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Antimicrobial Activity of Sorbate

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ABSTRACT

During the last 30 years sorbate has been tested and used widely in the preservation of various food products throughout the world. Currently it has received increased attention as a potential replacer of nitrite for botulism control in processed meat products. Previous reports, however, had suggested sorbate as a selective agent for clostridia in laboratory media. Recent developments as well as the need for safe, practical and effective food preservatives in current and future food processing have generated intense interest in preservatives such as sorbate. This paper reviews the significant developments relating to use of sorbate as a food preservative - its antimicrobial effects, applications, advantages and limitations. A summary of the current status as well as unanswered questions relevant to the mechanism(s) through which the compound exerts its antimicrobial activity also is presented.

The antimicrobial properties of sorbic acid were first discovered in the 1940s. The compound itself, however, was isolated in 1859 in London by the German chemist, A. W. Hoffmann, from the berries of the mountain ash tree (rowan berry) through reaction with alkali. The structure of the compound was clarified in the period of 1870-1890 and it was first synthesized by O. Doebner in 1900. A U.S. patent was awarded to C. M. Gooding and The Best Foods, Inc. in 1945 (*32*) for the antifungal properties of sorbic acid. They discovered that the compound is a good fungistatic agent for foods and food wrappers. Since then the testing and use of the compound as an antifungal agent have been widespread.

CHEMISTRY

Sorbic acid is a straight chain, *a*, *β*-unsaturated trans-trans, 2, 4-hexadienoic monocarboxylic aliphatic acid and has the molecular formula CH_3-CH=CH-CH=CH-COOH. The carboxyl group of sorbic acid reacts readily and forms salts and esters. The salts of sorbic acid, especially the potassium salt, are very important in applications due to high solubility in water. The low water solubility is a disadvantage for sorbic acid. At 25 C, the solubility of the acid in water is 0.16% and of potassium sorbate in water is over 50%. Solubility in water increases with pH and temperature. In vegetable oil, the acid is more soluble than the potassium salt (Table 1). Increased concentrations (> 10%) of soluble food components, such as glucose, sucrose, and NaCl reduce the solubility of sorbic acid in water. Presence of such water, soluble components may increase the sorbic acid solubility ratio in oil to water from 3.0 to as high as 5.0 (*33*). An increase in fat content will also lower the amount of sorbic acid in the aqueous phase where it is needed for microbial control (*66*).

TABLE 1. *Solubility of sorbate* (%).

Solvent	Sorbic acid	Potassium sorbate
Water (25 C, pH 3.1)	0.16	58.20
Water (25 C, pH 4.4)	0.22	-
Water (25 C, pH 5.9)	1.02	-
Water (100 C)	4.00	64.00
Oil (20 C)	1.00	0.01
Oil (100 C)	10.00	0.19

APPLICATIONS

Since the first report 35 years ago on its antimicrobial properties, sorbic acid has been tested and used as an antimicrobial preservative in a wide range of products. A survey of the literature demonstrates the extensive number of products in which sorbate has been used or tested as a preservative. Many of these reports come from Japan, the U.S.S.R., and Germany. In 1976, Dr. Eric Lück of Germany reported that the number of publications on sorbic acid and its derivatives exceeded 3,000 in more than 20 languages (*51*). All this occurred in less than 30 years, and demonstrates the great response the use of sorbic acid has received in preservation.

Sorbic acid and its potassium salt are the most widely used forms of the compound and are collectively known as sorbates. Practical applications of sorbates include preservation of human food, animal feed, pharmaceuticals, cosmetic products and packaging materials. The practical applications of sorbate as a food preservative (Table 2) include dairy products (cheese and cheese products, yogurt, sour cream, cheese spreads and dips), bakery products (cakes and cake mixes, pies and pie fillings, doughnuts, fudges, icings, toppings), fruit and

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vegetable products (wines, beverages, fruit juices and syrups, jams and jellies, dried fruits, confections, salads, fermented and pickled vegetables) and other food products (certain meat and fish products, mayonnaise, margarine, salad dressing). In the U.S., sorbate is a GRAS substance and its use may be requested in any food product which allows preservatives. It can also be used in more than 70 food products having Standards of Identity. Methods of application of sorbate include direct addition into the product, dipping in or spraying with a sorbate solution, dusting and incorporation in the wrapping or packaging material. Levels of incorporation into the product vary from 0.01 to 0.30% sorbic acid (Table 3).

