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Anexo al presente:

1. Copia del documento SOFOS ET AL. "Interactions and Synergistic Effects".
Sorbate food preservatives, 1989, págs. 55 – 194

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INTERACTIONS AND SYNERGISTIC EFFECTS

I. INTRODUCTION

Environmental, compositional, and processing factors, such as pH of the substrate, concentration of sorbate, water activity, temperature of processing and storage, gas atmosphere and packaging, type of microbial flora, initial microbial load, composition of the food, and other additives and certain food components, singly or in combination, can influence the antimicrobial activity of sorbates.

These factors, acting in combination with sorbate, may broaden, intensify, enhance, or negate its antimicrobial activity. Thus, they can act synergistically or be antagonistic to sorbate. This, of course, depends on individual factors, levels, and combinations. If growth of spoilage or pathogenic microorganisms is inhibited in a system but the organisms remain viable, then growth may eventually resume when environmental factors and other conditions change and become supportive to growth or antagonistic to sorbate. The length of microbial inhibition and product shelf life will vary with the intensity and importance of these factors that influence the activity of sorbate. The complexity of the various interactions in nonhomogeneous food systems makes it difficult to estimate or predict the antimicrobial contribution of sorbate under commercial conditions. Variation in interactions may also be responsible for discrepancies in the antimicrobial activity of sorbate reported in the literature.

Combining food preservatives may enhance, reduce, or cause no change in their individual antimicrobial effects. Thus, the interaction of two or more preservatives may be synergistic, additive, or antagonistic.¹ An interaction is synergistic when a combination of smaller amounts (e.g., 40%) than the minimum inhibitory concentration (MIC) of each of two preservatives results in 100% inhibition of the test organism. In contrast, the relationship is antagonistic when 50% of the MIC of the compound and a larger amount (i.e., 85%) of the second preservative are needed for 100% inhibition of growth. The additive effect occurs when a combination of 50% of each preservative results in 100% inhibition.^{1,2} Although an additive effect does not permit a reduction in the total amount of chemicals needed for preservation, its usefulness is usually related to a broader spectrum of microorganisms being inhibited by the combination. The actual type of interaction between two chemical food preservatives can be variable, depending on amounts used, microorganisms being inhibited, type of food, and environmental conditions.

Several examples in the literature have indicated synergistic and antagonistic effects of sorbate with other chemicals. A mixture of sorbate and sulfur dioxide has acted synergistically to inhibit *Aspergillus niger*,³ while their relationship may have been additive against *Escherichia coli*.² The effects of combinations of preservatives on *E. coli* have been classified into three groups.² Benzoic acid and ethyl and propyl parabens tended to be additive or slightly antagonistic when used in combination with other preservatives. Sorbic acid, boric acid, and sodium formate tended to be antagonistic when used in combination with other preservatives, while sodium sorbate tended to be synergistic. Additive interactions also exist against *E. coli* between sorbate and formic acid, benzoic acid, and parabens.² Sorbate and formic acid combinations are antagonistic against *Saccharomyces cerevisiae* and synergistic against *A. niger*.^{1,3,4}

The influence of various natural food components and added chemical compounds in food on the antimicrobial action of sorbic acid and other preservatives has been studied against *A. niger*.⁵ Sodium, potassium, and magnesium chlorides at 0.01 to 0.25% and sugars were without influence; isobutyric acid, gluconic acid, and cysteine-HCl were synergistic.

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and calcium chloride, malonic acid, and malic acid were antagonistic to sorbate. The binary combinations of nisin or tylosin with parabens were synergistic with sorbic and benzoic acids against *Lactobacillus buchneri*, additive against *Staphylococcus aureus*, and antagonistic against *E. coli*.^{6,7}

II. INTRINSIC PARAMETERS

A. FOOD COMPONENTS

Since solubility, distribution, and partition of sorbate in foods varies with composition and types of individual components, its antimicrobial activity will also be variable. Factors that influence the selection of a food preservative and the concentration to be used include the composition of the food; its pH; water content; presence of other inhibitors (e.g., salt, sugar, spices, smoke, acids, chemical additives); initial level and type of contamination, which is influenced by type of processing, ingredients, and sanitation of premises and equipment; and handling and distribution of the food (e.g., length and temperature of storage, frequency of consumer use, and expected shelf life).⁸

Compared to other common food preservatives, sorbate has a more favorable fat-to-water partition ratio (Chapter 3).^{1,9,10} This enhances the usefulness of sorbate in lipid-containing foods, such as mayonnaise, margarine, and salad dressings, because in these fat-in-water emulsions a relatively higher level of sorbate, compared to other preservatives, is found in the microbiologically susceptible water phase. Increased amounts of fat, however, will reduce the concentration of sorbate in the aqueous phase where microbial control is necessary.¹¹ Salt, sugars, and other soluble food components will also reduce the concentration of sorbate in the aqueous phase.^{1,9,12} The synergistic activity of sorbate with sugar and salt; however, may offset the negative effects of these solutes on the solubility of sorbate.¹²⁻²⁰

Presence of acids in the food system may reduce the water solubility of sorbate (Chapter 3), but they also enhance microbial inhibition by increasing the amount of undissociated sorbic acid, by increasing the hydrogen ion concentration and by their own direct antimicrobial effects.^{1,12} The specific type of acid present in the food will also be influential on the antimicrobial activity of sorbate. Acetic acid decreases the fat-to-water partition ratio of sorbate.¹² Citric and lactic acids enhance the antimicrobial action of sorbate to a greater extent than inorganic acids.^{21,22} In complex food systems, however, it is usually difficult to predict multifactor interactions and their positive or negative contribution to the antimicrobial activity of sorbate.

In certain instances, microbial inactivation or inhibition is more efficient in simpler substrates, while in others the opposite is true. For example, inactivation of *Salmonella typhimurium* by sorbate was more rapid in nutrient broth and required substantially more time in food systems (i.e., skim milk and evaporated milk).^{23,24} Inhibition of *Aspergillus parasiticus* growth and aflatoxin production, however, was more pronounced in an enriched medium compared to a basal medium.²⁵ The inhibitory effect (Table 1) of sorbate on sublethally injured (i.e., freezing, heat) pseudomonads was greater in basal broth than in a rich medium (i.e., tryptone-peptone-extract).²⁶ Similarly, inhibition of growth of *Pseudomonas aeruginosa* by sorbate was less in substrates containing bouillon, yeast extract, casaminoacids, or peptone than in a medium containing ammonium nitrate.²⁷ These discrepancies may be due to variation in other factors involved in inhibition among different experimental conditions. Thus, studies should be designed to examine microbial inhibition by sorbate under conditions encountered in specific applications.

B. MICROBIAL FLORA

The influence of the type of microbial flora and variation in inhibition among microbial strains are discussed in Chapters 4 and 6. In general, the types and number of microorganisms

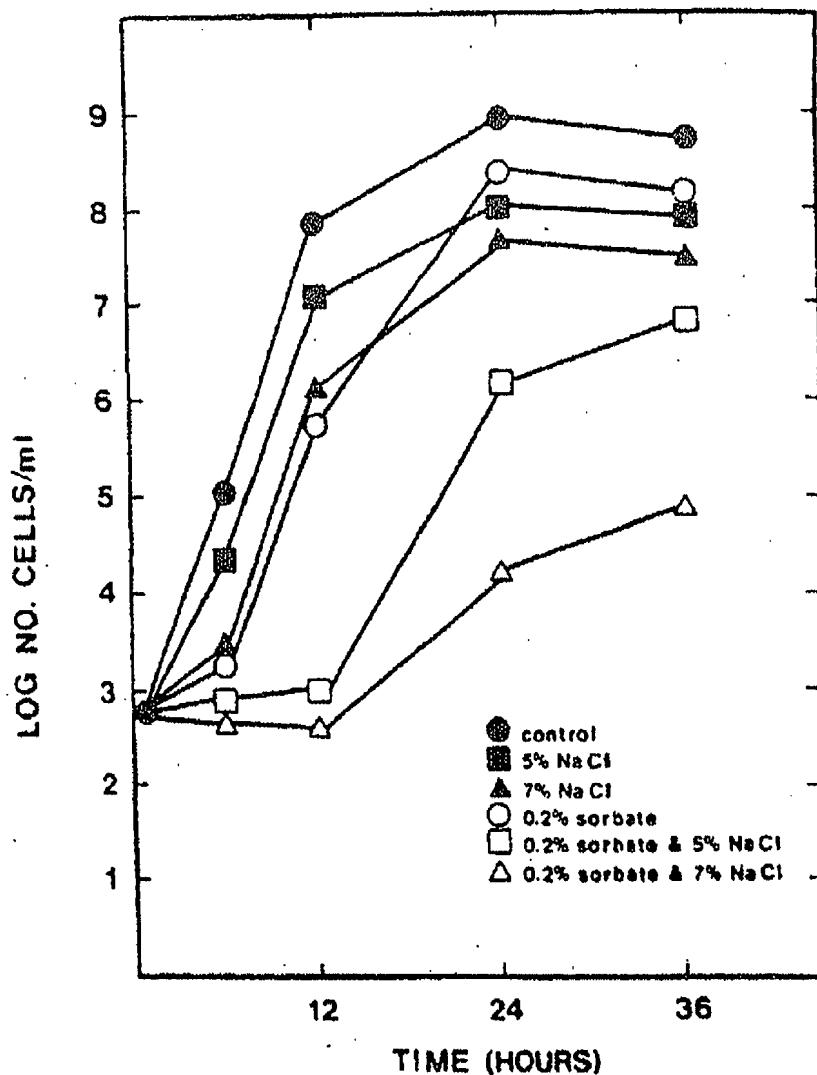


FIGURE 1. Interaction of potassium sorbate and sodium chloride against growth of *Staphylococcus aureus* 12600 in pH 6.0 trypticase soy broth at 37°C. (From Robach, M. C. and Stateler, C. L., *J. Food Prot.*, 43, 208, 1980. With permission.)

spp.)^{13,30-35} and bacteria such as *S. aureus* (Fig. 1),¹⁹ (*Staphylococcus epidermidis*,¹³ *Staphylococcus* spp.,³⁶ *Brochothrix thermosphacta*, *Serratia liquefaciens*, *Bacillus cereus*, *Yersinia enterocolitica*, *Lactobacillus* sp.,³⁷ *Micrococcus* sp.,³⁶ *Clostridium sporogenes*,³⁸⁻⁴¹ *Salmonella typhimurium*,^{42,43} and *Clostridium botulinum*.^{44,45}

In addition to nutrient media, increased inhibition at higher solute levels has been reported in egg yolk,³⁶ uncured poultry products,³⁹ strawberry jam,³⁰ raspberry syrup,⁴⁶ butter,^{31,47} whey,⁴⁸ grape juice,³³ prunes,³⁴ mango pulp,⁴⁹ biltong,³⁵ processed cheese spreads,⁴⁵ comminuted beef/pork^{37,38} and in a pork slurry.⁴⁴ Use of 1% sorbate has permitted reduction of the sodium chloride level from 11% to 7% to achieve the same inhibition of staphylococci and micrococci in egg yolks of pH 6.5.³⁶ Stable, uncured poultry products can be formed with very low salt levels (0.65%) when sorbate (0.20%) is included in the formulation.³⁹

The lack of inhibition of lactic-acid-producing bacteria and clostridia by sorbate reported in early studies (Chapter 6) may have also been influenced by the absence of sodium chloride from the culture media. Studies reporting inhibition of *C. botulinum* in cured meat products have used 1.5 to 2.5% sodium chloride in the formulation, which may have been critical in inhibition.⁵⁰

It is interesting that solutes, such as sodium chloride and sucrose, increase the inhibitory activity of sorbate, considering the fact that they reduce sorbic acid solubility in water and increase the partition ratio in oil-to-water from 3.0 to 5.0, when they are present at con-

centrations >10% (Chapter 3).^{9,12} Apparently, their inhibitory activity through lowering of water activity and the increased activity of sorbate at lower water activities compensate for any changes in solubility.

The influence of reduced water activity on the action of sorbate is not always positive. Presence of sucrose or sodium chloride in certain situations has reduced the synergistic effect of sorbate and heat on the inactivation of microorganisms.²⁰ Cells of *S. typhimurium* remained viable for a longer period of time in a soy-starch system of water activity 0.79 than 0.97. In general, as storage time and temperature (22°C vs. 40°C) increased, the cells were stressed at a faster rate at the higher water activity values.⁴³

In nutrient broth, sodium chloride (1.25 and 2.5%) decreased the effects of potassium sorbate and sodium acid pyrophosphate against *C. botulinum*.⁵¹⁻⁵³ Decreasing the sodium chloride level from 2.5% to 1.25% reduced the antibotulinal effects of phosphate and sorbate, but elimination of sodium chloride increased the inhibitory effect. At these low levels, however, sodium chloride may have acted to stimulate growth of the organism.⁵⁴ Levels of 1.25 to 2.50% (w/w) sodium chloride in foods of 50% solids are in the range of 2.5 to 5.0% brine (w/v), while in the broth system the brine level was essentially equivalent to the incoming salt level (1.25 to 2.50%).

Preconditioning of *Saccharomyces rouxii* cells in 60% sucrose + 0.1% sorbate rendered the cells more sensitive to inhibition by sorbate compared to cells preconditioned in 0% sucrose + 0.1% sorbate.⁵⁵ Lowering of the water activity, however, enhanced the resistance of *S. rouxii* to increasing concentrations of sorbate.^{56,57}

In general, it is believed that solutes increase the inhibitory activity of sorbate by reducing the water activity. A potential mode of action for this phenomenon may be that the solutes induce cellular swelling, which increases the sensitivity of microorganisms to preservatives.^{1,4}

D. INFLUENCE OF pH

It has been well established that the antimicrobial activity of weak, short-chain, lipophilic acid preservatives such as sorbate is dependent on the pH of the substrate.^{1,4,9,10,50,58} In general, antimicrobial activity increases as the pH of the environment decreases and approaches the dissociation constant (pK_a value) of the compound. Thus, sorbate is more effective as a preservative in food products with lower pH (<6.5) values.^{8-10,59-61}

Increased inhibition by sorbate with reduced pH has been reported for several species of microorganisms, including *Bacillus subtilis*,⁶² *C. botulinum*,^{44,45,63-66} *Staphylococcus aureus*,^{67,68} aflatoxin production by *A. parasiticus*,⁶⁹ *Pseudomonas fluorescens*,⁷⁰ *P. aeruginosa*,²⁷ *Salmonella enteritidis*,⁷¹ and *Salmonella typhimurium*.⁷² Increased inhibition at reduced pH values has been observed in both nutrient media and foods.

The maximum pH for inhibition by sorbate is believed to be in the range of 6.0 to 6.5.^{1,9,10} Optimum effectiveness, however, is at pH values below 6.0, while it is relatively ineffective at pH values of 7.0 and above.^{10,62,63,66,73}

Sorbates have the advantage of being effective at pH values higher (6.0 to 6.5) than those for propionate (5.0 to 5.5) and benzoate (4.0 to 4.5), which are also commonly used as food preservatives.^{9,10} The ability of sorbate to act as an antimicrobial agent at higher pH values (>5.5) allows use of the preservative in more foods than benzoates and propionates and in foods where esters of parahydroxy benzoic acid (i.e., parabens) may not be useful because of solubility and water-to-fat partition problems. Sorbates can also be used in combination with benzoates or propionates in foods of reduced pH. This is done to minimize off-flavors by using lower concentrations and to broaden the scope of microorganisms inhibited.^{9,10,12,28}

Overall, it is very important to use sorbate and other chemical food preservatives in products of appropriate pH values. The pH at which inhibition becomes significant, however, is dependent on factors such as concentration of sorbate, species and strain of microorganism.

ganisms, temperature of storage, type and properties of the substrate, acidulant used to adjust the pH, etc.

Inhibition of *S. typhimurium* by 0.3% sorbate was observed at pH values below 6.7 in nutrient broth and at pH 6.4 in skim milk.⁷³ Overall, however, inactivation was greater at pH 5.0 than 5.5. The lower the pH of the substrate, the lower the concentration of sorbate needed for microbial inhibition.⁷⁴ At pH 5.5, sorbate was bacteriostatic and bacteriocidal to various bacteria at a concentration of 0.1%, but at pH 7 to 8, 10% of sorbate was ineffective.⁷⁵ This has also been demonstrated in the inhibition of ochratoxin A production by *Aspergillus sulphureus* and *Penicillium viridicatum*.⁷⁶ At pH values below 4.0, very low concentrations of sorbate (0.002%) may be inhibitory to fungi, yeasts, and bacteria.^{69,61}

Depending on microorganisms or substrate, low sorbate concentrations can be inhibitory even at higher pH values. A concentration of 0.05% sorbate delayed growth of *Vibrio parahaemolyticus* in crab meat and flounder homogenates at pH 6.2.⁷⁷ In emulsified chicken meat products, however, levels of 0.2% sorbate were needed to delay growth of *C. botulinum* at pH values of less than 6.0 or 6.2, depending on the absence or presence of nitrite in the formulation, respectively.⁶³ No inhibition was observed in chicken meat products with pH values above these levels. The minimum inhibitory concentration of sorbate against *Penicillium* molds at pH 4.75 was 0.020 to 0.025%, while at pH 5.5 it was 0.06 to 0.08%.⁷⁸ In nutrient broth, a level of 0.05% sorbate inhibited growth of *Pseudomonas fluorescens* at pH 5.5, while at pH 6.0 a concentration of 0.2% sorbate was necessary to delay growth.⁷⁰ The influence of pH on the antimicrobial activity of sorbate will also vary with individual microbial strains under consideration.^{79,80}

In addition to overall growth and production of metabolites (e.g., mycotoxins), inhibition of spore germination by sorbate is also more pronounced at lower pH values.^{66,79} Inhibition of uptake processes, such as uptake of amino acids (i.e., L-serine and L-histidine) by *S. typhimurium*, is also more pronounced at lower pH values.⁷² Oxygen uptake by *P. aeruginosa* with several substrates (i.e., acetate, pyruvate, lactate, α -ketoglutarate, and oxaloacetate) is lower (10-fold) in the presence of sorbate at pH 5.3 compared to pH 6.8.²⁷

While the antimicrobial activity of sorbic acid and its metal salts is increased with reduced pH values, other derivatives of sorbate show antimicrobial activity which is almost independent of pH. For example, esters of sorbic acid show antimicrobial activity at levels as low as 0.002%, independent of pH of the substrate.⁶¹

Depending on the type of sorbate added to a substrate, its pH may also change. Use of potassium sorbate is not expected to result in major changes (± 0.10 pH units) in the pH of the food, while sorbic acid may reduce pH values by 0.20 to 0.40 pH units.^{38,58,81} The influence of the type of acidulant used to adjust the pH on microbial inhibition is discussed in another section.

