseudomonas fluorescens* was inhibited by 0.05% sorbate in trypticase soy broth (pH 5.5) and 0.20% sorbate delayed growth of both strains at pH 6.0 (142). *P. putrefaciens* was inactivated by 0.20% sorbate in trypticase soy broth at pH 6.0 (144). *P. fragi* is also inhibited by sorbate (110). Growth of *S. aureus* in brain heart infusion (BHI) at pH 5.0 was inhibited by 1% sorbate, but at pH 7.0 the organism grew in the presence of 5% sorbate (88). Potassium sorbate (0.25%) had no effect on the growth of *S. aureus* in BHI at pH 6.0 (118). In pasteurized minced cod inoculated with *S. aureus*, 0.5% sorbate resulted in a markedly slower rate of growth of the pathogen as well as a delay in enterotoxin production compared to when sorbate was absent (98). Bacon containing 0.26% potassium sorbate did not support growth of *S. aureus* (125). Growth of *S. aureus* in pie fillings that are otherwise excellent microbiological media can be prevented by adding sorbate (129, 152).

Trypticase soy broth with 0.1 or 0.2% sorbate adjusted to pH 5.5 was bacteriostatic to salmonellae and *Yersinia enterocolitica* and extended the lag phase of *P. fluorescens* for 24 h (128). A reduction in numbers of *S. typhimurium* was noted in cooked turkey meat treated with 0.1% sorbate. Growth of *Vibrio parahaemolyticus* was inhibited by 0.1% sorbic acid in crab meat homogenates (145). Sorbate reduced the number or delayed growth of spoilage bacteria in cottage cheese (27, 41), poultry (47, 73, 109, 143, 146, 147, 149, 167, 168), frankfurters (67), beef (66, 67), seafood (4, 37, 62, 98, 163) and yogurt (68); sorbate inhibited *Bacillus cereus* and *B. subtilis* in rice filling of Karelian pastry (134).

Tomkin et al. (169) found that adding 0.1% potassium sorbate to cooked uncured sausage retarded growth and toxin production by *Clostridium botulinum*. The treatment also delayed or retarded growth of *Clostridium perfringens* and *S. aureus*. Several other reports have indicated that sorbate or sorbate-nitrite combinations are effective inhibitors of *C. botulinum* (69, 70, 74, 78, 100, 112, 113, 147, 162).

**RESISTANCE TO AND METABOLISM OF SORBATE BY MICROORGANISMS**

Some microorganisms can grow in the presence of large amounts of sorbate, and some actually metabolize or degrade it. Melnick et al. (105) reported in 1954 that high initial mold populations degraded sorbic acid in cheese. They hypothesized that the degradation occurred as a result of β-oxidation with CO₂ and H₂O as end-products. Marth et al. (101), in 1966, were the first investigators to find that some molds in the genus *Penicillium* were able to degrade sorbate to 1, 3-pentadiene, a volatile compound with an extremely strong kerosene-like odor. They proposed that 1, 3-pentadiene was produced as a result of a decarboxylation reaction, which eliminated inhibitory sorbate from the growth medium and allowed growth of the mold. Later, presence of 1, 3-pentadiene, probably because of microbial activity, was also observed in a sorbate-treated noncarbonated beverage (63) and in sorbate-treated Feta cheese (71).

Kuroguchi et al. (85, 86) reported that sorbic acid was metabolized by *Mucor* species to 4-hexenol and by *Geotrichum* species to 4-hexenoic acid and ethyl sorbate. Tahara et al. (165) reported that all *Mucor* species they tested metabolized sorbic acid to 4-hexenol. Takanami et al. (166) found a decrease in sorbic acid in “Yamagobozuke”, a pickled vegetable, which was associated with an increase in 4-hexenoic acid. The metabolite was presumably formed during the lactic fermentation of the vegetable. Losses of sorbate have been noted in cucumber fermentation, and may also be the result of lactic fermentation (3).