TABLE 2. *Sorbate applications in food products.*

Dairy products	*Fruits and vegetable products*
Cheeses	Wines
Cheese products	Beverages
Yogurt	Fruit juices
Sour cream	Syrups
Cheese spreads	Jams and jellies
Cheese dips	Dried fruits
	Salads
Bakery products	Fermented vegetables
Cakes	Confectionary
Cake mixes	
Pies	*Other food products*
Pie fillings	Dry sausages
Doughnuts	Smoked and salted fish
Fudges	Mayonnaise
Icings	Margarine
Toppings	Salad dressings

TABLE 3. *Levels of sorbic acid incorporation in food products* (%).

Cheeses	0.05-0.30
Wines	0.01-0.04
Beverages	0.03-0.10
Dried fruits	0.02-0.05
Bakery products	0.03-0.10
Salad dressings	0.05-0.10
Fish	0.03-0.15
Mayonnaise	0.10

ANTIMICROBIAL EFFECTS

Sorbate has been shown to inhibit growth of yeasts, molds and many bacteria. Its activity against bacteria, however, is not as comprehensive as that against yeasts and molds. After the patent of Gooding (*32*), it was reported by Phillips and Mundt (*72*) and Jones and Harper (*45*) that 0.1% sorbic acid prevented growth of surface yeasts in cucumber fermentations, with no interference with the desirable acid fermentation. Similar results for both film-forming and subsurface yeasts were reported by Costilow et al. (*11,12*). Borg et al. (*6*), however, observed that 0.1% sorbic acid not only inhibited growth of fermentative yeasts in cucumber fermentations, but it also retarded growth and acid production by the acid-forming bacteria. Curing and color of the fermented cucumbers were also impaired. Additional research by Costilow et al. (*13*) demonstrated that the inhibitory effect of sorbic acid on lactic acid-producing bacteria in cucumber fermentations depended upon brine strength. The higher the salt (NaCl) concentration, the greater the inhibition of lactic fermentation. Inhibitory effects of sorbic acid against yeasts have also been reported by numerous other researchers including Emard and Vaughn (*24*), Ferguson and Powrie (*25*), Geminder (*31*), Pederson et al. (*69*) and Huang and Armstrong (*41*).

The usefulness of sorbic acid as a mold inhibitor in cheeses has been demonstrated by several workers (*20,21,54-57,94,95*). Inhibitory effects against molds by sorbic acid have also been reported in other foods and laboratory media (*4,24,41,47*).

Even though the action of sorbate against bacteria is not as comprehensive as that against yeasts and molds, there are many bacteria that are inhibited by the compound. Vaughn and Emard (*109*) and Emard and Vaughn (*24*) reported that sorbate inhibited several species of bacteria in laboratory media. Results presented by Doell (*22*) indicated that sorbate concentrations as low as 0.075% were effective against *Salmonella typhimurium* and *Escherichia coli*. *S. typhimurium* was also inactivated by sorbate in laboratory media, milk and cheese (*67,68*). Sorbate has also inhibited total microbial growth, staphylococci, *Pseudomonas* sp., *Vibrio parahaemolyticus*, *Bacillus* sp., etc. (*8,24,34,59,73,78,81,82,85,109*). Several other reports have dealt with the preservative action of sorbate in a variety of products (*1,7,10,15,31,41,46,48,58,65,70,71,83,84,110*).

The effectiveness of sorbate as an inhibitory agent against key microorganisms is used to determine the use concentration of the compound and depends on factors such as the pH of the product, ingredients of the product, moisture content of the product, other additives present, product contamination, processing, packaging, storage temperature, length of storage and sanitation (Table 4).