Although it has been well accepted that the antimicrobial activity of sorbate is more pronounced at pH values below 6.0 to 6.5 with virtually no inhibition at pH values near neutrality, there is evidence in the literature that sorbate has inhibited microbial growth in nutrient media and foods of pH values above 6.5. Sorbate inhibited *B. subtilis* in the rice filling of Karelian (from Finland) pastry at pH 6.60⁸² and extended the shelf life of cod fish with an initial pH of 6.7 to 7.0.⁸³ At pH 7.0, sorbate (0.5%) has also inhibited *Moraxella* organisms isolated from seafood.³⁶ Sorbate has also inhibited *C. botulinum* at pH 6.5⁸⁴ and 6.9.⁶⁵ In addition, sorbate levels as low as 0.003% showed inhibition of *V. parahaemolyticus* in a broth of pH 6.7. Other studies, however, may have not been designed or their methodology may have been insufficient to detect minor inhibitory effects that may have taken place at higher pH values.⁶⁵

1. Dissociation

In general, the interaction of pH with sorbate is not completely understood. The increased inhibitory activity of sorbate at lower pH values has been attributed to the influence of pH

on the amount of undissociated acid present in the substrate (Chapter 3).^{9,10} In general, the antimicrobial activity of sorbate and other weak lipophilic acids has been attributed for a long time to the undissociated form and not the acid anion of the compound.^{4,11,29,60,64,85-93}

Sorbate and similar organic acids dissociate in aqueous solutions and release hydrogen ions. The extent of their dissociation depends on the pH value of the solution. Certain acids, including acetic, reduce the pH of the substrate and inhibit microbial growth through the high concentration of hydrogen ions.¹ Sorbate and similar weak acids, however, cause only minor decreases in pH and act as antimicrobials mostly through their undissociated species.^{4,87,94,95} Since the amount of the compound in the undissociated form is determined by the pH of the environment, antimicrobial activity is enhanced at pH values approaching the dissociation constant (pKa value), where 50% of the acid is in the undissociated form.

With antimicrobials such as sorbate, concentrations as high as 0.1 to 0.3% are needed for microbial inhibition, especially as the pH of the substrate is increased (>5.0). This is in contrast to lower levels (<0.1%) needed in substrates of low (<5.0) pH, and the small amounts (0.01 to 0.02%) of other preservatives (e.g., sodium nitrite) which are effective even at higher pH values. If we accept, however, that only the undissociated form of sorbate is effective as an antimicrobial, then the need for these higher ingoing concentrations can be easily explained. Since only part of the ingoing sorbate will be in the undissociated form, then, depending on pH, only a portion of the ingoing levels will be effective as antimicrobials.⁵⁸ In a product of pH 5.8 with 0.2% ingoing sorbate, only 0.014% of the compound will be in the undissociated form. This is theoretical and may be even lower if we consider other factors that will influence solubility, partition, and dissociation of sorbate in individual substrates. Other reasons for the need of higher sorbate levels may also be related to mechanisms of antimicrobial action by various preservatives (Chapter 7).

In addition to pH, dissociation of sorbate is influenced by its solubility and the water activity of the system (Chapter 3).^{1,96} Thus, in order to predict the effectiveness of sorbate in a food product, we need to know the pH, its solubility, and the dissociation constant for the particular system. Although there is variation in the literature concerning the pKa value of sorbic acid, the most commonly quoted number (pKa) is pH 4.76.^{9,10,99} This value is based on the dissociation constant of $1.73 \times 10^{-5} \text{ mol dm}^{-3}$. As indicated above, however, the pKa value may vary because it is influenced by other factors, including water activity.⁹⁷ This is because dissociation requires the association of the hydrogen ion of the carboxylic group with water to form a hydroxonium ion.⁹⁸ Information on the dissociation constant of sorbic acid in presence of solutes is limited, with the exception of work on dissociation in water-glycerol mixtures.^{97,99}

The pKa value for sorbic acid at 25°C in water-glycerol mixtures can be fitted, with a standard deviation of fit of 0.017, by the equation: $\text{pKa} = 4.680 + 0.0123 M - 2.66 \times 10^{-4} M^2$, where $M = \% \text{ (w/w) glycerol in the solution}$.⁹⁷ Values for pKa determined at 25°C include 4.674 in absence of glycerol, 4.807 in 20% glycerol, and 4.625 in 50.02% glycerol. The dissociation constant of sorbic acid in water at 25°C, determined by Pethybridge et al.,⁹⁷ is $2.12 \times 10^{-5} \text{ mol dm}^{-3}$ (pKa = 4.67), which is similar to the original value of $1.73 \times 10^{-5} \text{ mol dm}^{-3}$ (pKa = 4.76) and the commonly reported pKa value of 4.8.²⁹

Considering the nonhomogeneity of food systems, the difference between the above dissociation values should not be considered significant enough to result in major variations in microbial inhibition by sorbate. Also, it has been demonstrated that with changes in water activity between 0.80 and 1.00, pKa values vary only between 4.60 and 4.80.⁹⁷ Thus, changes in pKa values with reduction in water activity are not large enough to result in major differences in inhibition due to differences in the amount of undissociated acid available in complex nonhomogeneous food systems.⁹⁷

Overall, the dissociation properties of sorbic acid appear to be relatively constant if

sorbate. Variations in the pH of the food, however, will change the amount of sorbate in the undissociated form, and this will influence its antimicrobial activity. Thus, any factor that may influence product pH will also affect the antimicrobial activity of sorbate.

Although the antimicrobial activity of sorbate is generally believed to increase with reduced pH values, as indicated above, a few studies have demonstrated antimicrobial action even at pH values (>6.5) above its accepted maximum. A few studies have also shown that the activity of weak acids, including sorbic, is sometimes less influenced by pH changes than believed.¹⁰⁰⁻¹⁰⁴ Since at higher pH values the undissociated portion of the compound is minor or nonexistent, the question is whether the dissociated form of the compound is also antimicrobial.

Rehm and Lukas¹⁰⁰ attempted in 1963 to determine the inhibitory efficiency of the undissociated and dissociated forms of sorbate and indicated that the dissociated molecules may show antimicrobial activity, which can be variable among microbial species. Antimicrobial activity, however, was mostly due to the undissociated form, since the minimum inhibitory concentration of the anion was 20 to 40 times that of the free acid for *Saccharomyces cerevisiae*, 100 times for *A. niger*, and 300 to 400 times for *E. coli*.¹⁰⁰

The popular theory that growth is inhibited only by the undissociated acid was also questioned 20 years later by Eklund.¹⁰⁴ A study evaluated the minimum inhibitory concentration of sorbic acid for *B. subtilis*, *B. cereus*, *E. coli*, *P. aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* at different pH values. The author derived a mathematical model for ascribing antimicrobial activity to the undissociated acid, based on the traditional theory of inhibition only by the undissociated form of the compound. This equation,

$$MIC_{total} = \frac{(MIC_{undiss})pH(10^{pH})}{10^{pK}} + MIC_{undiss} \quad (1)$$

states that the minimum inhibitory concentration of the total acid increases exponentially with increasing pH. This, however, did not agree with his experimental data and, thus, Eklund¹⁰⁴ derived a new mathematical model,

$$MIC_{total} = \frac{1}{\frac{(1-a)}{MIC_{diss}} \frac{a}{MIC_{undiss}}} \quad (2)$$

$$a = \frac{[HA]}{[A^-] + [HA]} = \frac{1}{10^{pH-pK} + 1} \quad (3)$$

to fit the experimental total MIC values at different pH levels. Using this new model, Eklund¹⁰⁴ concluded that the dissociated acid had an effect on inhibition, but it was 10 to 600 times less than the effect of the undissociated acid. At pH values above 6.0, however, more than 50% of the preservative action of sorbic acid was attributed to the dissociated species.

These results indicate that the commonly held view that only the undissociated acid is effective may not be true. Therefore, the concept that sorbic acid causes acidification of the interior of the cell and that this is the main mechanism of inhibition cannot completely explain inhibition of growth by sorbic acid at pH values near neutrality. It would appear that more than one mechanism of inhibition is involved (Chapter 7).

III. PROCESSING VARIABLES

A. HEAT TREATMENT

Sorbate may interact with heat treatments and affect dormancy of heated spores, rate and extent of microbial destruction during heating, and recovery of heat-injured spores. Low levels (0.01%) of sorbate may have a stimulatory influence on breaking dormancy during heat activation, as indicated with ascospores of *Byssoschlamys nivea* in grape juice.¹⁰⁵ Low sorbate levels may also act synergistically with heat to inactivate yeasts, molds, and bacteria. Sorbate also can inhibit repair of thermally injured cells.

Increased thermal inactivation in the presence of sorbate has been demonstrated with several yeasts, including *Candida utilis*,¹⁰⁶⁻¹⁰⁸ *Saccharomyces cerevisiae*,^{109,110} *Candida krusei*, *Hansenula anomala*, *S. bailii*, *Torulopsis magnoliae*,¹¹⁰ *Debaryomyces hansenii*, *Pichia membranaefaciens*, *Kloeckera apiculata*, and *Rhodotorula rubra*.^{111,112} Increased thermal inactivation of molds in the presence of sorbate has been reported for *Aspergillus flavus*, *Geotrichum candidum*, *Penicillium puberulum*,^{20,113} *Byssoschlamys nivea*,¹¹³ and *A. niger* (Figure 2).¹⁰⁶

Also, certain bacteria have undergone enhanced thermal inactivation in the presence of sorbate. These include *Pseudomonas fluorescens*,^{109,114} *Bacillus licheniformis*,¹¹⁵ *E. coli*,^{106,108,116} *Lactobacillus viridescens*,¹¹⁷ and *V. parahaemolyticus*.¹¹⁸

In addition to enhanced thermal destruction of yeasts, molds, and bacteria in the presence of sorbate, recovery of heat-injured organisms is also inhibited by presence of sorbate in the substrate (Table 2). Thermally injured yeasts inhibited by sorbate in the recovery medium include *C. utilis*,^{106,107} *D. hansenii*, *Pichia membranaefaciens*, *S. cerevisiae*, *C. krusei*, *K. apiculata*, and *R. rubra*.^{111,112} Inhibition of repair in molds has been demonstrated with *A. parasiticus*,⁶⁹ *A. flavus*, and *Penicillium puberulum*.^{20,113,119}

Shibasaki and Tsuchido¹⁰⁶ also reported inhibition of repair of *E. coli*. Sorbate, however, had no effect on the recovery of heat-treated (70 to 95°C, 50 min) *Bacillus firmus* and *B. pulvifaciens* spores.¹²⁰ It is also interesting to note that a significant interaction between heat treatment and sorbate existed in a pork slurry inoculated with *C. botulinum* spores. The result of the interaction, however, was to increase spoilage and probability of toxin production.⁴⁴ Thermally injured pseudomonads cells were more sensitive to sorbate during recovery than freeze-injured cells.²⁶ The importance of a report¹²¹ indicating that sorbate retarded thermal inactivation of the enzyme polyphenoloxidase is unknown. Sorbic acid has also reduced the heat treatment necessary for canning of blue-green algae for feed or other uses.¹²² Addition of sodium chloride (8%) and sorbate (0.3%) reduced the heat treatment and minimized destruction of product quality.

Thus, several microorganisms with different physiological characteristics show enhanced thermal destruction and injury in the presence of sorbate, and sorbate retards or prevents repair of their injury.

Concentrations of sorbate effective in increasing thermal destruction and inhibition of repair range from 0.0025% to 3.0%.^{71,108-111,113,114,118} The higher levels are usually needed for the inactivation of bacteria. Increasing sorbate levels also increase the extent of inactivation or inhibition.

The influence of sorbate on thermal inactivation and recovery of injured organisms is variable with species and strains of microorganisms. A higher level (0.01%) was needed to cause a significant increase in thermal inactivation of *P. puberulum* compared to the amount (0.005%) needed for *A. flavus* and *G. candidum*.¹¹³ Certain species (e.g., *P. thomii*) may be insensitive to sorbate during thermal inactivation.¹⁰⁶

Resistance of microorganisms to heat is increased in the presence of solutes (e.g., sodium chloride, sucrose) that lower the water activity of the substrate. Regardless of solute con-

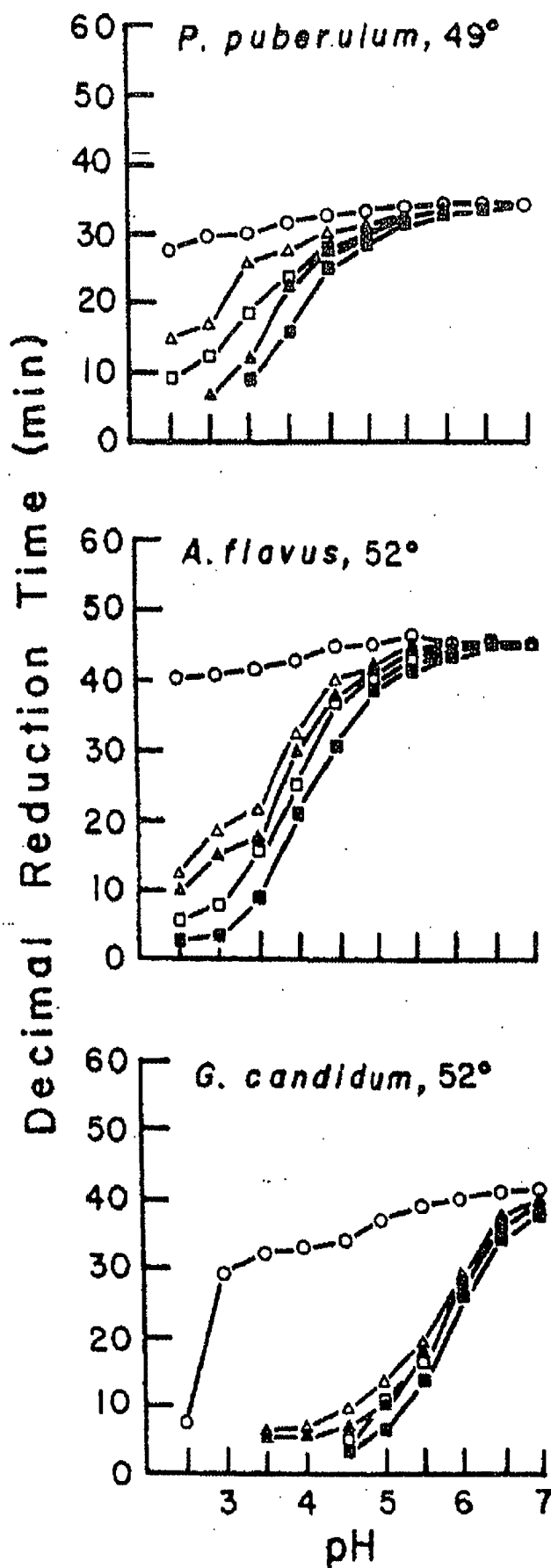


FIGURE 2. Influence of pH and preservatives in the heating medium on the rate of thermal inactivation of molds. 0, no preservative; Δ , 0.05% potassium sorbate; $\square$, 0.1% potassium sorbate; $\blacktriangle$, 0.05% sodium benzoate; $\blacksquare$, 0.1% sodium benzoate. (From Beuchat, L. R., *J. Food Prot.*, 44, 450, 1981. With permission.)

TABLE 2
Effect of Sorbate on Growth Characteristics of Heated *Pseudomonas* I spp.²⁶

Heating treatment		Potassium sorbate in broth (%)	Tryptone-peptone extract broth	
(°C)	(min)		Lag(h)	GT(h)
—	—	0	0	3.2
—	—	0.3	0.5	3.4
45	5	0	2	3.3
45	5	0.3	3.5	3.4
50	3	0	13.5	3.6
50	3	0.3	19	4
50	5	0	18	3.6
50	5	0.3	20	4

Note: GT, generation time.

TABLE 3
Effect of Sorbate and pH on Heat Resistance of Molds¹¹³

Organism	Potassium sorbate (%)	Decimal reduction time (min)		
		pH 2.5	pH 3.5	pH 4.5
<i>Penicillium puberulum</i>	0	29	30	33
	0.01	23	29	31
<i>Aspergillus flavus</i>	0	41	43	45
	0.01	19	37	43
<i>Geotrichum candidum</i>	0	7	29	31
	0.01	5	9	14

Note: GT, generation time.

instances, however, the detrimental effects of sorbate are reduced in the presence of high concentrations (30 to 50%) of sucrose.¹¹⁰ The influence of sorbate on the heat resistance of molds and yeasts may be influenced by the type of solute (e.g., sodium chloride, sucrose) present in the substrate.^{20,111} In general, the protective effect of sucrose on the thermal destruction of yeasts and molds is diminished by sorbate. Presence of organic acids (e.g., citric, tartaric, malic) in fruit juices does not cause a consistent effect on the lethality of sorbate during heating of yeasts.¹²³ Thermal destruction of molds, however, increases in the presence of sorbate and with reduced pH (Table 3).

In addition to sorbate, other food preservatives, such as benzoate, enhance thermal destruction and inhibit recovery of injured yeasts and molds. It is not clear, however, which of the two compounds (i.e., sorbate or benzoate) is more effective. With molds, sorbate was more effective than benzoate in inhibiting colony formation by heat-injured cells, and benzoate appeared to be more destructive to certain species during heating.^{20,113} With yeasts, however, benzoate was less effective than sorbate, which increased the rate of thermal inactivation at concentrations of $\leq 0.005\%$ in culture broth.^{112,113} In fruit juices, benzoate was generally more effective than sorbate in thermal inactivation of yeasts.¹¹⁰

The mechanism of increased thermal destruction and inhibition of recovery by sorbate is unknown. Beuchat¹¹³ has speculated that the higher sensitivity of molds to heat treatment may be due to an increased permeability caused by an expansion of the cell wall and cytoplasmic membrane with increased temperatures. In that case, the preservative might enter the cell more easily at higher temperatures simply because of a weakening of the physical barriers inherent in the cell wall and membrane. Differences in the structural

transport into cells, which would influence their effectiveness at various temperatures.¹¹¹ Tsuchido et al.¹⁰⁷ found no efflux of intracellular substances and no inhibition of nucleic acid synthesis with sorbate in thermally injured cells of *Candida utilis*. They attributed inhibition of recovery to inhibition of protein synthesis and respiratory activity.

Increased thermal destruction of yeasts in presence of sorbate takes place not only in culture media, but also in foods, such as fruit juices (e.g., apple, apricot, grape, orange, and pineapple), other fruit products (e.g., apple cider, peach slices, and fruit salad), and in wine.^{1,110,123,124} Addition of sorbate to fruit products before thermal processing can afford reductions in the heat treatment (e.g., temperature and/or time) necessary for product preservation.^{117,125} This may help in maintaining better sensory qualities and nutritional value in the products without compromising their shelf life. Energy costs for processing may also be reduced. Appropriate studies are needed, however, to determine the combined effects of solutes and preservatives in combination with heat and their influence on the shelf life and quality of individual products. More research is also needed on the influence of sorbate on thermal destruction and recovery of bacteria and especially their spores.

Overall, temperatures outside the range of growth of a given microorganism will enhance the antimicrobial effectiveness of sorbate. Influence of higher temperatures was discussed here, while the effect of low storage temperatures is discussed in a subsequent section.

B. IRRADIATION

Preservation of foods with ionizing radiation has been studied since the 1940s and certain uses have been approved in several countries.¹²⁶ Combination treatments of low-dose irradiation and sorbate application may be useful in extending the shelf life of food products. Such treatments, however, must not cause any adverse effects on product quality and safety. An important question to be answered is whether irradiation affects sorbate by causing radiolysis of the compound, which might result in the formation of undesirable compounds. Treatment of foods such as fish with low-dose (i.e., pasteurization) radiation to extend shelf life may be more useful when combined with sorbate, since the compound may provide safety from *Clostridium botulinum* outgrowth and toxin production.