Raduchev and Rizvanov (133) also reported that some microorganisms, including *Lactobacillus plantarum*, *Streptococcus lactis*, *Acetobacter* species, and penicillia could use sorbic acid as a carbon source. Lukas (7, 9, 96) reported that *A. niger* and molds in the genus *Penicillium* could decompose sorbic acid. It was shown that sorbic acid was not stored in the cells or decomposed by extracellular enzymes. It was probably taken up into the cells and metabolized to CO₂ and H₂O. Rehm et al. (139) reported that *A. niger* and *P. fluorescens* were able to metabolize sublethal concentrations of sorbic acid. Lawrence (89, 90) showed that spores of *P. roqueforti* were able to oxidize fatty acids to methyl ketones.

Wines treated with sorbic acid to prevent yeast spoilage often develop a geranium-like odor (75, 79). Crowell and Guymon (46) identified ethyl sorbate; 2, 4-hexadien-1-ol; 1-ethoxyhexa-2, 4-diene; 3, 5-hexadien-2-al; and 2-ethoxyhexa-3, 5-diene in a sorbate-treated red table wine spoiled by lactic acid bacteria. The latter compound was responsible for the geranium-like odor and was thought to result from the lactic fermentation. Wurdig et al. (180) studied the geranium-like odor in wines and concluded...
that it was a result of degradation of sorbic acid to 2, 4-hexadien-1-ol. The geranium-like odor has also been reported by Burkhardt (35) and Radler (132), both of whom attributed it to degradation of sorbic acid by lactic acid bacteria.

There have been numerous reports of sorbate-resistant yeasts (22, 77, 126, 127, 178), but none of these has indicated that degradation of sorbate occurs. Warth (178) found that resistance to preservatives by S. bailii resulted primarily from an inducible, energy-requiring system which transports preservatives from the cell.

MECHANISM OF INHIBITION BY SORBATE

The process by which sorbate inhibits microbial growth is not clear and defined. Some work has implicated inhibition of various enzyme systems. In 1954, Melnick et al. (105) proposed that sorbic acid inhibits certain dehydrogenase enzymes which are involved in the β-oxidation of fatty acids. Other workers have postulated that sorbate may inhibit respiration through a competitive action with acetate at the site of acetyl-CoA formation (70). Sorbate would competitively combine with coenzyme A and acetate, causing inhibition of the enzyme reaction related to coenzyme A (161).

Sulfhydryl-containing enzymes have been proposed to be the site of inhibition by sorbic acid (183). Azukas (8) reported that sorbate inhibits enolase. Troller (173) proposed that the inhibitory action of sorbate resulted from inhibiting catalase, which would cause an increase of toxic hydrogen peroxide within the cell. It has been proposed that sorbic acid uncouples oxidative phosphorylation by inhibiting enzymes within the cell (184, 185). Borst et al. (25) noted that long-chain fatty acids had uncoupling activity. Uncoupling of active transport systems from the cellular energy supply was suggested as the mechanism of yeast inhibition under anaerobic conditions (178). Sorbic acid causes a decrease in ATP content of mold spores (92, 130), which may be a result of the uncoupling activity of sorbic acid. It has been proposed that a proton or charge gradient is involved in energizing membrane transport. The undissociated form of sorbate could discharge the gradient by diffusing through the membrane and ionizing when it reaches an interior compartment of the cell with a higher pH (76). Freese et al. (60) have proposed that sorbate prevents microbial growth by inhibiting the transport of substrate molecules into cells. Yousef and Marth (187) concluded that sorbate inhibited aflatoxin biosynthesis in the same manner. Inhibition of transport of carbohydrates has also been suggested as the inhibitory mechanism of sorbic acid (50, 51).

From the variety of systems implicated in the antimicrobial action of sorbate, it is likely that there is more than one mechanism of action and the inhibition is relatively nonspecific. Furthermore, the mechanism of inhibition may differ in bacteria, yeasts and molds.

SUMMARY

While sorbic acid is among the most commonly used of the antimicrobial food preservatives, it does have limitations in its effectiveness. The maximum permissible dosage in most foods is 0.3%. At this concentration, sorbic acid is effective against a variety of microorganisms; however, some grow at concentrations much greater than 0.3%. Some molds and bacteria are able to degrade sorbic acid. Environmental factors such as pH, water activity, temperature, atmosphere, microbial load, type of microbial flora, and certain food components can influence the effectiveness of sorbic acid. All of these factors should be considered when using sorbic acid as an antimicrobial preservative. Each situation should be considered on an individual basis and environmental factors should be controlled to give optimal antimicrobial activity.

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