TABLE 4. *Factors influencing antimicrobial effectiveness of sorbate.*

pH of product	Processing
Product ingredients	Packaging
Other additives	Storage temperature
Product moisture	Storage length
Product contamination	Sanitation

ADVANTAGES

A considerable amount of research has dealt with the toxicity, metabolism, stability, method of measurement and effectiveness of sorbate as a preservative. Early in its use, sorbate was classified as relatively non-toxic. It was reported that sorbate can be metabolized by the organism in a way similar to the naturally occurring fatty acids (*20,21*). It was also reported that sorbate was more efficient and less toxic than benzoate (*32,94*). Unlike the metabolizable sorbate, benzoate must first be detoxified in the liver before it can be excreted. Some people have

regarded sorbate as a food used to preserve another food. The LD_{50} for sorbic acid is about 10 g/kg of body weight, while that for common salt (NaCl) is 5 g/kg of body weight. Among food preservatives, the World Health Organization has stipulated for sorbate the highest Acceptable Daily Intake (ADI) which is 25 mg/kg of body weight.

Gooding et al. (*33*) indicated that sorbate has a fat-to-water partition greater than benzoate (3.0 vs. 6.1). Sorbate was found at least three times more effective than benzoate in preserving cheese, fish and bakery products (*7,33,94*). The fat-to-water distribution of propionate was found to be better than that of sorbate (0.17 vs. 3.0), but sorbate is considered a more inhibitory preservative than propionate as well (*58*). Sorbate was about four times as effective as propionate in preserving cheese, fish and bakery products (*33*). *S. typhimurium* was inactivated more rapidly in refrigerated cheese-food formulated with sorbate than with propionate (*68*). It should be emphasized that the partioning of these preservatives is very important to the extent of their activity (*92*).

pH EFFECTS

The antimicrobial effectiveness of fatty acids containing 1-14 carbon atoms has been associated with their undissociated fraction and not the acid anion (*14,40,79*). Sorbate is more effective at pH values approaching its dissociation constant (pKa) which is 4.75. At this pH value, 50% of the acid is in the effective undissociated form (Table 5). Consequently, sorbate is more effective in low pH foods (*5,24,64,101*). The more favorable antimicrobial effects at higher pH levels obtained with sorbate as compared to benzoate and propionate may be partly explained by considering their pKa values. Sorbate has a maximum pH for activity around 6.0-6.5, while those for propionate and benzoate are 5.0-5.5 and 4.0-4.5, respectively. Sorbate can also replace benzoate, partially or totally, in more acidic foods to avoid off-flavors and extend the spectrum of microorganisms inhibited.

TABLE 5. *Effect of pH on sorbic acid dissociation.*

pH	Undissociated (%)
7.00	0.6
6.00	6.0
5.80	7.0
5.00	37.0
4.75 (pKa)	50.0
4.40	70.0
4.00	86.0
3.70	93.0
3.00	98.0

INTERACTIONS

Soluble food components such as sugars and salt increase the fat-to-water ratio of sorbate partition, which would mean that a lower level of sorbate remains in the water phase of the system to act as an antimicrobial agent. Studies, however, have indicated that salt and sugars act synergistically with sorbate and increase the antimicrobial effects (*11,33,88*). Other reports have also indicated synergistic interactions among sorbate, pH level and salt and sugar concentrations (*11,12,13,33*). Recently, Robach and Stateler (*86*) reported that sorbate exhibited synergistic activity with salt (NaCl), BHA, and TBHQ against certain strains of *Staphylococcus aureus*. Acidity may depress the solubility of sorbate in water, but, in general, acids increase its antimicrobial activity, mainly by lowering the pH of the substrate to levels closer to the dissociation constant. Some acids, such as acetic, may be used in conjunction with sorbate in sweet cucumber pickles to prevent growth of lactics (*88*). Acetic acid bacteria, which were favored by control of yeasts with sorbate in apple juice, were inhibited by inclusion of ascorbic acid in the product (*25*). Synergistic sorbate-temperature interactions have also been reported. A mild heat treatment (49 C) and a sorbate concentration of 0.06-0.12% increased greatly the shelf-life of fruit products (*87*). Also, at low temperatures (1.1 C) sorbate increased the storage life of grape juice (*69*). Shibasaki and Tsuchido (*93*) reported that sorbic acid inhibited the cellular repair mechanisms after heating of *Escherichia coli* and *Candida utilis*, and thus enhanced the destructive effects of heat treatment on the cells.