Research on the combined effect of irradiation and sorbate treatment of foods is rather limited. Certain studies, however, have indicated extended product shelf life when both treatments are combined. Most of these studies have examined fish and to a lesser extent fruit juices and cheese. A sorbate residue of 0.1% followed by irradiation at ≤ 4 kGy retarded mold growth on cured fish and extended shelf life compared to commonly processed fish.^{127,128} Kamaboko fish products (i.e., fish paste for surimi products) preserved with sorbate (0.2%) and irradiated at 3 kGy had a shelf life at 20°C, which was extended by 15 d compared to product treated only with sorbate.¹²⁹ Dipping of fish fillets for 40 s in a 5% sorbate solution and irradiation with 1 kGy resulted in extended product shelf life.¹³⁰ It has also been reported that an integrated treatment of shrimp consisting of a 60-min dip in 0.5% sorbate, blanching, salting, dehydration, and irradiation (2.5 kGy) stabilized the shrimp, which was free of mold and bacterial spoilage for 4 months at room temperature.¹³¹

An irradiation dose of 5 kGy in the presence of sorbate (0.005%) completely sterilized apple juice, while 10 kGy were needed for pear and peach juices. The sterilized apple juice had a shelf life of 190 d at 20 to 30°C, while the control juice (i.e., irradiated, without sorbate treatment) was fermented within 3 to 6 d.¹³² A lower dose (1 kGy) of irradiation in presence of 0.002% sorbate destroyed all yeasts in apple juice, but the fungi remained active.¹³³

One study with cheddar cheese has indicated that irradiation may supplement the anti-fungal properties of sorbate and extend product shelf life. Irradiation of cheddar cheese with 2 kGy inhibited mold growth at 28 to 30°C for 17 to 18 d, compared to 3 to 4 days for unirradiated controls. Spraying the cellophane wrapper with 0.5% sorbate increased the time

for onset of mold growth to 24 to 26 d postirradiation. This synergistic action was attributed to radiation-induced changes in cell wall permeability, which increase the inhibitory action of sorbate.¹³⁴ With such low doses of radiation and sorbate, the eating quality of the products does not appear to be adversely affected as was reported for fish.¹²⁷

Even though all published studies have indicated an increase in product shelf life by the combination of irradiation and sorbate treatment, one study reported that sorbate did not sensitize resting cell of *Saccharomyces cerevisiae* to destruction by irradiation.¹³⁵ It may be, however, that irradiation of sorbate results in formation of more potent antimicrobial activity. An irradiated solution of sorbate at 7 to 13 kGy showed stronger activity against *E. coli* and other bacteria than the nonirradiated solution.¹³⁶ This was true in spite of the fact that the sorbate level was reduced after irradiation of the solution. It was, thus, assumed that a new antimicrobial compound was formed in the solution by irradiation. Presence of oxygen was essential for the production of the new hypothetical antimicrobial substance. Hamada and Ishio¹²⁹ also found that after irradiation of fish the amount of sorbate present was 0.098% compared to 0.145% which was the initial level.

The potential chemical changes and radiolytic products caused by irradiation are of concern and have caused delays in acceptance and approval of radiation treatments in food preservation. A few studies have specifically examined radiolysis of sorbate. Radiolysis is expressed in G values, which indicate molecules decomposed per absorbed energy in eV.¹⁴⁰ An aqueous solution of sorbate was irradiated at 0.248 kGy/h and the G value was 1.85.¹³⁶ Hamada and Ishio have published three reports on the subject of sorbate radiolysis.¹³⁷⁻¹³⁹ G values were 4.69 (97.3%) for 0.2% sorbate when irradiated with a dose of 1 kGy. G values in a mackerel homogenate were 2.18 and in a whale homogenate 0.48 at 0.1 to 5 kGy. Sodium nitrite depressed radiolysis values of sorbate, while glucose was ineffective. It is clear that more studies are needed to examine potential benefits and disadvantages of combination treatments involving sorbate and low doses of irradiation in food products.

IV. ENVIRONMENTAL FACTORS

A. STORAGE TEMPERATURE

Sorbate is generally a more effective inhibitor of microbial growth at lower storage temperatures (Figure 3). This has been demonstrated with several microbial species and substrates. In general, storage of sorbate-treated foods at refrigerated temperatures improves their shelf life more than nonrefrigerated or untreated foods.¹⁴⁰

Increased inhibition or destruction of microorganisms by sorbate at lower temperatures has been demonstrated with clostridia,^{41,44} molds,¹⁴¹ *Salmonella typhimurium*,^{23,24,72} and *B. nivea*.^{142,143} Sorbate in combination with low temperature storage (e.g., 12° vs. 24°C) has increased the shelf life of grape juice and wine and has reduced loss of ascorbic acid from pasteurized strawberry juice.¹⁴⁰ Combinations of sorbate and nitrite were more inhibitory to bacterial growth in frankfurters at 7 to 9°C than at 20°C. Inhibition of *C. botulinum* toxin in a pork slurry by sorbate was more pronounced at lower (15°C vs. 35°C) temperatures.⁴⁴ Shelf life of butter was better in presence of sorbate at refrigerated than higher temperatures.⁴⁷ The effect of sorbate on shelf-life of vacuum-packaged, fresh, poultry pieces was also temperature dependent, and it was completely negated at higher (12°C) compared to lower (2°C) temperatures (Table 4).¹⁴⁴ Increased storage temperature also resulted in a greater proportion of Gram-negative, facultative anaerobic bacteria in the spoilage association.

However, death of *S. typhimurium* in cold-pack cheese food was more rapid with sorbate at 12.8°C compared to 4.4°C.²⁴ Inactivation of *S. typhimurium* was more rapid at higher temperature (7 to 13°C vs. 37°C), and it required more time at reduced temperatures of storage of milk and nutrient broth.²³ Destruction of *E. coli* by sorbate was also slower at

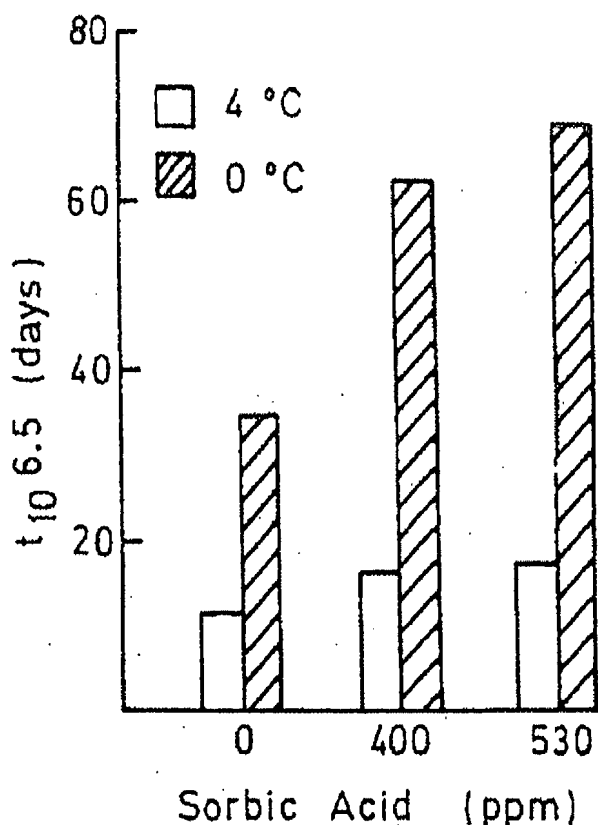


FIGURE 3. Influence of storage temperature and sorbic acid on microbial growth ($t_{10\ 6.5}$: d to reach $10^{6.5}$ CFU/cm²) in vacuum packaged beef samples of pH 5.90. (Reprinted from *J. Food Sci.*, 1987, Vol. 52, 257. © by Institute of Food Technologists.)

TABLE 4
Effect of Sorbate (Immersion 5%, 30 s) and Temperature on Bacterial Numbers (Log/6 cm²) on Vacuum-Packaged Poultry Breast Pieces

Potassium sorbate (%)	Storage temperature (°C)	Days of storage		
		7	14	21
0	2	4.9	6.8	7.7 ^a
5	2	5.7	4.6	5.5
0	5	6.9 ^a	4.6	—
5	5	5.0	7.6 ^a	—
0	12	8.4 ^a	—	—
5	12	7.9 ^a	—	—

^a Time at which off-odors were first detected.

From McMeekin, T. A., Pennington, P. I., and Thomas, C. J., *J. Food Saf.*, 6, 261, 1984. With permission.

these and other microorganisms, however, appears to be more pronounced at lower temperatures.^{145,146} Tuncan and Martin⁷² reported that L-serine and L-histidine uptake by *S. typhimurium* was inhibited more by sorbate at lower temperatures. Production of histamine by *Proteus morganii* and *Klebsiella pneumoniae* in presence of sorbate was greater at 10°C compared to 32°C.¹⁴⁷ Inhibition of growth (Figure 4) and patulin production by *B. nivea* in apple and grape juice was also slower at reduced temperature (21, 30, 37°C).^{142,143}

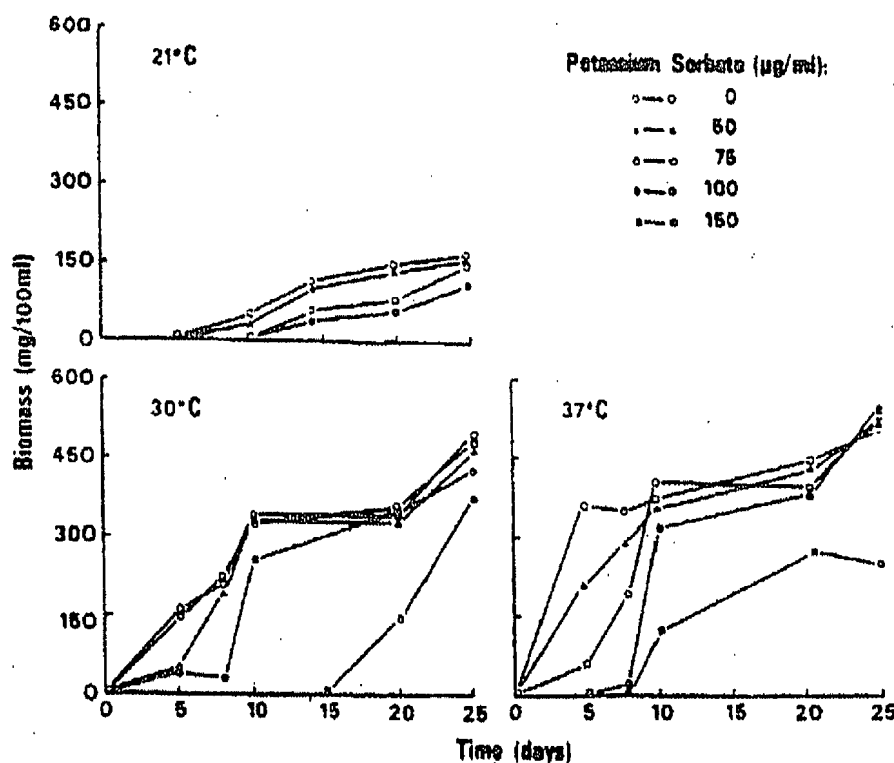


FIGURE 4. Influence of potassium sorbate and storage temperature on production by biomass by *Byssoschlamys nivea* in apple juice. (Reprinted from *J. Food Sci.*, 1984, Vol. 49, 402. © by Institute of Food Technologists.)

B. GAS ATMOSPHERE AND PACKAGING

The antimicrobial activity of sorbate is influenced by the type of packaging and gas atmosphere surrounding the product. Vacuum packaging (i.e., reduction or elimination of oxygen) and modified gas atmosphere (e.g., increased level of carbon dioxide) usually enhance the inhibitory effect of sorbate treatments (Figure 5). Reduced inhibitory activity of sorbate against *Candida albicans* under aerobic compared to anaerobic conditions was attributed to detoxification of sorbate by the yeast culture.¹⁴⁸ Aerobic detoxification varied with the composition of the substrate. Cysteine and glutathione reduced detoxification of sorbate, and they increased antimicrobial activity.

Decreased rates of microbial growth and increased shelf life under vacuum-packaged conditions and treatment with sorbate have been demonstrated with refrigerated (2°C) poultry meat,¹⁴⁴ pork loins (5°C),¹⁴⁹ scallops (4°C),¹⁵⁰ Morwong fillets (4°C),¹⁵¹ and other fish products (Table 5). McMeekin et al.¹⁴⁴ reported that microbial growth was completely inhibited in fresh poultry for up to 35 d at 2°C under vacuum-packaged conditions and treatment with sorbate. Myers et al.¹⁴⁹ also indicated that sorbate improved the keeping quality of vacuum-packaged and refrigerated pork loins and reduced numbers of psychrotrophic and pectinolytic bacteria (mostly *Yersinia* spp. and some *Klebsiella oxytoca*) by 97 to 99%. It was thus concluded that sorbate is effective in inhibiting growth of *Yersinia* spp. in vacuum-packaged, refrigerated, fresh pork. Increased evacuation also improved the shelf-life of sorbate-treated refrigerated bacon.¹⁵² Permeability of the packaging film, however, did not affect the influence of sorbate on growth of *Clostridium sporogenes* in sliced bologna.¹⁵³

Inhibition of microbial growth and delay of spoilage in refrigerated and vacuum-packaged fish^{150,151,154} is desirable from a marketing standpoint. The potential of growth and toxin production by nonproteolytic strains of *C. botulinum* under these conditions, however, should be considered, especially if toxin production will precede development of objectionable spoilage defects. Since sorbate is an effective antitoxigenic agent (Chapter 9), its use may

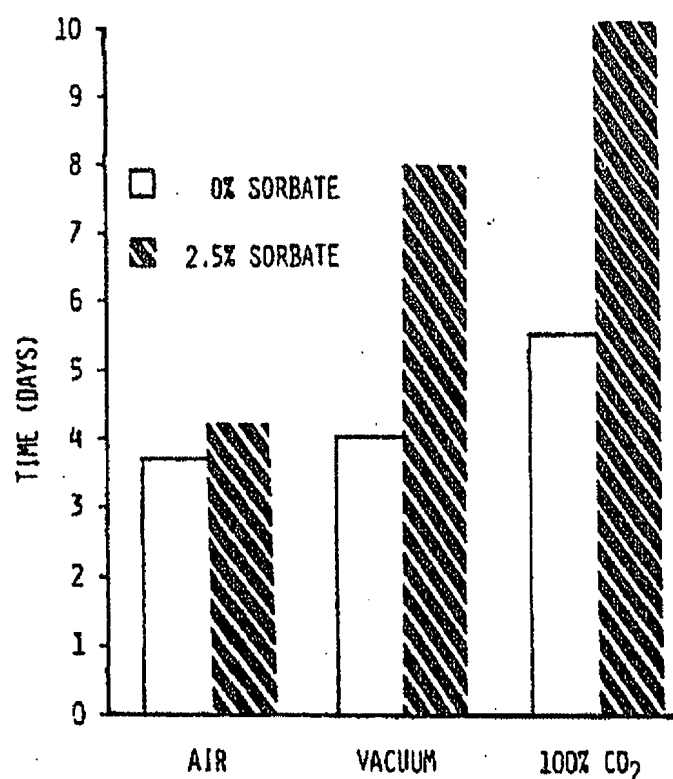


FIGURE 5. Influence of storage atmosphere and potassium sorbate on the shelf-life (d to reach 10⁷ organisms/cm²) of fresh poultry samples stored at 10°C. (Reprinted from *J. Food Sci.*, 1985, Vol. 50, 1360. © by Institute of Food Technologists.)

TABLE 5
Effect of Sorbate, Packaging and Storage Temperature on Trimethylamine Production (mg/100 g) in Blue Grenadier Fish Fillets²⁸⁹

Potassium sorbate (%)	Packaging	Storage temperature (°C)	Days of storage		
			0	11	18
0	Vacuum	4	0.3	2.9	78
0.1	Vacuum	4	0.4	0.7	1
0	Aerobic	4	0.3	14.6	83
0.1	Aerobic	4	0.2	1.1	3.6
0	Vacuum	-18	0.3	0.4	0.2

studies are needed, however, to verify its potential antimicrobial activity in the products and specified conditions.

Several studies have also shown that combinations of carbon dioxide and sorbate can be effective inhibitors of microbial growth.¹ Sorbate and carbon dioxide can inhibit mold spoilage of grains.¹⁵⁵ Modified atmosphere (CO₂) and sorbate are inhibitory, not only to spoilage organisms, but also to bacterial pathogens, such as *Salmonella enteritidis* and *Staphylococcus aureus* (Figure 6). Synergistic effects have been demonstrated in model systems^{68,71} and in poultry meat.^{156,157} The synergistic relationship between carbon dioxide and sorbate is demonstrated by the fact that by increasing carbon dioxide levels, the concentration of sorbate necessary for equivalent inhibition is reduced. The synergistic antimicrobial effect is more pronounced with increasing concentration of carbon dioxide and sorbate and with decreasing pH.^{68,71,156,157}

Concentrations of 1.5 to 5% sorbate and 100% carbon dioxide at pH 5.5 can be com-

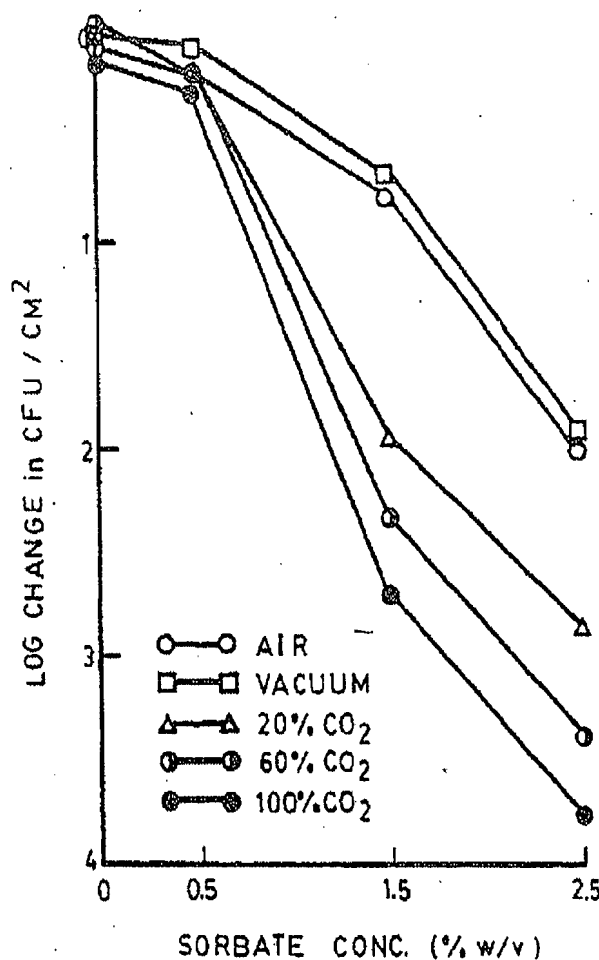


FIGURE 6. Growth of *Staphylococcus aureus* in trypticase soy agar of pH 5.5, containing potassium sorbate, after 60 h of exposure to different gas atmospheres. (From Elliot, P. H., Tomilins, R. I., and Gray, R. J. H., *J. Food Prot.*, 45, 1112, 1982. With permission.)

pletely bacteriostatic.¹⁵⁶ The inhibitory effect of sorbate in combination with a carbon dioxide atmosphere is more pronounced compared to that in vacuum environments.⁶⁸ No synergistic effect is observed at pH 6.5, but it increases as the pH is reduced and it is maximized at pH 5.5.¹⁵⁷

Sensitivity of microorganisms to carbon dioxide and sorbate combinations is variable with species. Elliot et al.⁶⁸ found that in a nutrient medium a combination treatment (i.e., carbon dioxide and sorbate) was up to 100 times more effective against *S. aureus* than *Salmonella enteritidis*. Although combinations of carbon dioxide and sorbate in poultry meat were effective against both *S. enteritidis* and *Staphylococcus aureus*, the former was more sensitive to sorbate, and the latter, to carbon dioxide, when tested individually.¹⁵⁶ Spoilage microorganisms in fresh poultry meat were less sensitive to sorbate than *Salmonella enteritidis* and *Staphylococcus aureus* under similar conditions.¹⁵⁷ The combination of carbon dioxide and sorbate provided the most inhibitory system against poultry spoilage organisms, especially *Pseudomonas* spp., without creating a selective environment for lactic-acid-producing microorganisms.¹⁵⁷

Although the lactic-acid-producing bacteria are believed to be resistant to inhibition by modified atmospheres and sorbate, the combination of carbon dioxide and sorbate does not appear to create conditions for selection of these species that would result in early souring of the product.¹⁵⁷ Thus, combining sorbate and carbon dioxide atmospheres has a good potential for improving the shelf life of fresh poultry. This application would be more

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type items. Combinations of carbon dioxide and sorbate are also effective in extending the shelf life of fish.^{158,159}

The antimicrobial activity of carbon dioxide-sorbate combinations may be even more pronounced if lactic or acetic acid are introduced to adjust the pH of low acid systems (i.e., fish, poultry), because these acids potentiate the action of sorbate more than hydrochloric acid.⁵⁷

V. ADDITIVES

A. ACIDS

The type of acid naturally present or added to the food may influence the antimicrobial activity of sorbate. It is not only the activity of the acid as an acidulant, but also the specific acid anion itself may be influential.¹⁶⁰ Several studies have demonstrated that individual acids affect differently the antimicrobial activity of sorbate. In general, however, preservation of foods with sorbate is more effective in presence of acids which lower the pH and increase the amount of the more effective undissociated form of sorbate.

Publications and patents have presented information indicating preservation of products, such as sweet alcoholic beverages, with combinations of sorbic and lactic acids;¹⁶¹ fish paste (kamaboko) with potassium sorbate and glucono-delta-lactone;¹⁶² and cooked noodles with several carboxylic acids (e.g., lactic, glutamic, caproic) and sorbate.¹⁶³ Earlier (1964) Perry et al.¹⁶⁴ reported that the shelf life of eviscerated, refrigerated, cut-up, "dry-pack", poultry meat was extended by the synergistic action of a two-step process consisting of acidic hydration and sorbic acid application. Poultry parts were hydrated in an ice slush solution of sodium dihydrogen phosphate for 2 h, which lowered the surface pH to below 5.5. After draining, the parts were sprayed with a hot (i.e., 71°C) solution of 7.5% sorbic acid. The solvent consisted of 70% propylene glycol, 20% water, and 10% glycerol. These ingredients increase the solubility of sorbic acid at 71°C to 5 to 10%.

The specific type of acid present will also affect the extent of antimicrobial action by sorbate. Inactivation of *Salmonella typhimurium* by sorbate was greater in the presence of acetic than lactic acid, which were used to adjust the pH.²³ Acidification in general permitted the use of lower sorbate levels for similar extent of antimicrobial activity in poultry frankfurters, bacon, and chopped ham.¹⁶⁵⁻¹⁶⁷ The type of substrate may also influence the potentiating effect of the acid on antimicrobial activity, since the effect was higher in ham than in bacon. Citric acid increased the antimicrobial activity of sorbate in turkey but not in chicken frankfurters.¹⁶⁵

Acetic and citric acids potentiated the inhibitory effect of sorbate on mold in raw, cured, Italian dry salami, while lactic and succinic acids showed little or no effect.¹⁶⁸ In general, inorganic acids and lactic acid appear to be less effective potentiators of antimicrobial activity than other acids, such as acetic and citric. Gilliland and Ewell,¹⁶⁹ however, reported that cells of *Lactobacillus lactis* added to refrigerated raw milk in combination with sorbate were more inhibitory to psychrotrophs than either lactobacilli or sorbate tested alone.

The relative influence of specific acids on antimicrobial activity may depend on specific microorganisms under consideration. Restaino et al.⁵⁷ examined the effect of sorbate in combination with citric, lactic, phosphoric, and hydrochloric acids on the growth of six food-related bacteria in culture media of constant pH (5.5). Sorbate (0.1 to 0.2%) with any of the acids tested was bacteriostatic to *Y. enterocolitica* (Figure 7) and *Salmonella* group D and extended the lag phase of *P. fluorescens* by 24 h. The combination of sorbate with the acids also reduced the growth rate of *Lactobacillus plantarum* and an unidentified *Lactobacillus* isolated from frankfurters. *Pseudomonas aeruginosa*, however, was unaffected by any sorbate-acid combination. Overall, citric and lactic potentiated the antimicrobial action of sorbate more than other acids.

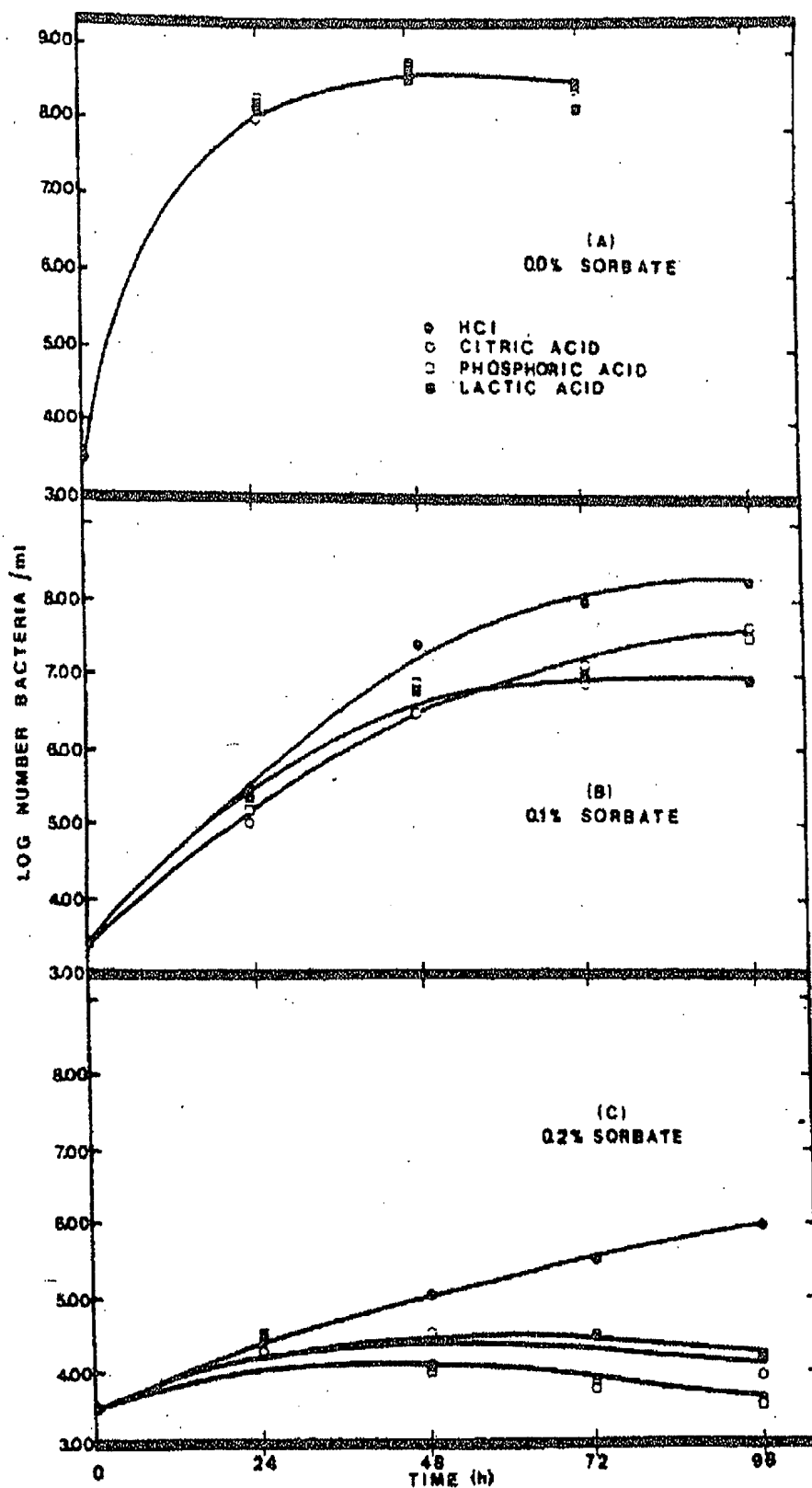


FIGURE 7. Influence of sorbate and acids on growth of *Yersinia enterocolitica* 0:17 in trypticase soy broth of pH 6.0 at 25°C. (Reprinted from *J. Food Sci.*, 1981, Vol. 46, 134. © by Institute of Food Technologists.)

Restaino et al.²² tested citric, lactic, malic, phosphoric, and hydrochloric acids in combination with sorbate on growth of osmophilic yeasts, including *Saccharomyces bailii*, *S. acidifaciens* (*S. bailii* var. *osmophilus*), *S. rouxii*, and *S. bisporous* in nutrient agar at pH 5.0 and water activity 0.88 to 0.89. Citric acid potentiated the antimicrobial activity of

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as a longer lag phase or a reduction of the rate of growth. The lack of activity by lactic acid was attributed to either the acid being antagonistic to sorbate or the specific yeast strains tested having been adapted to the acid, if it was a natural component in their source of isolation. Thus, use of acids, other than those naturally present in a food, may be advisable when potentiation of antimicrobial activity is the target. Additional studies are needed, however, to develop combinations of sorbate with acids and other additives.

B. ANTIOXIDANTS

Lipid oxidation and rancidity development in food products is inhibited by addition of several chemical compounds which act as antioxidants. The most commonly used antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroxyquinone (TBHQ), and propyl gallate (PG). Several of these compounds have shown inhibitory activity against various microorganisms.¹⁷⁰ Thus, they could be valuable by serving the dual function of antioxidant and antimicrobial.

Several studies have shown that certain combinations of sorbate with these antioxidants inhibit microorganisms, such as *Staphylococcus aureus* and *Salmonella typhimurium*, under certain conditions.¹ Combinations of sorbate with BHA or TBHQ delayed growth of *Staphylococcus aureus* in culture media, while the sorbate-BHA combination did not delay initiation of growth of *Salmonella typhimurium*.¹⁷¹ Overall, however, all combinations with sorbate increased the antimicrobial activity. Synergistic effects of sorbic acid and BHA against *Clostridium perfringens* have also been reported.¹⁷² Sorbate (1 to 5%) in combination with BHA, BHT, and PG (50 to 100 ppm) exerted greater bactericidal and bacteriostatic effect at pH 5 and 7 against *Staphylococcus aureus*, while TBHQ was highly inhibitory both in the presence and absence of sorbate. BHA in combination with sorbate was more effective than BHT and PG.⁶⁷ Combinations of sorbate and TBHQ were synergistic in inhibiting one of two strains (12600) of *S. aureus*, while certain combinations of BHA and sorbate were synergistic against both strains (S-6 and 12600) (Figure 8).¹⁹

Certain combinations of sorbate with antioxidants (i.e., BHA, TBHQ, and PG) resulted in synergistic inhibition of *Salmonella seftenberg* in culture media of pH 5.5.¹⁷³ The most effective combination consisted of 0.2% potassium sorbate and 200 ppm BHA. All sorbate/antioxidant combinations also significantly inhibited the test organism in ground beef compared to control samples. The antimicrobial effectiveness of sorbate/antioxidant combinations, however, was markedly diminished in ground beef compared to culture media. The diminished effect can probably be attributed to the antioxidant being used up to prevent autoxidation of the lipids.

Sorbate (0.1%) was tested alone and in combination with BHA (150 ppm) against growth of the natural flora in raw turkey meat and against *S. typhimurium* inoculated into cooked turkey meat.¹⁷⁴ There was no inhibition of the natural flora by BHA or sorbate tested alone, but there was a slight inhibition when the two compounds were combined. Sorbate and sorbate-BHA combinations did not differ in their effectiveness and were more effective than BHA alone in reducing numbers of *S. typhimurium* in cooked turkey. Overall, there was no synergistic effect evident, but a combination of sorbate and BHA was more effective than sorbate alone in inhibiting Gram-negative organisms and in reducing survival of *Salmonella*.

Earlier studies, however, had indicated that combinations of sorbate with antioxidants were successful in preserving foods. Nickerson and Starr¹⁷⁵ were awarded a patent for preserving fresh pork and beef sausage, hamburger, fresh fish fillets, eviscerated cut up poultry, etc. Combinations of fat soluble (e.g., BHA, BHT, catechol, quercetin, and 2,6-dimethoxyphenol) and water soluble (e.g., ascorbic, citric, and phosphoric acids) compounds with sorbate inhibited rancidity and bacteria, respectively.

In addition to bacteria, sorbate/BHA combinations have inhibited growth of *Aspergillus*

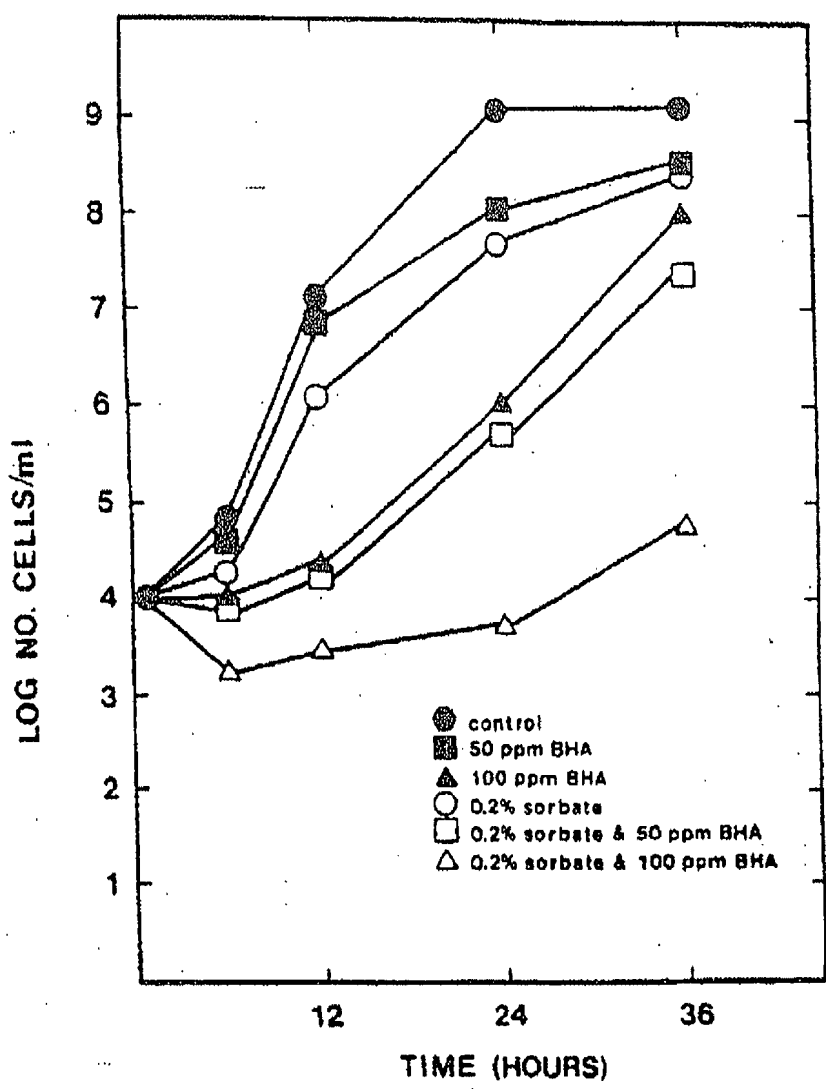


FIGURE 8. Effect of butylated hydroxyanisole (BHA) and potassium sorbate on growth of *Staphylococcus aureus* S-6 in trypticase soy broth of pH 6.0 at 37°C. (From Robach, M. C. and Stateler, C. L., *J. Food Prot.*, 43, 208, 1980. With permission.)

flavus in a liquid culture medium.¹⁷⁶ One advantage of such combinations is that sorbate levels needed for inhibition may be reduced. In addition, rancidity of the fat can also be inhibited.

C. PHOSPHATES

Phosphates used in foods have several functions, including their effects on water and fat binding, emulsification, antioxidant activity, and overall improvement of cooking yields, texture, flavor, and color.¹⁷⁷ Phosphates are especially useful in low-salt meat products, where they improve the technological and quality properties and allow reductions in the sodium content. Their effect as antimicrobials, however, depends on the specific type of phosphate used, the composition of the product, its pH, the type of microorganisms, and presence of other chemicals.^{54,177-179}

Certain studies have indicated interaction of phosphates with sorbate and improvement of antimicrobial activity when both compounds are used in a formulation. A patent was issued for sausage preparation with a mixture of alkaline phosphates with potassium sorbate or sorbic acid.¹⁸⁰ Ivey and Robach¹⁸¹ reported that either sodium acid pyrophosphate (SAPP) or sodium hexametaphosphate (SHMP) displayed statistically significant interactions with

found that only SAPP, not SHMP or sodium tripolyphosphate (STPP), showed anticlostridial activity in combination with nitrite and sorbic acid in a similar product.

Increased inhibition of *C. botulinum* by SAPP/sorbate combinations in beef-pork frankfurters was also reported.¹⁸² SAPP was more effective than other phosphates (SHMP, and especially STPP) in inhibiting *C. botulinum* in frankfurters made with mechanically deboned chicken meat.¹⁸³ Effectiveness, however, was present only in combinations of the phosphate with sorbic acid or potassium sorbate and sodium nitrite and not in treatments without nitrite. A polyphosphate, consisting of 53% STPP and 47% sodium pyrophosphate, had no apparent additional effect on preservation of fish fillets treated with or without sorbate.¹⁵¹ Bacteria isolated from spoiled kamaboko were inhibited by combinations of sorbic acid, with SHMP and sodium ultrapolyphosphate, but not by STPP or sodium pyrophosphate.

Interactions of phosphates with sorbate have also been observed in studies with culture media. Seward et al.⁶⁶ observed interactions of STPP (0.5%) and potassium sorbate (0.5%) at pH 7.1 in the inhibition of *C. botulinum* type E. Treatments containing SAPP and potassium sorbate in culture broth were also more effective than other formulations in delaying growth of *C. botulinum*.⁵¹ SAPP/sorbate combinations in culture media at pH 5.85 did not prevent *C. botulinum* spore germination and outgrowth but may prevent cell division. The same treatment at pH 5.5, however, inhibited spore germination.⁵³ This demonstrates the influence of pH on the inhibitory activity of phosphate/sorbate combinations. Sorbate/SAPP combinations were also inhibitory against *S. aureus*.¹⁸⁴ Thus, alkaline phosphates (e.g., STPP), which raise the pH of the substrate, are usually ineffective as antimicrobials,¹⁷⁷ with or without sorbate in the formulation. However, acidic phosphates (e.g., SAPP) are usually more effective as antimicrobial agents either alone or in combination with sorbate.

D. ANTIMICROBIALS

1. Benzoate and Propionate

Combinations of sorbate with benzoate can be useful in the preservation of food products because they can expand the range of microorganisms inhibited at lower concentrations of each preservative.^{9,10,185} Use of lower concentrations also reduces any potential undesirable effects on product flavor. Graham and Chaparro,¹⁸⁶ however, indicated that a combination of 0.05% sodium benzoate and 0.05% potassium sorbate was less effective than 0.07 to 0.1% sodium benzoate in preserving "mabi", a fermented drink, that needs refrigeration, and is derived from the bark of the mabi tree (*Colubrina elliptica*). In contrast, rubber and cork gaskets used in car and tractor engines and in food industry equipment under warm weather conditions were made fungus resistant by treating with a combination of 1% sorbic acid and 1% benzoic acid.¹⁸⁷ Such materials can be used in manufacturing containers for citrus fruit, which are susceptible to *Penicillium* and *Aspergillus* rot during refrigerated storage. Sodium benzoate (0.5%) was most effective in preserving hake fish in ice than EDTA or sorbate tested individually; all, however, increased shelf life. Combinations of two preservatives failed to result in better shelf life than sodium benzoate used alone.¹⁸⁸ Mixtures of sorbate with propionate are also useful in the preservation of bread and other yeast-leavened bakery products. The interactions of sorbate with nitrite are discussed in Chapters 9 and 11.