DERIVATIVES

Of the derivatives of sorbic acid, only its salts have been commercially developed and used as antimicrobial agents. Some other derivatives, however, have also been tested and found effective. One compound found to be 1,000 times more inhibitory against yeast alcohol dehydrogenase was sorbamide (*53*), while sorbohydroxamic acid was found effective against molds in a pH range of 3.6-9.2 (*23,108*). The pKa of sorbohydroxamic acid is 8.8 and, therefore, it is undissociated over a wider pH range than sorbic acid. Sorbic aldehyde was also found effective by Troller and Olsen (*108*), but its very undesirable flavor and odor eliminated its usefulness. The undissociable esters of sorbic acid also maintain their activity over a wide pH range, but their insolubility in water limits their use (*23,64*).

METABOLISM AND RESISTANCE

It is known that certain mold species are more resistant to sorbic acid and they result in occasional mold spoilage of foods preserved with the compound (*9,107*). It was reported by Melnick et al. (*57*) that high initial mold populations degraded sorbic acid in cheese. This would indicate that sorbate will not adequately preserve cheese which was produced under unsanitary conditions. Yeasts and lactic acid bacteria did not utilize sorbic acid (*11*). However, York and Vaughn (*113*) reported that species of *Clostridium* utilized the compound as a carbon source. Marth et al. (*52*) conducted systematic studies with molds in cheese and reported that certain mold species degraded sorbate and that sorbate degradation was

enhanced by a nutritious substrate and retarded by a poor medium. The degradation of sorbate was accompanied by the formation of a volatile compound with a hydrocarbon-like odor, which was identified as 1,3-pentadiene. It was postulated that the molds degraded sorbate through decarboxylation:

Potassium sorbate + tartaric acid → potassium tartrate + sorbic acid

Sorbic acid decarboxylation → 1,3-pentadene + CO_2

Marth et al. (*52*) suggested that in addition to the mechanism through fatty acid metabolism identified by Melnick et al. (*57*), the above mechanism might also be involved in sorbate metabolism by molds. Deak and Novak (*18*) reported that sorbic acid was metabolized by yeasts at low intracellular concentrations. At high concentrations, however, it suppressed yeast metabolism and growth. Warth (*111*) indicated that *Saccharomyces bailii* is resistant to sorbic, benzoic, and other short-chain monocarboxylic acid preservatives, and suggested that resistance to these preservatives results primarily from an inducible energy-requiring system which transports the preservative out of the cell. This mechanism is induced when the organisms are previously grown in the presence of low concentrations of the preservative.

SELECTIVE INHIBITION

Several reports have suggested that sorbic acid exerts a selective inhibition against all types of catalase-positive microorganisms, and it could be used as a selective agent for catalase-negative lactic acid bacteria and clostridia (*24,72,109,113,114*). However, there are major indications that these conclusions were incomplete. It is clear from some of these studies that the pH of the medium affected the selective power of sorbic acid. Generally, the effectiveness of sorbic acid in these studies was dependent on factors such as concentration of the compound, laboratory medium used, pH of the medium and type and strain of microorganisms examined. There are also data that contradict the above conclusions. Emard and Vaughn (*24*) showed that some catalase-positive strains of *S. aureus* grew as well as catalase-negative lactobacilli. In the studies of York and Vaughn (*113,114*), there was variation in tolerance to sorbic acid concentrations among species and strains of clostridia tested. Hamdan et al. (*36*) reported that both growth and acid production by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (both catalase-negative) were reduced by 0.05-0.10% sorbic acid. Also, Costilow et al. (*11*) reported that two strains of *Pediococcus cerevisiae* tested were weakly catalase-positive and appeared to be as tolerant to sorbic acid as the catalase-negative bacteria. Finally, Hansen and Appleman (*37*) concluded that sorbic acid did not inhibit or stimulate the growth of clostridia in microbiological media of pH 6.7.

MEAT PRODUCTS

At the present time, the only approved use of sorbate in meats in the U.S. is that of dipping the casings of stuffed dry sausages in a 2.5% potassium sorbate solution to prevent mold growth on the surface of the product during the drying period. The reported lack of inhibition for clostridia in laboratory media and the observations that its activity against bacteria was selective must have contributed as major reasons for the limited use of sorbate in meat products. A study was conducted by Tompkin et al. in 1974 (*105*), which determined the effects of potassium sorbate on *Clostridium botulinum*, salmonellae, *S. aureus* and *Clostridium perfringens* in an uncured, cooked sausage, temperature-abused at 27 C. The objectives of the study were to determine whether treating sausages with sorbate to prevent mold growth would stimulate pathogenic bacterial growth. The results indicated that 0.1% potassium sorbate delayed or retarded total microbial growth, salmonellae, and *S. aureus* growth, while *C. perfringens* declined to undetectable levels in both control and sorbate-treated samples. Sorbate also retarded botulinal growth and toxin production. This, however, was unexpected due to the above-mentioned reports that had suggested sorbic acid as a selective agent for clostridia in laboratory media.

Publication of this work coincided with reports implicating nitrite as a potential precursor of carcinogenic nitrosamines in cured meat products, and especially some crisp-fried bacon. Recently another, still unverified, report implicated nitrite as being a direct carcinogen to laboratory animals (*61*). Nitrite, however, has been used for many years in the curing of meat where it performs some very important functions. It reacts with myoglobin to form the characteristic cured meat color; it has an effect on cured meat flavor; it acts as an antioxidant, preventing off-flavors; and more importantly, it retards botulinal toxin production if the product is temperature-abused. The need for means to minimize nitrosamine formation in meat products while maintaining their excellent botulinal safety record led to extensive research testing sorbate as a total or partial replacer of nitrite in meat products for botulism control. Studies were conducted in several laboratories, which basically demonstrated that sorbate (0.20%) has an effect in delaying toxin production in several products. More importantly, a combination of sorbate (0.20%) with decreased nitrite levels (40-80 ppm) acted synergistically and extended the time necessary for toxin to be produced in the products under abuse conditions (*43,44,74-76, 96-103*). On May 16, 1979, a USDA regulation allowing the use of 0.26% potassium sorbate in combination with 40 ppm of sodium nitrite in bacon production was scheduled to become operational. Recent USDA studies with bacon, although verifying the antibotulinal activity of sorbate and nitrite combinations, implicated the product in some allergic reactions of certain individuals conducting sensory evaluation tests. This was an unanticipated and still unverified development, and has caused a delay in any action on the use of sorbate in bacon production.

SPORE GERMINATION

The inhibitory effects of sorbate have been reported as fungistatic as well as fungicidal (*11,57*). Slow death of yeasts in fruit juices treated with sorbate was reported by Pederson et al. (*69*), while only a fungistatic effect was reported by Harada et al. (*38*). Przybylski and Bullerman (*77*) reported that conidia of *Aspergillus parasiticus* lost viability in presence of sorbate, possibly due to ATP depletion. Low concentrations (0.1-1.0 ppm) of some C_{18} fatty acids (oleic, linoleic, linolenic) inhibited germination of *C. botulinum* spores, and did not affect *Bacillus* spores (*26*). Concerning the effects of sorbic acid on spore-forming bacteria, the evidence is contradictory. It was reported by Curran and Knaysi (*16*) that 0.01-0.10% sorbic acid did not affect the germination of *Bacillus subtilis* spores. Gould (*34*), however, indicated that the rate of spore germination of six *Bacillus* spp. was depressed at sorbate concentrations of more than 0.04% at pH 6.0. The work in our laboratory with comminuted meat products has consistently indicated that sorbic acid concentrations of 0.15-0.20% tested alone or in combination with nitrite, inhibit botulinal spore germination in comminuted meat products (*97,100,101*). However, since some samples eventually become toxic, it would appear that a small number of spores germinate with eventual outgrowth and toxin production.