2. Antibiotics

Sorbate has been found to be synergistic, additive, antagonistic or has shown no interaction with antibiotics in inhibiting microbial growth. The specific effect depends on individual microorganisms, antibiotics, and substrates.¹⁸⁹

Rehm et al.⁶ tested the interaction of sorbic acid with various antibiotics for inhibition of bacteria. The preservative generally had an additive effect with tetracycline, but in some cases, especially with *S. aureus*, there was an antagonistic effect. Antagonistic interactions

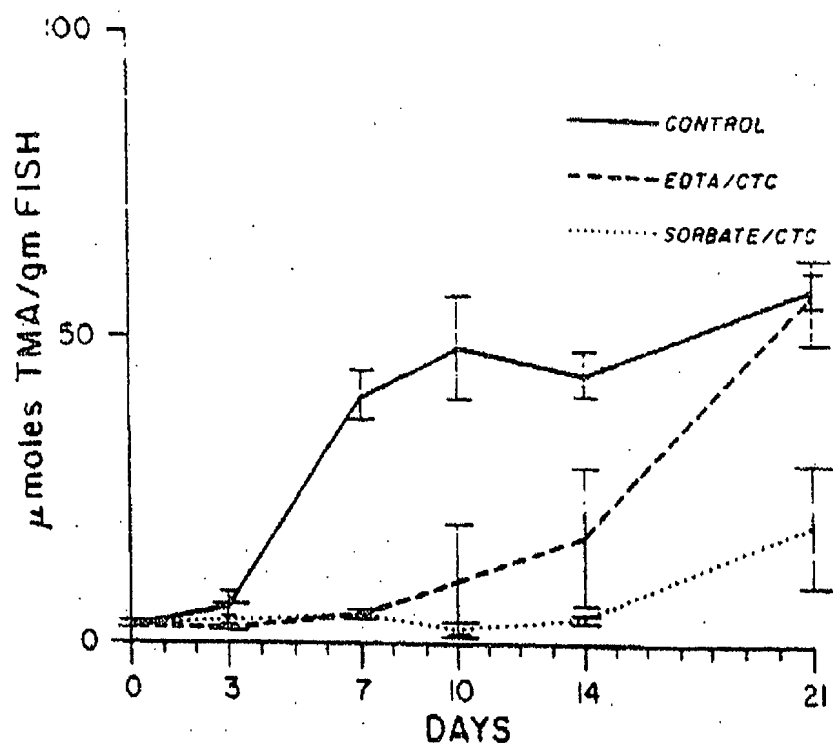


FIGURE 9. Influence of a potassium sorbate (1%), ethylene diamine tetraacetic acid (EDTA, 1%) and chlortetracycline (CTC, 5ppm) immersion (1 min.) solution on trimethylamine production in vacuum packaged rockfish fillets stored at 2°C. (Reprinted from *J. Food Sci.*, 1984. Vol. 49, 188. © by Institute of Food Technologists.)

were also found with novobiocin and with polymyxin, bacitracin, and colistin against *S. aureus*. There was no interaction with penicillin, and the interaction was synergistic with chloramphenicol, streptomycin, and neomycin.

Interactions of sorbate with antibiotics in food systems are also variable. The approved-for-use-in-foods antibiotic nisin when used in combination with sodium sorbate (0.06%) retarded mold growth on the surface of process cheese and improved its storage stability.¹⁹⁰ Nisin (150 units/g) and sorbic acid (0.075%) also decreased growth of butyric acid bacteria and fungi, and increased shelf life of process cheese at 20 to 35°C by 2 to 3 times.¹⁹¹ Sorbic acid (0.07%) in combination with 0.02% nisin may also be used for preserving apple pectin carboxymethyl cellulose.¹⁹²

Combinations of other antibiotics with sorbate have also been tested in food systems. Oxytetracycline (4 ppm) and 0.04% sorbic acid had no additional effect on the shelf life of herring compared to either compound tested by itself.¹⁹³ Antimicrobial activity was also not improved with a combination of sorbic acid and aueromycin against *E. coli*, *B. subtilis*, *Proteus vulgaris*, and *Staphylococcus candidus*.¹⁹⁴ Improved antimicrobial activity was found, however, with a combination of sorbate and chlorotetracycline in rockfish fillets (Figure 9);¹⁹⁵ with kasugamycin in cucumbers;¹⁹⁶ and with tylosin in fish sausage.¹⁹⁷ Use of most antibiotics in food products, however, is not permitted.

E. OTHER INTERACTIONS

1. Ascorbate

Fruit products may be preserved more effectively with combinations of sorbate and ascorbate. A patent has described preservation of strawberry jam with combinations of sorbic acid (0.09 g/kg) and ascorbic acid (0.5 g/kg).¹⁹⁸ This treatment allows storage of the product at temperatures of up to 25°C instead of the necessary storage at less than 10°C in the

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for inhibition of microbial spoilage in raw or partially cooked foods (e.g., marmalade) stored at room temperature.

Combinations of sorbic and ascorbic acids were also more effective than sorbic acid by itself in inhibiting microbial spoilage of carbonated fruit drinks. The optimum combination consisted of 50 to 100 mg/l sorbic acid and 100 to 200 mg/l ascorbic acid. Svorcova²⁰⁰ also reported that potassium sorbate and ascorbic acid were more effective than either one used singly in preserving mixtures of fruit juices with sugar and mineral water. Fresh unpasteurized apple juice with 0.35% sorbic acid and 50 mg per 100 ml ascorbic acid may be preserved without refrigeration for a minimum of 2 weeks. Sorbic acid inhibits yeast growth, and ascorbic acid prevents oxidation of sorbic acid and inhibits *Acetobacter* growth. Oxygen, however, must be excluded from the product.²⁰¹

2. Sulfur Dioxide

Combinations of sorbate and sulfur dioxide have proven useful in the preservation of wines where they control undesirable yeasts and bacteria and maintain its quality (Chapter 8). Several studies have reported on their interaction in expanding the range of microorganisms being inhibited in wine environments at reduced concentrations. Although sorbic acid by itself may give the wine an unfavorable taste, a reduced level (≤ 200 ppm) in combination with sulfur dioxide (100 ppm) gives an increased shelf-life by suppressing yeasts such as *Candida*, *Pichia*, and *Hansenula*, as well as bacteria and chemical deterioration.²⁰² The wine is preserved for long periods of time with no adverse effects on its taste. Other combinations of sorbate and sulfur dioxide that preserve wines have used 0.013 to 0.026% potassium sorbate and 30 mg/l sulfur dioxide, or 100 to 250 mg/l sorbic acid and "small" amounts of free sulfur dioxide.²⁰³ Combinations of sorbic acid (25 to 75 mg/l) and sulfur dioxide (100 to 125 mg/l) prevent chemical, microbiological, and sensory changes even in sweetened red or white table wines,²⁰⁴ while each compound used individually is usually ineffective. A combination of sorbic acid and sulfur dioxide is also effective in preserving other fruit products, such as strawberry pulp.

3. Emulsifiers

Sucrose esters of fatty acids are compounds derived from sucrose esterified with a mixture of palmitic and stearic acids and are effective as emulsifiers. These sucrose fatty acid esters also possess antimicrobial properties.²⁰⁵⁻²⁰⁷ A patent indicates that a composition containing sorbic acid, water, and emulsifiers (i.e., sucrose fatty acid ester, sorbitan fatty acid ester, lactate fatty acid ester, and lecithin) was a preservative for noodles.²⁰⁸ The antimicrobial activity of sorbate or propionate with sucrose esters was also investigated by Marshall and Bullerman²⁰⁹ against growth and aflatoxin production by *Aspergillus parasiticus*. A synergistic effect on inhibition of growth was observed at high levels of propionate or sorbate in combination with the esters (Figure 10), while aflatoxin production remained relatively unaffected. Thus, sucrose esters in combination with sorbate or propionate may provide added antimycotic activity at pH values higher than the optimum for either inhibitor alone. Thus, various combinations may be useful in low acid food commodities.

Other emulsifiers (e.g., Tween® 20, 40, 60, 80) neither suppressed nor potentiated the action of sorbic acid in meat-peptone broth inoculated with *S. aureus*.²¹⁰ In contrast, both sodium dodecyl sulfate and sucrose monopalmitate showed antibacterial activities by themselves, which were higher than that of sorbic acid.

4. Propylene Glycol

The interaction of sorbate and propylene glycol on antimicrobial activity appears to be variable. Propylene-glycol caused partial antagonism of 0.1% sorbate in creams, as well as an increase in the minimum concentration of sorbate needed to inhibit strains of *Pseudomonas aeruginosa* in broth of pH 6.5 to 7.0.²¹¹

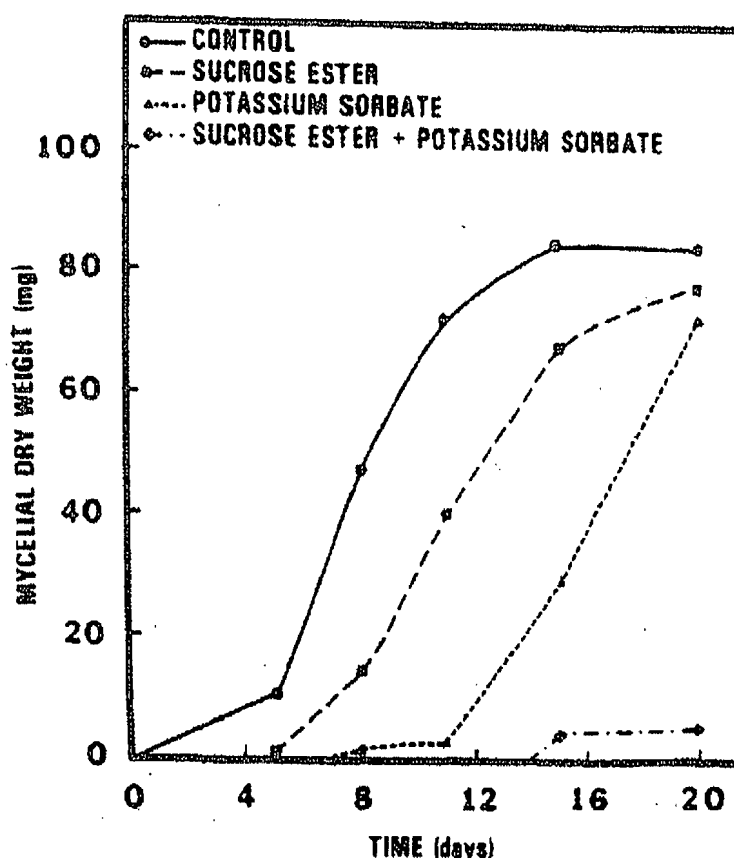


FIGURE 10. Influence of potassium sorbate (0.3%) and sucrose fatty acid ester (1%) on growth of *Aspergillus parasiticus* in a culture broth of pH 6.0 at 25°C. (From Marshall, D. L. and Bullerman, L. B., *J. Food Prot.*, 49, 378, 1986. With permission.)

In foods, however, sorbate-propylene glycol combinations appear to be effective antimicrobials. Sorbic acid and propylene glycol prevented growth of fungi on dried seasoned saury for 1 month at 30°C.²¹² A patent was also awarded for preservation of cooked noodles by soaking in a solution of sorbic acid and propylene glycol, draining, and packaging.²⁰⁸

5. Fatty Acids

Mixtures of sorbate with other fatty acids have the potential of improving the shelf life of various foods, as indicated by several patents. The preservative activity of sorbates in raw meat and fish has been enhanced by mixing the compound with an unsaturated fatty acid of chain length $\leq C_{18}$.^{213,214} A composition containing sorbate and C_{1-10} and C_{16-20} saturated fatty acids was a preservative for processed meats.²¹⁵

Derivatives of fatty acids have also enhanced the antimicrobial activity of sorbate. A composition of sorbate, caprylic acid monoglyceride, and sodium nitrite was effective in inhibiting growth and toxin production by *Clostridium botulinum* in meat.²¹⁶ Sorbic acid and several derivatives of fatty acids (i.e., amides, aminimides, and monoglycerides) were inhibitory to toxigenic *A. flavus* and *A. parasiticus*.²¹⁷ Monolaurin, a fatty acid derivative emulsifier, inhibited *S. aureus* to a greater extent when used in combination with sorbic acid.²¹⁸ Robach et al.,²¹⁹ however, found no synergistic effect of these compounds against *Salmonella*.

6. Amino Acids

Foods can also be preserved by mixing sorbate with amino acids and other compounds.

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indicates that fish and meat products can be preserved by treatment with an acidic amino acid coated with an edible fat or oil and sorbate, and/or 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide. Glutamic and aspartic acid were more effective in the mixture than other amino acids.

7. Glucose Oxidase

Glucose oxidase can also enhance preservation of foods with sorbate. A combination of sorbic acid with glucose oxidase inhibited grape juice fermentation more than sorbic acid alone.²²² The natural properties of apple and cherry juices were also preserved for more than 6 months by the synergistic antimicrobial action of sorbic acid (0.02 to 0.04%) and glucose oxidase (50 to 100 units/l).²²³ Glucose oxidase (5 units) and sorbate (0.03%) also suppressed growth of microflora in sweetened condensed milk.²²⁴

8. Other

Several patents and publications indicate interactions which have enhanced the antimicrobial activity by sorbate when used in combination with several compounds in foods, culture media, or other materials. Several of these interactions are mentioned here.

Sorbic acid (0.03%) and diethyl ester of polycarbonic acid (0.04%) inhibited molds in grape juice.²²⁵ Mixtures of diethyl ester of polycarbonic acid and sorbic acid inhibited yeast cells in a fermentation broth with 12.4% alcohol, but when used singly the ester was more inhibitory than sorbic acid.²²⁶ A food preservative was formulated with cinnamic acid and one or more acids, including sorbic acid.²²⁷

Compositions containing sorbic acid or its salts and 2-(4-thiazolyl) benzimidazole were microbiocides and were effective in controlling *Spherothea fuliginosa* on cucumbers.²²⁸ Potassium sorbate in combination with benzimidazole reduced *Penicillium* decay in citrus fruits.²²⁹

A patent was issued on the use of acyloxyalkanoic acid together with sorbic acid to preserve foods and feeds.²³⁰ A combination of sorbic acid (0.02%) and vitamin K₃ (0.03%) completely inhibited growth of wine yeasts and acid-forming bacteria for 30 d.²³¹

Sulphydryl-containing compounds (e.g., cysteine-HCl, glutathione) in combination with sorbic acid inhibited molds for up to 220 d at room temperature. Such compositions were suggested as preservatives for a variety of food products, including meats, fish, dairy, fruits, vegetables, oils, and confectionary.²³²

Carboxymethylcellulose was ineffective in preserving apple jam which spoiled in 8 to 13 d at 30 to 37°C and 90% relative humidity. Sorbic acid alone preserved the product for 4 months, while a combination of carboxymethylcellulose (1%) and sorbic acid (0.05%) inhibited spoilage for 5 months at 30°C.²³³

Dihydroxyacetone has also shown increased antimicrobial activity in combination with sorbate or other carboxylic acids in foods and feeds.²³⁴ Mashed potatoes with 5% dihydroxyacetone and 0.3% sorbate did not spoil for 40 months at room temperature, while controls spoiled in less than 1 month, and with dihydroxyacetone alone they were preserved for less than 3 months.

Sorbic acid in combination with 5-hydroxy-1-methyl-2-isopropyl-benzene or 5-nitro-2-furylacrylic amide were bacteriostatic in sausages.²³⁵ Sodium bicarbonate (0.01%) has also increased the effectiveness of sodium sorbate (0.1%) in preserving apple cider.²³⁶

Sorbic acid has promoted the preservative effect of gelatin (1%) and egg white lysozyme (0.05%) on fish paste (kamaboko).²³⁷ Kamaboko is also preserved with a combination of sorbate and furylfuramide.²³⁸ Furylfuramide (5 ppm) and sorbic acid (0.2%) used together were also more effective against several organisms isolated from Vienna sausage.²³⁹ The combination, however, was ineffective against *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Comamonas* spp.

TABLE 6
Effect of Sorbate, Sodium Chloride, pH, Heating Treatment, Inoculum Level
and Storage Temperature on Probability of Toxin Production by *Clostridium*
botulinum in Pork Slurries⁴⁴

Potassium sorbate (%)	Sodium chloride (%)	pH	Inoculum (spores/bottle)	Unheated		Heated (80°C)	
				Storage temp (°C)		Storage temp (°C)	
				15	35	15	35
0	2.5	5.7	10	21	38	34	53
0.26	2.5	5.7	10	6	13	11	22
0	2.5	5.7	1000	54	90	68	94
0.26	2.5	5.7	1000	11	50	19	65
0	2.5	6.6	10	87	100	53	97
0.26	2.5	6.6	10	62	98	22	33
0	4.5	5.7	10	2	26	4	40
0.26	4.5	5.7	10	1	8	1	14
0	4.5	6.6	10	34	99	8	95
0.26	4.5	6.6	10	12	97	2	83

Sorbic acid (0.15%) and *o*-phenylphenol (0.05%) were also synergistic as antimicrobials in wheat-starch paste.²⁴⁰ When the combination was used as an antiseptic for starch adhesives, there was no decay at 30°C for 240 d, compared to a shelf life of less than 15 d for the control.²⁴¹ Extremely low (0.00001%) concentrations of 1,5-pentanediol and 0.1% sorbic acid in isotonic buffered saline were destructive to *Pseudomonas aeruginosa* and can be useful as preservatives for contact lenses.

Meat-based animal feeds with 15 to 30% moisture can be stabilized by incorporation of a combination of 0.1% sorbate and 0.1% levulinic acid to prevent mold growth. Inhibition by the combination is better than with the individual compounds.²⁴² Sorbate in combination with smoke has also inhibited mold spoilage in fish.²⁴³ Treating peas with a 1:5 to 1:3 dilution of an emulsion of paraffin, wax, and sorbic acid in water decreased spoilage and weight losses. The same solution also preserved cucumbers and peppers but not onions and radishes.^{224,225}

VI. MULTIFACTOR INTERACTIONS

In most practical applications of food preservation, the action of sorbate and other chemical food preservatives is to interact with various other factors and together to extend the shelf life of the product (Table 6). These factors have been discussed in other parts of this chapter and include processing variables such as heat, drying, irradiation; parameters associated with the raw ingredients, such as pH and composition; chemical additives used in the formulation; and environmental parameters, such as gas atmosphere, temperature of storage, and packaging. The interaction of such factors constitutes the "total preservation system", which maintains product quality and/or safety. Each one of these factors, including sorbate, is important and, if changed, may compromise product preservation.

In addition to interactions of sorbate with one or more factors commonly used in food processing, the literature contains numerous publications and patents of interactions of sorbate with multiple other factors. These multifactor interactions may include both commonly used and approved variables and treatments, as well as compounds that are not approved for common use or may have undesirable effects on product quality and safety. It is interesting,

Combinations of tetracycline antibiotics, irradiation, and sorbic acid were found effective in delaying microbial spoilage of prepackaged meats.²⁴⁶ Before the widespread testing of sorbate as an antibotulinal agent in meat products (Chapter 9), a patent was issued,²⁴⁷ indicating that sausages could be preserved by incorporation of mixtures of sodium metaphosphate, glucono-delta-lactone, sodium chloride, and sorbic acid. Application of a solution combining 2% gelatin, 2% liquid smoke, 0.2% corn starch, and 1% potassium sorbate on the inside of regenerated cellulose casings inhibited mold growth during curing of sausages.²⁴⁸ A composition containing sorbate, C₁₆₋₂₀ fatty acid monoglycerides, nitrite and/or hexametaphosphate could preserve meat products, especially against *Clostridium botulinum*.

Wieners and fish paste have been preserved with a combination of sorbic acid and one or more substances selected from organic and inorganic or metallic ions. Propylene glycol caproic acid ester (5 parts), sorbitan caproic acid ester (10 parts), and glycerol stearate (15 parts) were liquefied by heating and to the mixture was added sorbic acid (20 parts), citric acid (10 parts), sodium polyphosphate (35 parts), and exsiccated alum (5 parts). Wieners containing this mixture (1%) and a conventional sorbic acid preservative (1%) were preserved for 16 d, while controls spoiled within 3 d.^{249,250} Wieners were also well preserved by adding mainly sorbic acid and sodium polyphosphate, and if necessary alum, gluconolactone, arabolactone, and antifungal organic acids.²⁵¹

Preservatives for meat products containing 15 to 50% moisture were formulated with compounds selected from sodium chloride, potassium chloride, alkali metal salts of hydroxycarboxylic acids, orthophosphates, metaphosphates, ultraphosphates, and polyphosphates.²⁵² Diced ham, boiled in a solution of sodium chloride (5%), potassium citrate (5%), potassium sorbate (0.8%), and water (89.2%) for 7 min, then cooled to 30 to 40°C, left in the salt solution for 1 h and dried at 38 to 43°C to 39% water, was preserved. Meat products were also preserved by dipping in or spraying with a 5 to 18% solution of sodium, potassium, calcium, or ammonium sorbate in water or in an aqueous solution of gelatin, starch, or cellulose derivatives. The treated product resisted microbial growth for 5 weeks at 10°C and 90% relative humidity.²⁵³

Ground meat and fish products were preserved at pH < 6.7 with 0.1 to 1.0% long-chain ($\geq C_{18}$) unsaturated fatty acids, optionally containing a hydroxyl group or their alkali metal salts together with sorbate.²¹⁴ Addition of unsaturated fatty acids markedly increased the effectiveness of sorbates as preservatives.

Three chemical dips consisting of combinations of potassium sorbate (10 to 20 g), sodium acetate (5 to 10 g), sodium chloride (5 g), water (100 ml), with or without propylene glycol (20 ml), glycerol (10 ml), sodium citrate (10 g), and 0.5 N sodium hydroxide (to pH 6), were tested against *E. coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Clostridium perfringens* at 20 and 30°C in unchilled fresh beef.²⁵⁴ All treatments inhibited bacterial growth to a similar extent, and shelf life was extended to 44 h at 30°C or 68 h at 20°C. Control samples spoiled in less than 32 h.

The shelf life of beef carcasses was doubled by spraying with a mixture of sorbate, acetate, and sodium chloride.²⁵⁵ Ice containing glycol diformate (0.02 to 0.04%) and sorbic acid (0.1%) was superior in preserving chickens and crawfish to plain ice or ice and tetracycline.²⁵⁶ A 5-cycle rapid-freeze/rapid-thaw process was used in conjunction with chemicals to reduce *Salmonella typhimurium* counts on poultry meat.²⁵⁷ Chemicals used included chlorine, sorbate, lactic acid, and propionate.

A proposed mold inhibitor for application in the dry form on meat and cheese products consists of a mixture of 10 to 90% sodium bicarbonate and 10 to 90% sodium chloride with which are mixed 0.5 to 3.5 g sodium diacetate and 1 to 4 g sorbic acid per pound of dry mix.²⁵⁸ Fish, such as salmon, were preserved by smoking and treatment with a solution containing sodium sorbate and sodium erythorbate to inhibit yeast and mold growth and enhance and stabilize the desirable pink-red color of the fish. The preservative solution could be applied either before or after smoking.²⁵⁹ Sturgeon soft caviar was preserved with sodium

benzoate, sorbic acid, and sodium hexametaphosphate, and it was rated equivalent to pasteurized caviar in microbial stability, nutrition, and flavor.²⁶⁰

Potassium sorbate (0.26%), ethyl alcohol (5%), glycine (2%), and/or serine (2%) were effective in preventing deterioration of packaged kamaboko for more than 6 d at 25°C.²⁶¹ The mixture was effective at pH values of 6.13 to 6.90, and the most effective combination consisted of 0.26% sorbate, 3% ethanol, and 1% glycine. Fish paste or kamaboko was also preserved without pH adjustment by a mixture of propylene glycol (1 to 1.5%), glycine (0.5%), and sorbate (0.26%).²⁶²

Mackerel fish was dry-cured with sodium chloride + sodium benzoate + sodium acid pyrophosphate + sodium bicarbonate + sodium hexametaphosphate + ascorbic acid and subsequently dipped in a solution of sorbate prior to drying and it was stable for 6 to 7 months at room temperature.²⁶³ Sodium or potassium phosphates, sorbate, and citric acid also improved the shelf life and quality of fish.²⁶⁴ Dipping of ice-stored herring in a mixture of oxytetracycline, sorbic acid, and propyl gallate, however, did not improve its shelf life.¹⁹³

Dehydro-irradiation has been described as useful for ambient storage of shell-on tropical shrimp.²⁶⁵ The process integrates treatments such as reduced water activity by drying to 40% moisture; incorporation of sodium chloride (3.5 to 4.0%) and sorbic acid (0.03 to 0.04%); packaging; and irradiation (0.25 Mrad). The product retained its color, texture, and flavor during storage.

A solution containing citric acid (3 to 5 parts), sorbate (0.5 to 1.5 parts) and optionally saccharin (0.1 to 0.5 parts), and sufficient water to give 500 to 1000 parts was found to be a preservative for prepackaged vegetables and had only a slight acid taste.²⁶⁶ Raw, cleaned potatoes could be stored for 50 h without browning after they were soaked for 5 min in any of the following preservative solutions: sodium bicarbonate; benzoic acid and 0.1% sodium bicarbonate; sodium bicarbonate + sorbic acid + ascorbic acid; or sorbic acid + nisin.²⁶⁷ Cut lettuce could be stored for up to 4 weeks under refrigeration by dipping for about 1 min in a 5% solution of $\text{Na}_2\text{S}_2\text{O}_5$ (0.9%), citric acid (0.6%), ascorbic acid (0.03%), and potassium sorbate (0.2%). It was packaged in film and closed tightly after draining.²⁶⁸ A preservative for pickles consisted of finely powdered sorbic acid suspended in an organic acid solution (e.g., malic), optionally in the presence of sucrose esters.²⁶⁹ Yeast growth on sauerkraut was inhibited with 0.05% sodium benzoate or 0.02% sorbic acid in the presence of 3% sodium chloride and 1.5% lactic acid.²⁷⁰ A mixture of glucose oxidase, sorbic acid, and ethanol ensured stabilization and preservation of the high quality of thermally sealed strawberry, raspberry, and black currant juices over a period of 6 to 8 months.²²³

The inhibitory effect of sorbic acid, sulfur dioxide, and formic acid on alcoholic fermentation with *Saccharomyces ellipsoideus* was enhanced when ultrasound was applied simultaneously.²⁷¹ A mixture containing tannin, sorbic acid, and ethanol was mixed with, sprayed onto, or impregnated into food products in an acidic pH. The preservative effect of tannin was increased synergistically with sorbic acid and ethanol.²⁷²

Rice was prepared by cooking in a stabilizing solution consisting of 3 to 7% polyhydric alcohol, sodium chloride, and sufficient antimycotic (e.g., sorbic acid) to inhibit mold growth.²⁷³ The same solution could be used to preserve pasta products.²⁷⁴

The stability of low-fat mayonnaise was improved by chemical stabilization with a mixture of sodium benzoate and potassium sorbate and 45 min of heating at 90°C. The product was suitable for storage at 15°C for 12 weeks, while without the treatment maximum storage was only 4 weeks.²⁷⁵ Incorporation of citric acid, sucrose, and sorbic acid increased the effectiveness of sorbic acid as an inhibitor of molds in chapaties.²⁷⁶

Best results for preserving milk samples in storage pending microbiological testing were obtained with a mixture consisting of boric acid (50 g), potassium sorbate (0.75 g), glycerol (10 g), and 1% methylene blue (0.5 ml). The mixture was dissolved in 1 liter of water,

boric acid, glycerol, potassium sorbate, and nystatin was also suitable for preventing the multiplication of microorganisms in milk and caused the least change in the direct epifluorescent filter technique count of bacteria and somatic cells.²⁷⁸

Concentrated (about 100 g solids/100 g water) sweet whey was stabilized against microbial spoilage by a combination of a slight reduction of water activity, lowered pH, and addition of potassium sorbate. This product could be stored for up to 3 months at 30°C without bacterial or mold deterioration and with no need for spray drying.²⁷⁹

An aqueous composition containing propionic acid, sorbic acid, and preferably dehydroacetic acid and methyl paraben preserved hides from bacterial growth for 30 d when stored in a 40% aqueous solution containing 0.4% of the mixture.²⁸⁰

Sorbic acid, *o*-phenylphenol, and sodium benzoate prevented microbial growth and physical deterioration (i.e., water separation and coagulation) of a starch paste (glue).²³⁸

A combination of starch, hydroxypropyl cellulose, sorbate, and an antioxidant gave water-dispersible, biodegradable products, which were resistant to fungal attack and useful for production of tampon applicators.²⁸¹

Several other patents also indicate synergistic antimicrobial effects of complex compositions containing sorbate. One of these includes sorbic acid, dehydroacetic acid, or their salts; 2-pyridinethiol-1-oxide or its salts; tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione; or tetracyldi (aminoethyl) glycine.²⁸² A series of five patents was based on compositions containing N-(fluorodichloro-methyl thio) phthalimide and one or more of dehydroacetic acid, sorbic acid, and their salts. In other patents, the former compound was replaced with methyl-1-(butyl carbomoyl)-2-benzimidazole carbamate, *o*-phenylphenols, *p*-chloro-*m*-xylenol, or 2,4,5,6-tetrachloro isophthalonitrile, respectively.²⁸³⁻²⁸⁸

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INTERACTIONS AND SYNERGISTIC EFFECTS

I. INTRODUCTION

Environmental, compositional, and processing factors, such as pH of the substrate, concentration of sorbate, water activity, temperature of processing and storage, gas atmosphere and packaging, type of microbial flora, initial microbial load, composition of the food, and other additives and certain food components, singly or in combination, can influence the antimicrobial activity of sorbates.

These factors, acting in combination with sorbate, may broaden, intensify, enhance, or negate its antimicrobial activity. Thus, they can act synergistically or be antagonistic to sorbate. This, of course, depends on individual factors, levels, and combinations. If growth of spoilage or pathogenic microorganisms is inhibited in a system but the organisms remain viable, then growth may eventually resume when environmental factors and other conditions change and become supportive to growth or antagonistic to sorbate. The length of microbial inhibition and product shelf life will vary with the intensity and importance of these factors that influence the activity of sorbate. The complexity of the various interactions in nonhomogeneous food systems makes it difficult to estimate or predict the antimicrobial contribution of sorbate under commercial conditions. Variation in interactions may also be responsible for discrepancies in the antimicrobial activity of sorbate reported in the literature.

Combining food preservatives may enhance, reduce, or cause no change in their individual antimicrobial effects. Thus, the interaction of two or more preservatives may be synergistic, additive, or antagonistic.¹ An interaction is synergistic when a combination of smaller amounts (e.g., 40%) than the minimum inhibitory concentration (MIC) of each of two preservatives results in 100% inhibition of the test organism. In contrast, the relationship is antagonistic when 50% of the MIC of the compound and a larger amount (i.e., 85%) of the second preservative are needed for 100% inhibition of growth. The additive effect occurs when a combination of 50% of each preservative results in 100% inhibition.^{1,2} Although an additive effect does not permit a reduction in the total amount of chemicals needed for preservation, its usefulness is usually related to a broader spectrum of microorganisms being inhibited by the combination. The actual type of interaction between two chemical food preservatives can be variable, depending on amounts used, microorganisms being inhibited, type of food, and environmental conditions.

Several examples in the literature have indicated synergistic and antagonistic effects of sorbate with other chemicals. A mixture of sorbate and sulfur dioxide has acted synergistically to inhibit *Aspergillus niger*,³ while their relationship may have been additive against *Escherichia coli*.² The effects of combinations of preservatives on *E. coli* have been classified into three groups.² Benzoic acid and ethyl and propyl parabens tended to be additive or slightly antagonistic when used in combination with other preservatives. Sorbic acid, boric acid, and sodium formate tended to be antagonistic when used in combination with other preservatives, while sodium sorbate tended to be synergistic. Additive interactions also exist against *E. coli* between sorbate and formic acid, benzoic acid, and parabens.² Sorbate and formic acid combinations are antagonistic against *Saccharomyces cerevisiae* and synergistic against *A. niger*.^{1,3,4}

The influence of various natural food components and added chemical compounds in food on the antimicrobial action of sorbic acid and other preservatives has been studied against *A. niger*.⁵ Sodium, potassium, and magnesium chlorides at 0.01 to 0.25% and sugars were without influence; isobutyric acid, gluconic acid, and cysteine-HCl were synergistic;

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and calcium chloride, malonic acid, and malic acid were antagonistic to sorbate. The binary combinations of nisin or tylosin with parabens were synergistic with sorbic and benzoic acids against *Lactobacillus buchneri*, additive against *Staphylococcus aureus*, and antagonistic against *E. coli*.^{6,7}

II. INTRINSIC PARAMETERS

A. FOOD COMPONENTS

Since solubility, distribution, and partition of sorbate in foods varies with composition and types of individual components, its antimicrobial activity will also be variable. Factors that influence the selection of a food preservative and the concentration to be used include the composition of the food; its pH; water content; presence of other inhibitors (e.g., salt, sugar, spices, smoke, acids, chemical additives); initial level and type of contamination, which is influenced by type of processing, ingredients, and sanitation of premises and equipment; and handling and distribution of the food (e.g., length and temperature of storage, frequency of consumer use, and expected shelf life).⁸

Compared to other common food preservatives, sorbate has a more favorable fat-to-water partition ratio (Chapter 3).^{1,9,10} This enhances the usefulness of sorbate in lipid-containing foods, such as mayonnaise, margarine, and salad dressings, because in these fat-in-water emulsions a relatively higher level of sorbate, compared to other preservatives, is found in the microbiologically susceptible water phase. Increased amounts of fat, however, will reduce the concentration of sorbate in the aqueous phase where microbial control is necessary.¹¹ Salt, sugars, and other soluble food components will also reduce the concentration of sorbate in the aqueous phase.^{1,9,12} The synergistic activity of sorbate with sugar and salt, however, may offset the negative effects of these solutes on the solubility of sorbate.¹²⁻²⁰

Presence of acids in the food system may reduce the water solubility of sorbate (Chapter 3), but they also enhance microbial inhibition by increasing the amount of undissociated sorbic acid, by increasing the hydrogen ion concentration and by their own direct antimicrobial effects.^{1,12} The specific type of acid present in the food will also be influential on the antimicrobial activity of sorbate. Acetic acid decreases the fat-to-water partition ratio of sorbate.¹² Citric and lactic acids enhance the antimicrobial action of sorbate to a greater extent than inorganic acids.^{21,22} In complex food systems, however, it is usually difficult to predict multifactor interactions and their positive or negative contribution to the antimicrobial activity of sorbate.

In certain instances, microbial inactivation or inhibition is more efficient in simpler substrates, while in others the opposite is true. For example, inactivation of *Salmonella typhimurium* by sorbate was more rapid in nutrient broth and required substantially more time in food systems (i.e., skim milk and evaporated milk).^{23,24} Inhibition of *Aspergillus parasiticus* growth and aflatoxin production, however, was more pronounced in an enriched medium compared to a basal medium.²⁵ The inhibitory effect (Table 1) of sorbate on sublethally injured (i.e., freezing, heat) pseudomonads was greater in basal broth than in a rich medium (i.e., tryptone-peptone-extract).²⁶ Similarly, inhibition of growth of *Pseudomonas aeruginosa* by sorbate was less in substrates containing bouillon, yeast extract, casaminoacids, or peptone than in a medium containing ammonium nitrate.²⁷ These discrepancies may be due to variation in other factors involved in inhibition among different experimental conditions. Thus, studies should be designed to examine microbial inhibition by sorbate under conditions encountered in specific applications.

B. MICROBIAL FLORA

The influence of the type of microbial flora and variation in inhibition among microbial strains are discussed in Chapters 4 and 6. In general, the types and number of microorganisms

TABLE 1
Effect of Sorbate and Recovery Media on Growth Characteristics of Heat-Stressed *Alteromonas putrefaciens*²⁶

Heating at 45°C (s)	Potassium sorbate in broth (%)	Minimal broth		Tryptone-peptone extract broth	
		Lag(h)	GT(h)	Lag(h)	GT(h)
0	0	5	12	2	3
0	0.05	15	33	5	4
10	0	38	12	10	4
10	0.05	NG	NG	18	4
120	0	55	12	15	4
120	0.05	NG	NG	26	5

Note: GT, generation time; NG, no growth.

present in a food product will affect the potential of sorbate to inhibit growth and preserve the product.

Sorbate is an inhibitor of all types of microorganisms (i.e., yeasts, molds, and bacteria), but it is not effective against all species and strains (Chapter 6). Some microorganisms can grow in the presence of high concentrations of sorbate (>0.3%); some can actually metabolize the compound while others are inhibited with very low (<0.05%) concentrations. Sorbate concentrations above 0.3%, however, are impractical in food preservation because of adverse effects on its sensory characteristics (Chapter 8). In foods contaminated with a mixed flora of microorganisms, some microorganisms may be inhibited, while the resistant ones may grow and spoil the food. In certain instances, the resistant strains may actually grow faster than normal because of lack of competition. In general, preservation of food products will vary with concentration of sorbate and type and level of contamination.

The antimicrobial activity of sorbate is reduced with increasing levels of contamination. Low initial contamination usually permits sorbate to extend the lag phase of microbial growth, while greater concentrations of the preservative are needed for inhibition of the logarithmic phase of growth.^{1,4} Actually, sorbate levels permitted in foods ($\leq 0.3\%$) are inadequate for preservation of grossly contaminated products.²⁸ Thus, use of sorbate is not recommended as a substitute for appropriate sanitation practices and good manufacturing procedures.

C. WATER ACTIVITY

The action of antimicrobial food preservatives is generally increased as the water activity of the system is reduced.²⁹ Thus, addition of solutes that reduce water activity enhances antimicrobial action.¹ Common solutes present in foods or added to control water activity are sodium chloride and sugar. Several studies have reported increased antimicrobial activity by sorbate with addition of sugar or sodium chloride to the substrate.¹

In systems with high amounts of solutes (e.g., sodium chloride, sucrose, glucose), sorbate is even effective at higher pH (e.g., 6.5) values.¹² Sodium chloride increases the activity of sorbate against undesirable yeasts in cucumber fermentations, while sucrose also increases inhibition by sorbate in sweet cucumber pickles.¹⁵⁻¹⁷ Increased sorbate and brine levels, however, may also inhibit the desirable lactic-acid-producing bacteria in pickle manufacture (Chapter 8). Thus, optimum combinations of these ingredients should be selected for acceptable fermentation and preservation.