Nitrite does not affect spore germination but it exerts its effect during outgrowth and before release of the toxin. The latest reports indicate that residual nitrite is a key factor in the botulinal safety of cured meat products (*104*). Sorbate is shown to delay residual nitrite depletion in communited meat products (*96*). Since the sorbate-nitrite effects on botulism control in meat products are synergistic, the postulation can be made that the presence of low nitrite levels in sorbate-nitrite formulations for a longer time may be capable of delaying the outgrowth and toxin production by the few spores that do germinate in the presence of sorbate. Another possibility is that nitrite and sorbate react and form some compound(s) possibly more inhibitory than the parent substances. However, any compounds of this type that have been observed have been associated only with non-food systems of very low pH values (< 4.3) and with very high (0.5 M) nitrite and sorbate concentrations (*39, 49,60*). Nevertheless, the results have conclusively shown that sorbic acid retards botulinal spore germination in comminuted meat products to an extent that exceeds its effect on outgrowth. The mechanism for this action is still unknown.

MECHANISMS

Knowing that sorbate is an antimicrobial preservative that prevents or delays growth through a static or cidal effect either on the spore or the cell, it is of importance to understand the mechanism(s) through which it exerts these effects. An understanding of these mechanisms will enable us to improve and maybe expand its applications and it may be helpful in the search for new, safe and effective preservatives that the world needs to assure its continuous supply of safe, wholesome, nutritious and adequate food.

Some work has implicated the inhibition of various enzyme systems and their reactions as the mechanism of microbial growth inhibition by sorbate. Little or no agreement exists among these reports implicating several enzymes as the site of inhibition. As early as 1954, it was postulated (*57*) that sorbic acid inhibits certain dehydrogenases which are involved in the β-oxidation of fatty acids. α, β-Unsaturated fatty acids are intermediate products in the oxidation of fatty acids by molds. An accumulation of α, β-unsaturated fatty acid, such as that occurring by addition of sorbic acid would prevent the function of the dehydrogenase enzymes, and therefore would inhibit metabolism and growth of the molds. This mechanism, however, was a hypothesis derived from work dealing with the degradation of sorbic acid by molds.

Sulfhydryl-containing enzymes have been implicated again in relation to sorbic acid inhibition. Fumerase was reported as the site of inhibition of oxidative metabolism of catalase-positive bacteria, yeasts and molds in the presence of sorbic acid (*115*). Other sulfhydryl enzymes reported as inhibited by sorbate include aspartase, succinic dehydrogenase and yeast alcohol dehydrogenase (*53,112,117*). It has been postulated (*116,117*) that sorbic acid uncouples oxidative phosphorylation by inhibiting the enzymes within the cell, and that anaerobic inhibition may be related by higher sorbic acid concentrations inhibiting amination of α-ketoglutarate (*117*). As a mechanism of sulfhydryl enzyme inhibition, it was suggested (*117*) that sorbic acid reacts slowly with cysteine through an addition reaction with the thiol group of cysteine and that this is the mechanism of inhibition of the sulfhydryl enzymes. However, Rehm (*80*) reported that the activity of sorbic acid against *Aspergillus niger* was actually increased by cysteine. Another report (*112*) suggested that the action of sorbate is similar to that of maleic acid, which forms stable complexes with sulfhydryl-containing enzymes through a thiohexenoic acid derivative (CH_3-CH=CH-RSCH-CH_2-COOH). A subsequent report (*53*) postulated that sorbate inhibits the enzymes by either the formation of a covalent bond between sulfur of the essential sulfhydryl group or the $ZnOH^-$ of the enzyme and the δ and/or β carbons of the sorbate ion. Another report (*3*) disagreed with the above and postulated that the site of inhibition of fermentation of glucose by Baker's yeast was between 2-phosphoglyceric acid and phosphoenolpyruvate, which involves the enzyme enolase. Another enzyme reported as being inhibited by sorbate is proteinase (*17*), while Harada et al. (*38*) suggested that sorbate inhibited respiration through its competitive action with acetate on the site of acetyl-CoA formation. Sorbate would competitively combine with coenzyme A and acetate and would consequently inhibit the enzyme reaction relating