Increased inhibition by sorbate in presence of higher solute concentrations has been reported in both nutrient media and several foods against various microorganisms. Examples of microorganisms inhibited by sorbate in combination with solutes, such as sugars or sodium chloride, include yeasts and molds (e.g., *Aspergillus* spp., *Oospora* spp., *Mucor*

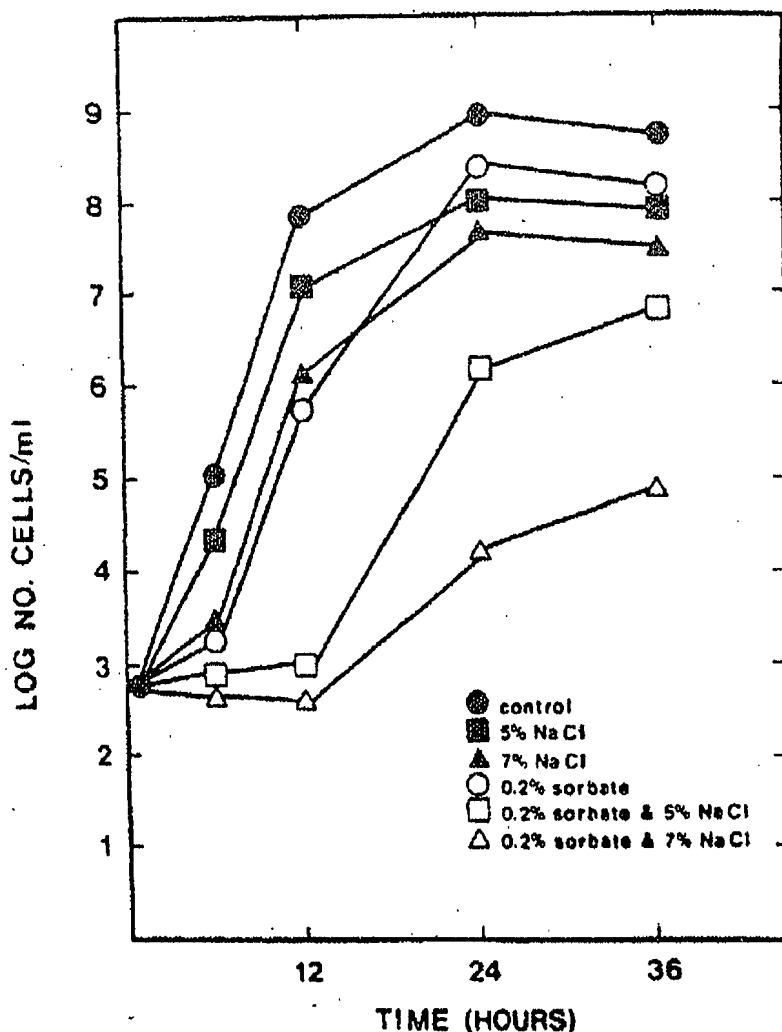


FIGURE 1. Interaction of potassium sorbate and sodium chloride against growth of *Staphylococcus aureus* 12600 in pH 6.0 trypticase soy broth at 37°C. (From Robach, M. C. and Stateler, C. L., *J. Food Prot.*, 43, 208, 1980. With permission.)

spp.)^{13,30-35} and bacteria such as *S. aureus* (Fig. 1),¹⁹ (*Staphylococcus epidermidis*,¹³ *Staphylococcus* spp.,³⁶ *Brochothrix thermosphacta*, *Serratia liquefaciens*, *Bacillus cereus*, *Yersinia enterocolitica*, *Lactobacillus* sp.,³⁷ *Micrococcus* sp.,³⁶ *Clostridium sporogenes*,³⁸⁻⁴¹ *Salmonella typhimurium*,^{42,43} and *Clostridium botulinum*.^{44,45}

In addition to nutrient media, increased inhibition at higher solute levels has been reported in egg yolk,³⁶ uncured poultry products,³⁹ strawberry jam,³⁰ raspberry syrup,⁴⁶ butter,^{31,47} whey,⁴⁸ grape juice,³³ prunes,³⁴ mango pulp,⁴⁹ biltong,³⁵ processed cheese spreads,⁴⁵ comminuted beef/pork^{37,38} and in a pork slurry.⁴⁴ Use of 1% sorbate has permitted reduction of the sodium chloride level from 11% to 7% to achieve the same inhibition of staphylococci and micrococci in egg yolks of pH 6.5.³⁶ Stable, uncured poultry products can be formed with very low salt levels (0.65%) when sorbate (0.20%) is included in the formulation.³⁹

The lack of inhibition of lactic-acid-producing bacteria and clostridia by sorbate reported in early studies (Chapter 6) may have also been influenced by the absence of sodium chloride from the culture media. Studies reporting inhibition of *C. botulinum* in cured meat products have used 1.5 to 2.5% sodium chloride in the formulation, which may have been critical in inhibition.⁵⁰

It is interesting that solutes, such as sodium chloride and sucrose, increase the inhibitory activity of sorbate, considering the fact that they reduce sorbic acid solubility in water and increase the partition ratio in oil-to-water from 3.0 to 5.0, when they are present at con-

centrations $>10\%$ (Chapter 3).^{9,12} Apparently, their inhibitory activity through lowering of water activity and the increased activity of sorbate at lower water activities compensate for any changes in solubility.

The influence of reduced water activity on the action of sorbate is not always positive. Presence of sucrose or sodium chloride in certain situations has reduced the synergistic effect of sorbate and heat on the inactivation of microorganisms.²⁰ Cells of *S. typhimurium* remained viable for a longer period of time in a soy-starch system of water activity 0.79 than 0.97. In general, as storage time and temperature (22°C vs. 40°C) increased, the cells were stressed at a faster rate at the higher water activity values.⁴³

In nutrient broth, sodium chloride (1.25 and 2.5%) decreased the effects of potassium sorbate and sodium acid pyrophosphate against *C. botulinum*.⁵¹⁻⁵³ Decreasing the sodium chloride level from 2.5% to 1.25% reduced the antibotulinal effects of phosphate and sorbate, but elimination of sodium chloride increased the inhibitory effect. At these low levels, however, sodium chloride may have acted to stimulate growth of the organism.⁵⁴ Levels of 1.25 to 2.50% (w/w) sodium chloride in foods of 50% solids are in the range of 2.5 to 5.0% brine (w/v), while in the broth system the brine level was essentially equivalent to the ingoing salt level (1.25 to 2.50%).

Preconditioning of *Saccharomyces rouxii* cells in 60% sucrose + 0.1% sorbate rendered the cells more sensitive to inhibition by sorbate compared to cells preconditioned in 0% sucrose + 0.1% sorbate.⁵⁵ Lowering of the water activity, however, enhanced the resistance of *S. rouxii* to increasing concentrations of sorbate.^{56,57}

In general, it is believed that solutes increase the inhibitory activity of sorbate by reducing the water activity. A potential mode of action for this phenomenon may be that the solutes induce cellular swelling, which increases the sensitivity of microorganisms to preservatives.^{1,4}

D. INFLUENCE OF pH

It has been well established that the antimicrobial activity of weak, short-chain, lipophilic acid preservatives such as sorbate is dependent on the pH of the substrate.^{1,4,9,10,50,58} In general, antimicrobial activity increases as the pH of the environment decreases and approaches the dissociation constant (pKa value) of the compound. Thus, sorbate is more effective as a preservative in food products with lower pH (<6.5) values.^{8-10,59-61}

Increased inhibition by sorbate with reduced pH has been reported for several species of microorganisms, including *Bacillus subtilis*,⁶² *C. botulinum*,^{44,45,63-66} *Staphylococcus aureus*,^{67,68} aflatoxin production by *A. parasiticus*,⁶⁹ *Pseudomonas fluorescens*,⁷⁰ *P. aeruginosa*,²⁷ *Salmonella enteritidis*,⁷¹ and *Salmonella typhimurium*.⁷² Increased inhibition at reduced pH values has been observed in both nutrient media and foods.

The maximum pH for inhibition by sorbate is believed to be in the range of 6.0 to 6.5.^{1,9,10} Optimum effectiveness, however, is at pH values below 6.0, while it is relatively ineffective at pH values of 7.0 and above.^{10,62,63,66,73}

Sorbates have the advantage of being effective at pH values higher (6.0 to 6.5) than those for propionate (5.0 to 5.5) and benzoate (4.0 to 4.5), which are also commonly used as food preservatives.^{9,10} The ability of sorbate to act as an antimicrobial agent at higher pH values (>5.5) allows use of the preservative in more foods than benzoates and propionates and in foods where esters of parahydroxy benzoic acid (i.e., parabens) may not be useful because of solubility and water-to-fat partition problems. Sorbates can also be used in combination with benzoates or propionates in foods of reduced pH. This is done to minimize off-flavors by using lower concentrations and to broaden the scope of microorganisms inhibited.^{9,10,12,28}

Overall, it is very important to use sorbate and other chemical food preservatives in products of appropriate pH values. The pH at which inhibition becomes significant, however, is dependent on factors such as concentration of sorbate, species and strains of microor-

ganisms, temperature of storage, type and properties of the substrate, acidulant used to adjust the pH, etc.

Inhibition of *S. typhimurium* by 0.3% sorbate was observed at pH values below 6.7 in nutrient broth and at pH 6.4 in skim milk.³³ Overall, however, inactivation was greater at pH 5.0 than 5.5. The lower the pH of the substrate, the lower the concentration of sorbate needed for microbial inhibition.⁷⁴ At pH 5.5, sorbate was bacteriostatic and bacteriocidal to various bacteria at a concentration of 0.1%, but at pH 7 to 8, 10% of sorbate was ineffective.⁷⁵ This has also been demonstrated in the inhibition of ochratoxin A production by *Aspergillus sulphureus* and *Penicillium viridicatum*.⁷⁶ At pH values below 4.0, very low concentrations of sorbate (0.002%) may be inhibitory to fungi, yeasts, and bacteria.^{60,61}

Depending on microorganisms or substrate, low sorbate concentrations can be inhibitory even at higher pH values. A concentration of 0.05% sorbate delayed growth of *Vibrio parahaemolyticus* in crab meat and flounder homogenates at pH 6.2.⁷⁷ In emulsified chicken meat products, however, levels of 0.2% sorbate were needed to delay growth of *C. botulinum* at pH values of less than 6.0 or 6.2, depending on the absence or presence of nitrite in the formulation, respectively.⁶³ No inhibition was observed in chicken meat products with pH values above these levels. The minimum inhibitory concentration of sorbate against *Penicillium* molds at pH 4.75 was 0.020 to 0.025%, while at pH 5.5 it was 0.06 to 0.08%.⁷⁸ In nutrient broth, a level of 0.05% sorbate inhibited growth of *Pseudomonas fluorescens* at pH 5.5, while at pH 6.0 a concentration of 0.2% sorbate was necessary to delay growth.⁷⁰ The influence of pH on the antimicrobial activity of sorbate will also vary with individual microbial strains under consideration.^{79,80}

In addition to overall growth and production of metabolites (e.g., mycotoxins), inhibition of spore germination by sorbate is also more pronounced at lower pH values.^{66,79} Inhibition of uptake processes, such as uptake of amino acids (i.e., L-serine and L-histidine) by *S. typhimurium*, is also more pronounced at lower pH values.⁷² Oxygen uptake by *P. aeruginosa* with several substrates (i.e., acetate, pyruvate, lactate, α -ketoglutarate, and oxaloacetate) is lower (10-fold) in the presence of sorbate at pH 5.3 compared to pH 6.8.²⁷

While the antimicrobial activity of sorbic acid and its metal salts is increased with reduced pH values, other derivatives of sorbate show antimicrobial activity which is almost independent of pH. For example, esters of sorbic acid show antimicrobial activity at levels as low as 0.002%, independent of pH of the substrate.⁶¹

Depending on the type of sorbate added to a substrate, its pH may also change. Use of potassium sorbate is not expected to result in major changes (± 0.10 pH units) in the pH of the food, while sorbic acid may reduce pH values by 0.20 to 0.40 pH units.^{38,58,81} The influence of the type of acidulant used to adjust the pH on microbial inhibition is discussed in another section.

Although it has been well accepted that the antimicrobial activity of sorbate is more pronounced at pH values below 6.0 to 6.5 with virtually no inhibition at pH values near neutrality, there is evidence in the literature that sorbate has inhibited microbial growth in nutrient media and foods of pH values above 6.5. Sorbate inhibited *B. subtilis* in the rice filling of Karelian (from Finland) pastry at pH 6.60⁸² and extended the shelf life of cod fish with an initial pH of 6.7 to 7.0.⁸³ At pH 7.0, sorbate (0.5%) has also inhibited *Moraxella* organisms isolated from seafood.²⁶ Sorbate has also inhibited *C. botulinum* at pH 6.5⁸⁴ and 6.9.⁶⁵ In addition, sorbate levels as low as 0.003% showed inhibition of *V. parahaemolyticus* in a broth of pH 6.7. Other studies, however, may have not been designed or their methodology may have been insufficient to detect minor inhibitory effects that may have taken place at higher pH values.⁶⁵

1. Dissociation

In general, the interaction of pH with sorbate is not completely understood. The increased inhibitory activity of sorbate at lower pH values has been attributed to the influence of pH

on the amount of undissociated acid present in the substrate (Chapter 3).^{9,10} In general, the antimicrobial activity of sorbate and other weak lipophilic acids has been attributed for a long time to the undissociated form and not the acid anion of the compound.^{4,11,29,60,64,85-93}

Sorbate and similar organic acids dissociate in aqueous solutions and release hydrogen ions. The extent of their dissociation depends on the pH value of the solution. Certain acids, including acetic, reduce the pH of the substrate and inhibit microbial growth through the high concentration of hydrogen ions.¹ Sorbate and similar weak acids, however, cause only minor decreases in pH and act as antimicrobials mostly through their undissociated species.^{4,87,94,95} Since the amount of the compound in the undissociated form is determined by the pH of the environment, antimicrobial activity is enhanced at pH values approaching the dissociation constant (pKa value), where 50% of the acid is in the undissociated form.

With antimicrobials such as sorbate, concentrations as high as 0.1 to 0.3% are needed for microbial inhibition, especially as the pH of the substrate is increased (>5.0). This is in contrast to lower levels (<0.1%) needed in substrates of low (<5.0) pH, and the small amounts (0.01 to 0.02%) of other preservatives (e.g., sodium nitrite) which are effective even at higher pH values. If we accept, however, that only the undissociated form of sorbate is effective as an antimicrobial, then the need for these higher ingoing concentrations can be easily explained. Since only part of the ingoing sorbate will be in the undissociated form, then, depending on pH, only a portion of the ingoing levels will be effective as antimicrobials.⁵⁸ In a product of pH 5.8 with 0.2% ingoing sorbate, only 0.014% of the compound will be in the undissociated form. This is theoretical and may be even lower if we consider other factors that will influence solubility, partition, and dissociation of sorbate in individual substrates. Other reasons for the need of higher sorbate levels may also be related to mechanisms of antimicrobial action by various preservatives (Chapter 7).

In addition to pH, dissociation of sorbate is influenced by its solubility and the water activity of the system (Chapter 3).^{1,96} Thus, in order to predict the effectiveness of sorbate in a food product, we need to know the pH, its solubility, and the dissociation constant for the particular system. Although there is variation in the literature concerning the pKa value of sorbic acid, the most commonly quoted number (pKa) is pH 4.76.^{9,10,99} This value is based on the dissociation constant of $1.73 \times 10^{-5} \text{ mol dm}^{-3}$. As indicated above, however, the pKa value may vary because it is influenced by other factors, including water activity.⁹⁷ This is because dissociation requires the association of the hydrogen ion of the carboxylic group with water to form a hydroxonium ion.⁹⁸ Information on the dissociation constant of sorbic acid in presence of solutes is limited, with the exception of work on dissociation in water-glycerol mixtures.^{97,99}

The pKa value for sorbic acid at 25°C in water-glycerol mixtures can be fitted, with a standard deviation of fit of 0.017, by the equation: $\text{pKa} = 4.680 + 0.0123 M - 2.66 \times 10^{-4} M^2$, where $M = \% \text{ (w/w) glycerol in the solution}$.⁹⁷ Values for pKa determined at 25°C include 4.674 in absence of glycerol, 4.807 in 20% glycerol, and 4.625 in 50.02% glycerol. The dissociation constant of sorbic acid in water at 25°C, determined by Pethybridge et al.,⁹⁷ is $2.12 \times 10^{-5} \text{ mol dm}^{-3}$ (pKa = 4.67), which is similar to the original value of $1.73 \times 10^{-5} \text{ mol dm}^{-3}$ (pKa = 4.76) and the commonly reported pKa value of 4.8.²⁹

Considering the nonhomogeneity of food systems, the difference between the above dissociation values should not be considered significant enough to result in major variations in microbial inhibition by sorbate. Also, it has been demonstrated that with changes in water activity between 0.80 and 1.00, pKa values vary only between 4.60 and 4.80.⁹⁷ Thus, changes in pKa values with reduction in water activity are not large enough to result in major differences in inhibition due to differences in the amount of undissociated acid available in complex nonhomogeneous food systems.⁹⁷

Overall, the dissociation properties of sorbic acid appear to be relatively constant if changes in water activity are within the logical range of foods needing preservation with

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sorbate. Variations in the pH of the food, however, will change the amount of sorbate in the undissociated form, and this will influence its antimicrobial activity. Thus, any factor that may influence product pH will also affect the antimicrobial activity of sorbate.

Although the antimicrobial activity of sorbate is generally believed to increase with reduced pH values, as indicated above, a few studies have demonstrated antimicrobial action even at pH values (>6.5) above its accepted maximum. A few studies have also shown that the activity of weak acids, including sorbic, is sometimes less influenced by pH changes than believed.¹⁰⁰⁻¹⁰⁴ Since at higher pH values the undissociated portion of the compound is minor or nonexistent, the question is whether the dissociated form of the compound is also antimicrobial.

Rehm and Lukas¹⁰⁰ attempted in 1963 to determine the inhibitory efficiency of the undissociated and dissociated forms of sorbate and indicated that the dissociated molecules may show antimicrobial activity, which can be variable among microbial species. Antimicrobial activity, however, was mostly due to the undissociated form, since the minimum inhibitory concentration of the anion was 20 to 40 times that of the free acid for *Saccharomyces cerevisiae*, 100 times for *A. niger*, and 300 to 400 times for *E. coli*.¹⁰⁰

The popular theory that growth is inhibited only by the undissociated acid was also questioned 20 years later by Eklund.¹⁰⁴ A study evaluated the minimum inhibitory concentration of sorbic acid for *B. subtilis*, *B. cereus*, *E. coli*, *P. aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* at different pH values. The author derived a mathematical model for ascribing antimicrobial activity to the undissociated acid, based on the traditional theory of inhibition only by the undissociated form of the compound. This equation,

$$MIC_{total} = \frac{(MIC_{undiss})pH(10^{pH})}{10^{pK}} + MIC_{undiss} \quad (1)$$

states that the minimum inhibitory concentration of the total acid increases exponentially with increasing pH. This, however, did not agree with his experimental data and, thus, Eklund¹⁰⁴ derived a new mathematical model,

$$MIC_{total} = \frac{1}{\frac{(1-a)}{MIC_{diss}} \frac{a}{MIC_{undiss}}} \quad (2)$$

$$a = \frac{[HA]}{[A^-] + [HA]} = \frac{1}{10^{pH-pK} + 1} \quad (3)$$

to fit the experimental total MIC values at different pH levels. Using this new model, Eklund¹⁰⁴ concluded that the dissociated acid had an effect on inhibition, but it was 10 to 600 times less than the effect of the undissociated acid. At pH values above 6.0, however, more than 50% of the preservative action of sorbic acid was attributed to the dissociated species.

These results indicate that the commonly held view that only the undissociated acid is effective may not be true. Therefore, the concept that sorbic acid causes acidification of the interior of the cell and that this is the main mechanism of inhibition cannot completely explain inhibition of growth by sorbic acid at pH values near neutrality. It would appear that more than one mechanism of inhibition is involved (Chapter 7).

III. PROCESSING VARIABLES

A. HEAT TREATMENT

Sorbate may interact with heat treatments and affect dormancy of heated spores, rate and extent of microbial destruction during heating, and recovery of heat-injured spores. Low levels (0.01%) of sorbate may have a stimulatory influence on breaking dormancy during heat activation, as indicated with ascospores of *Byssoschlamys nivea* in grape juice.¹⁰⁵ Low sorbate levels may also act synergistically with heat to inactivate yeasts, molds, and bacteria. Sorbate also can inhibit repair of thermally injured cells.