coenzyme A. Troller, in 1965 (*107*), considering the reports that sorbate was a selective inhibitor against catalase-positive bacteria, reasoned that perhaps the inhibitory effect of sorbate against molds is in inhibiting the activity or synthesis of the enzyme catalase. In the results, it was shown that sorbate inhibited germination of *A. niger* and catalase activity. It was therefore concluded that one of the mechanisms by which sorbic acid exerts its fungistatic effects is through inhibition of catalase. Inhibition of catalase would result in increased hydrogen peroxide concentration to a point where it could act on certain vital metabolic processes and prevent spore germination. Based on previous reports that catalase not only brings about destruction of hydrogen peroxide but itself is destroyed by this compound in the process, and assuming that sorbic acid is susceptible to autoxidative deterioration passing through the stage of a peroxide (sorbyl peroxides) it was postulated (*107*) that sorbic acid in its peroxide state may inactivate catalase or inhibit the activity of other enzymes or co-enzymes vital to mold cell development. It was also postulated by Tongue (*106*) that unsaturated fatty acids undergo oxidation through a free-radical mechanism which prevents outgrowth. There are reports, however, that sorbic acid is resistant to oxidation in foods (*51,56*). This theory also contradicts results of nitrite-sorbate combinations, because nitrite is known to act as an antioxidant and quench free-radicals and therefore retard oxidation. Dealing with a particular, frequently isolated, enzyme system or certain microorganisms under a controlled set of conditions, are limitations that prevent the results of the above studies from being generalized. The results may be perfectly correct under the particular conditions examined, but the mechanisms extracted constitute only postulations and hypotheses that may not be applicable under various conditions of testing.

Another attempt to understand the functions of sorbate is to briefly summarize some proposed mechanisms of action of fatty acids in general. Such generalizations, which utilize results of work with other similar compounds and with various microorganisms, may prove valuable in planning and coordinating future research which will enable us to better define the mechanisms of antimicrobial sorbate activity. Long-chain fatty acids have been reported as more inhibitory against gram-positive than against gram-negative bacteria (*27-30,62,89-92*). It was also reported (*62*) that the mechanism of antibacterial fatty acids varies, depending on the species and the structure of the fatty acid molecule. Nieman (*62*) concluded that the inhibitory effects of unsaturated fatty acids become more pronounced with increasing chain length and degree of unsaturation, and that the natural cis-forms exhibit a greater antibacterial activity than the corresponding trans-isomers. It was also reported (*62*) that saturated fatty acids can act also as growth inhibitors, with optimal antibacterial properties at a chain length of about 12 carbon atoms. Freese et al. (*27*) indicated that the amount of fatty acid necessary to produce inhibition of gram-positive *B. subtilis* decreases with increasing chain length and that unsaturated and saturated fatty acids are equally effective. Fatty acids with a chain length up to six carbon atoms produced similar inhibition against both gram-positive *B. subtilis* and gram-negative *E. coli* and *S. typhimurium*. As the number of carbon atoms increases, so does the concentration required to inhibit the gram-negative organisms, and the long-chain compounds are being inhibitory only to gram-positive organisms (*90*). Gram-positive and gram-negative organisms differ in that the latter possess an outer lipopolysaccharide structured membrane which is an effective barrier against hydrophobic substances, while the cytoplasmic membrane is very permeable (*63*). It has been suggested that the difference in fatty acid sensitivities between gram-positive and gram-negative bacteria may result from the outer membrane of gram-negative bacteria preventing fatty acids from reaching the inner fatty acid-sensitive cytoplasmic membrane (*27,90,92*). An increased permeability of the microbial membrane was also associated with inhibition of *S. aureus* by linoleic acid, and it was suggested that linoleic acid increases the permeability of the membrane by acting as a surfactant (*35*).

Freese et al. (*27*) and Sheu et al. (*92*) generalized that lipophilic acid preservatives uncouple both substrate transport and oxidative phosphorylation from the electron transport system. Growth is apparently depressed by inhibition of cellular uptake of amino acids, organic acids, phosphate and other compounds. Saturated compounds apparently only uncouple while unsaturated fatty acids also inhibit the electron transport system, and resistant organisms can prevent accumulation of the inhibitor on the membrane (*27*). Inhibition of transport of carbohydrates was also suggested as the inhibitory mechanism of sorbic acid (*18*). Interference with the transport of molecules was postulated by Kodicek (*50*) as a physicochemical mechanism. Molecules of unsaturated fatty acids are absorbed into the transport-active patches of the lipoprotein layer of the cytoplasmic membrane. The double bonding of unsaturated fatty acids sets up pressures which lead to steric disorganization and interference with the transport mechanism. Uncoupling of active transport systems from the cellular energy supply was suggested as the mechanism of yeast inhibition under anaerobic conditions (*111*). Permeation inhibition and inhibition via membrane disruption were discredited by Anderson and Costilow (*2*), who suggested that electron-transport inhibition is the mechanism with its primary site between cytochrome C and oxygen. Another report has suggested that a proton or charge gradient is involved in energizing membrane transport and that the undissociated form of sorbate could discharge this gradient by diffusing through the membrane and ionizing when it reaches an interior compartment of the cell with a higher pH (*42*).

It is obvious from the summarized work that there is

not agreement among scientists as to the mechanism(s) of sorbate action against microorganisms. The generalizations presented, as mentioned above, are postulations based on hypotheses and results of studies under specific conditions with particular strains of microorganisms or with isolated enzyme systems. The observations may be exact for one set of conditions but may not hold true for another, especially at the whole cell level. This, obviously, would suggest that there is no single mechanism known to date which can be held responsible for the antimicrobial activity of sorbate, but there may be a series of factors involved that bring about inhibition under one or more sets of circumstances. The diversity of microorganisms inhibited by sorbate; the variety of enzymes affected, and the variability of the results among groups, species, and even strains of the same species, strongly support this speculation. Generally, it can be concluded that no single mechanism has been reported that can claim full responsibility for food preservation by sorbate. There is a series of basic questions that remain unanswered, the understanding of which would greatly facilitate the search for mechanisms. Such general questions include: how the effects of sorbate differ from yeasts to molds and especially to bacteria, how these effect differ between spores and vegetative cells, between catalase-positive and -negative organisms, between gram-positive and -negative bacteria, under aerobic or anaerobic conditions, and from laboratory media to food systems. It is evident that we need extensive and basic research that is well coordinated among several laboratories, institutions, and groups of scientists, to be able to explain the inhibitory effects not only of sorbate, but of other food preservatives as well.

SUMMARY

Sorbate is an effective inhibitor of many microorganisms, including yeasts, molds and many bacteria. It is used in the preservation of a wide variety of products throughout the world. As with other food preservatives, it has advantages as well as limitations, but generally, when used with proper planning and under the correct conditions, the advantages outweigh the disadvantages. Recently, in an effort to reduce nitrite and potential nitrosamine levels in cured meat products, sorbate has been found effective in delaying *C. botulinum* toxin production, especially in combination with low nitrite (40-80 ppm) levels. Its antimicrobial effect appears to be on spore germination as well as outgrowth, but the mechanisms of action are not well understood and a concentrated effort is needed before they can be elucidated. Generally, sorbate is one of the very few preservatives that are permitted in food products, but food preservatives and additives in general have come under increased criticism in recent years. This is happening in a period during which we can monitor and regulate their use more closely, and when we need their assistance to enhance preservation with reduced energy to face economic and world population challenges. We can, therefore, conclude that sorbate as a food preservative is valuable and will remain in use. Our task is to ensure that it is used properly in appropriate situations.

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