Increased thermal inactivation in the presence of sorbate has been demonstrated with several yeasts, including *Candida utilis*,¹⁰⁶⁻¹⁰⁸ *Saccharomyces cerevisiae*,^{109,110} *Candida krusei*, *Hansenula anomala*, *S. bailii*, *Torulopsis magnoliae*,¹¹⁰ *Debaryomyces hansenii*, *Pichia membranaefaciens*, *Kloeckera apiculata*, and *Rhodotorula rubra*.^{111,112} Increased thermal inactivation of molds in the presence of sorbate has been reported for *Aspergillus flavus*, *Geotrichum candidum*, *Penicillium puberulum*,^{20,113} *Byssoschlamys nivea*,¹¹³ and *A. niger* (Figure 2).¹⁰⁶

Also, certain bacteria have undergone enhanced thermal inactivation in the presence of sorbate. These include *Pseudomonas fluorescens*,^{109,114} *Bacillus licheniformis*,¹¹⁵ *E. coli*,^{106,108,116} *Lactobacillus viridescens*,¹¹⁷ and *V. parahaemolyticus*.¹¹⁸

In addition to enhanced thermal destruction of yeasts, molds, and bacteria in the presence of sorbate, recovery of heat-injured organisms is also inhibited by presence of sorbate in the substrate (Table 2). Thermally injured yeasts inhibited by sorbate in the recovery medium include *C. utilis*,^{106,107} *D. hansenii*, *Pichia membranaefaciens*, *S. cerevisiae*, *C. krusei*, *K. apiculata*, and *R. rubra*.^{111,112} Inhibition of repair in molds has been demonstrated with *A. parasiticus*,⁶⁹ *A. flavus*, and *Penicillium puberulum*.^{20,113,119}

Shibasaki and Tsuchido¹⁰⁶ also reported inhibition of repair of *E. coli*. Sorbate, however, had no effect on the recovery of heat-treated (70 to 95°C, 50 min) *Bacillus firmus* and *B. pulvifaciens* spores.¹²⁰ It is also interesting to note that a significant interaction between heat treatment and sorbate existed in a pork slurry inoculated with *C. botulinum* spores. The result of the interaction, however, was to increase spoilage and probability of toxin production.⁴⁴ Thermally injured pseudomonads cells were more sensitive to sorbate during recovery than freeze-injured cells.²⁶ The importance of a report¹²¹ indicating that sorbate retarded thermal inactivation of the enzyme polyphenoloxidase is unknown. Sorbic acid has also reduced the heat treatment necessary for canning of blue-green algae for feed or other uses.¹²² Addition of sodium chloride (8%) and sorbate (0.3%) reduced the heat treatment and minimized destruction of product quality.

Thus, several microorganisms with different physiological characteristics show enhanced thermal destruction and injury in the presence of sorbate, and sorbate retards or prevents repair of their injury.

Concentrations of sorbate effective in increasing thermal destruction and inhibition of repair range from 0.0025% to 3.0%.^{71,108-111,113,114,118} The higher levels are usually needed for the inactivation of bacteria. Increasing sorbate levels also increase the extent of inactivation or inhibition.

The influence of sorbate on thermal inactivation and recovery of injured organisms is variable with species and strains of microorganisms. A higher level (0.01%) was needed to cause a significant increase in thermal inactivation of *P. puberulum* compared to the amount (0.005%) needed for *A. flavus* and *G. candidum*.¹¹³ Certain species (e.g., *P. thomii*) may be insensitive to sorbate during thermal inactivation.¹⁰⁶

Resistance of microorganisms to heat is increased in the presence of solutes (e.g., sodium chloride, sucrose) that lower the water activity of the substrate. Regardless of solute concentration, sorbate increases the sensitivity of microorganisms to heat.^{20,111,115} In certain

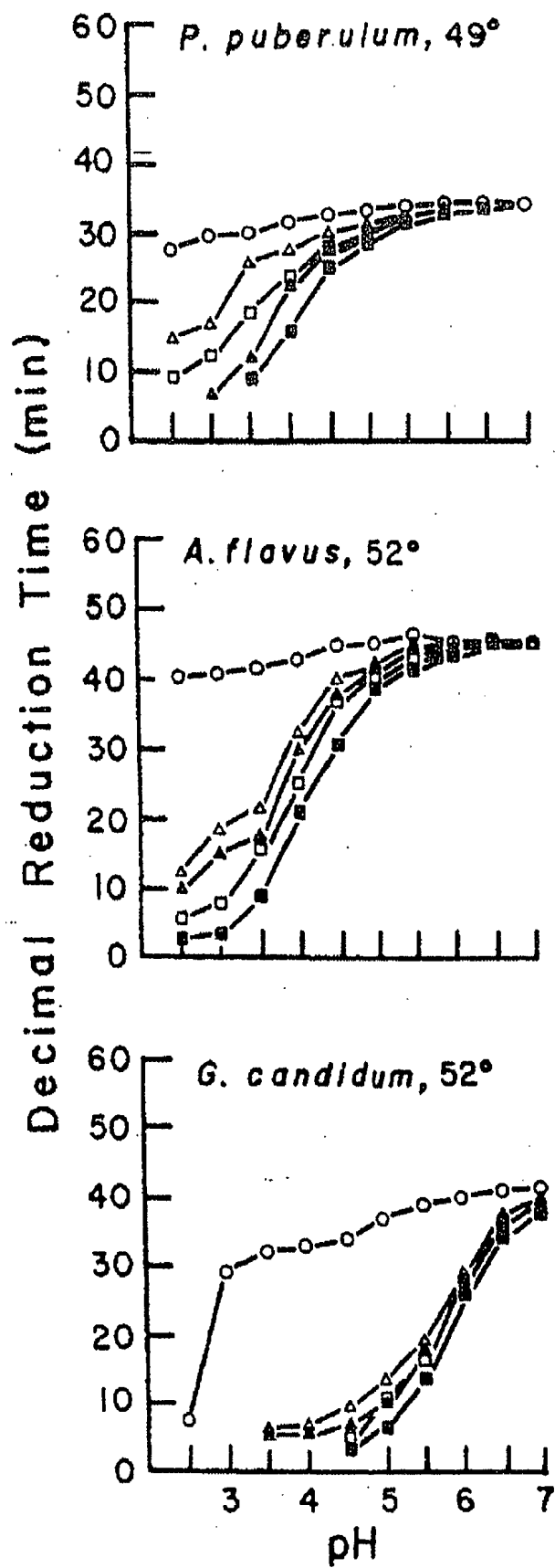


FIGURE 2. Influence of pH and preservatives in the heating medium on the rate of thermal inactivation of molds. 0, no preservative; Δ , 0.05% potassium sorbate; $\square$, 0.1% potassium sorbate; $\blacktriangle$, 0.05% sodium benzoate; $\blacksquare$, 0.1% sodium benzoate. (From Beuchat, L. R., *J. Food Prot.*, 44, 450, 1981. With permission.)

TABLE 2
Effect of Sorbate on Growth Characteristics of Heated *Pseudomonas* I spp.²⁶

Heating treatment		Potassium sorbate in broth (%)	Tryptone-peptone extract broth	
(°C)	(min)		Lag(h)	GT(h)
—	—	0	0	3.2
—	—	0.3	0.5	3.4
45	5	0	2	3.3
45	5	0.3	3.5	3.4
50	3	0	13.5	3.6
50	3	0.3	19	4
50	5	0	18	3.6
50	5	0.3	20	4

Note: GT, generation time.

TABLE 3
Effect of Sorbate and pH on Heat Resistance of Molds¹¹³

Organism	Potassium sorbate (%)	Decimal reduction time (min)		
		pH 2.5	pH 3.5	pH 4.5
<i>Penicillium puberulum</i>	0	29	30	33
	0.01	23	29	31
<i>Aspergillus flavus</i>	0	41	43	45
	0.01	19	37	43
<i>Geotrichum candidum</i>	0	7	29	31
	0.01	5	9	14

Note: GT, generation time.

instances, however, the detrimental effects of sorbate are reduced in the presence of high concentrations (30 to 50%) of sucrose.¹¹⁰ The influence of sorbate on the heat resistance of molds and yeasts may be influenced by the type of solute (e.g., sodium chloride, sucrose) present in the substrate.^{20,111} In general, the protective effect of sucrose on the thermal destruction of yeasts and molds is diminished by sorbate. Presence of organic acids (e.g., citric, tartaric, malic) in fruit juices does not cause a consistent effect on the lethality of sorbate during heating of yeasts.¹²³ Thermal destruction of molds, however, increases in the presence of sorbate and with reduced pH (Table 3).

In addition to sorbate, other food preservatives, such as benzoate, enhance thermal destruction and inhibit recovery of injured yeasts and molds. It is not clear, however, which of the two compounds (i.e., sorbate or benzoate) is more effective. With molds, sorbate was more effective than benzoate in inhibiting colony formation by heat-injured cells, and benzoate appeared to be more destructive to certain species during heating.^{20,113} With yeasts, however, benzoate was less effective than sorbate, which increased the rate of thermal inactivation at concentrations of $\leq 0.005\%$ in culture broth.^{112,113} In fruit juices, benzoate was generally more effective than sorbate in thermal inactivation of yeasts.¹¹⁰

The mechanism of increased thermal destruction and inhibition of recovery by sorbate is unknown. Beuchat¹¹³ has speculated that the higher sensitivity of molds to heat treatment may be due to an increased permeability caused by an expansion of the cell wall and cytoplasmic membrane with increased temperatures. In that case, the preservative might enter the cell more easily at higher temperatures simply because of a weakening of the physical barriers inherent in the cell wall and membrane. Differences in the structural

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transport into cells, which would influence their effectiveness at various temperatures.¹¹¹ Tsuchido et al.¹⁰⁷ found no efflux of intracellular substances and no inhibition of nucleic acid synthesis with sorbate in thermally injured cells of *Candida utilis*. They attributed inhibition of recovery to inhibition of protein synthesis and respiratory activity.

Increased thermal destruction of yeasts in presence of sorbate takes place not only in culture media, but also in foods, such as fruit juices (e.g., apple, apricot, grape, orange, and pineapple), other fruit products (e.g., apple cider, peach slices, and fruit salad), and in wine.^{1,110,123,124} Addition of sorbate to fruit products before thermal processing can afford reductions in the heat treatment (e.g., temperature and/or time) necessary for product preservation.^{117,125} This may help in maintaining better sensory qualities and nutritional value in the products without compromising their shelf life. Energy costs for processing may also be reduced. Appropriate studies are needed, however, to determine the combined effects of solutes and preservatives in combination with heat and their influence on the shelf life and quality of individual products. More research is also needed on the influence of sorbate on thermal destruction and recovery of bacteria and especially their spores.

Overall, temperatures outside the range of growth of a given microorganism will enhance the antimicrobial effectiveness of sorbate. Influence of higher temperatures was discussed here, while the effect of low storage temperatures is discussed in a subsequent section.

B. IRRADIATION

Preservation of foods with ionizing radiation has been studied since the 1940s and certain uses have been approved in several countries.¹²⁶ Combination treatments of low-dose irradiation and sorbate application may be useful in extending the shelf life of food products. Such treatments, however, must not cause any adverse effects on product quality and safety. An important question to be answered is whether irradiation affects sorbate by causing radiolysis of the compound, which might result in the formation of undesirable compounds. Treatment of foods such as fish with low-dose (i.e., pasteurization) radiation to extend shelf life may be more useful when combined with sorbate, since the compound may provide safety from *Clostridium botulinum* outgrowth and toxin production.

Research on the combined effect of irradiation and sorbate treatment of foods is rather limited. Certain studies, however, have indicated extended product shelf life when both treatments are combined. Most of these studies have examined fish and to a lesser extent fruit juices and cheese. A sorbate residue of 0.1% followed by irradiation at ≤ 4 kGy retarded mold growth on cured fish and extended shelf life compared to commonly processed fish.^{127,128} Kamaboko fish products (i.e., fish paste for surimi products) preserved with sorbate (0.2%) and irradiated at 3 kGy had a shelf life at 20°C, which was extended by 15 d compared to product treated only with sorbate.¹²⁹ Dipping of fish fillets for 40 s in a 5% sorbate solution and irradiation with 1 kGy resulted in extended product shelf life.¹³⁰ It has also been reported that an integrated treatment of shrimp consisting of a 60-min dip in 0.5% sorbate, blanching, salting, dehydration, and irradiation (2.5 kGy) stabilized the shrimp, which was free of mold and bacterial spoilage for 4 months at room temperature.¹³¹

An irradiation dose of 5 kGy in the presence of sorbate (0.005%) completely sterilized apple juice, while 10 kGy were needed for pear and peach juices. The sterilized apple juice had a shelf life of 190 d at 20 to 30°C, while the control juice (i.e., irradiated, without sorbate treatment) was fermented within 3 to 6 d.¹³² A lower dose (1 kGy) of irradiation in presence of 0.002% sorbate destroyed all yeasts in apple juice, but the fungi remained active.¹³³

One study with cheddar cheese has indicated that irradiation may supplement the antifungal properties of sorbate and extend product shelf life. Irradiation of cheddar cheese with 2 kGy inhibited mold growth at 28 to 30°C for 17 to 18 d, compared to 3 to 4 days for unirradiated controls. Spraying the cellophane wrapper with 0.5% sorbate increased the time

for onset of mold growth to 24 to 26 d postirradiation. This synergistic action was attributed to radiation-induced changes in cell wall permeability, which increase the inhibitory action of sorbate.¹³⁴ With such low doses of radiation and sorbate, the eating quality of the products does not appear to be adversely affected as was reported for fish.¹²⁷

Even though all published studies have indicated an increase in product shelf life by the combination of irradiation and sorbate treatment, one study reported that sorbate did not sensitize resting cell of *Saccharomyces cerevisiae* to destruction by irradiation.¹³⁵ It may be, however, that irradiation of sorbate results in formation of more potent antimicrobial activity. An irradiated solution of sorbate at 7 to 13 kGy showed stronger activity against *E. coli* and other bacteria than the nonirradiated solution.¹³⁶ This was true in spite of the fact that the sorbate level was reduced after irradiation of the solution. It was, thus, assumed that a new antimicrobial compound was formed in the solution by irradiation. Presence of oxygen was essential for the production of the new hypothetical antimicrobial substance. Hamada and Ishio¹²⁹ also found that after irradiation of fish the amount of sorbate present was 0.098% compared to 0.145% which was the initial level.

The potential chemical changes and radiolytic products caused by irradiation are of concern and have caused delays in acceptance and approval of radiation treatments in food preservation. A few studies have specifically examined radiolysis of sorbate. Radiolysis is expressed in G values, which indicate molecules decomposed per absorbed energy in eV.¹⁴⁰ An aqueous solution of sorbate was irradiated at 0.248 kGy/h and the G value was 1.85.¹³⁶ Hamada and Ishio have published three reports on the subject of sorbate radiolysis.¹³⁷⁻¹³⁹ G values were 4.69 (97.3%) for 0.2% sorbate when irradiated with a dose of 1 kGy. G values in a mackerel homogenate were 2.18 and in a whale homogenate 0.48 at 0.1 to 5 kGy. Sodium nitrite depressed radiolysis values of sorbate, while glucose was ineffective. It is clear that more studies are needed to examine potential benefits and disadvantages of combination treatments involving sorbate and low doses of irradiation in food products.

IV. ENVIRONMENTAL FACTORS

A. STORAGE TEMPERATURE

Sorbate is generally a more effective inhibitor of microbial growth at lower storage temperatures (Figure 3). This has been demonstrated with several microbial species and substrates. In general, storage of sorbate-treated foods at refrigerated temperatures improves their shelf life more than nonrefrigerated or untreated foods.¹⁴⁰

Increased inhibition or destruction of microorganisms by sorbate at lower temperatures has been demonstrated with clostridia,^{41,44} molds,¹⁴¹ *Salmonella typhimurium*,^{23,24,72} and *B. nivea*.^{142,143} Sorbate in combination with low temperature storage (e.g., 12° vs. 24°C) has increased the shelf life of grape juice and wine and has reduced loss of ascorbic acid from pasteurized strawberry juice.¹⁴⁰ Combinations of sorbate and nitrite were more inhibitory to bacterial growth in frankfurters at 7 to 9°C than at 20°C. Inhibition of *C. botulinum* toxin in a pork slurry by sorbate was more pronounced at lower (15°C vs. 35°C) temperatures.⁴⁴ Shelf life of butter was better in presence of sorbate at refrigerated than higher temperatures.⁴⁷ The effect of sorbate on shelf-life of vacuum-packaged, fresh, poultry pieces was also temperature dependent, and it was completely negated at higher (12°C) compared to lower (2°C) temperatures (Table 4).¹⁴⁴ Increased storage temperature also resulted in a greater proportion of Gram-negative, facultative anaerobic bacteria in the spoilage association.

However, death of *S. typhimurium* in cold-pack cheese food was more rapid with sorbate at 12.8°C compared to 4.4°C.²⁴ Inactivation of *S. typhimurium* was more rapid at higher temperature (7 to 13°C vs. 37°C), and it required more time at reduced temperatures of storage of milk and nutrient broth.²³ Destruction of *E. coli* by sorbate was also slower at lower temperatures¹¹⁶ with a temperature coefficient (Q_{10}) of 2.3. Inhibition of growth of

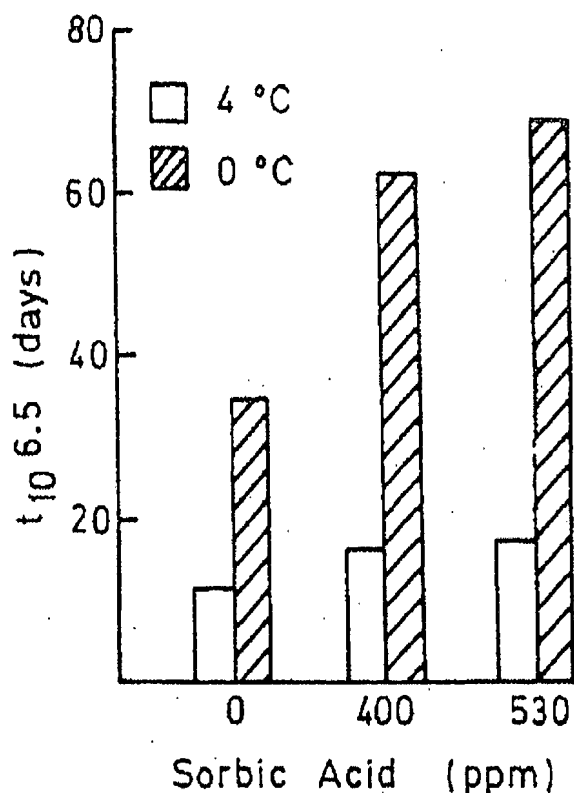


FIGURE 3. Influence of storage temperature and sorbic acid on microbial growth ($t_{10}^{6.5}$: d to reach $10^{6.5}$ CFU/cm²) in vacuum packaged beef samples of pH 5.90. (Reprinted from *J. Food Sci.*, 1987, Vol. 52, 257. © by Institute of Food Technologists.)

TABLE 4
Effect of Sorbate (Immersion 5%, 30 s) and Temperature on Bacterial Numbers (Log/6 cm²) on Vacuum-Packaged Poultry Breast Pieces

Potassium sorbate (%)	Storage temperature (°C)	Days of storage		
		7	14	21
0	2	4.9	6.8	7.7 ^a
5	2	5.7	4.6	5.5
0	5	6.9 ^a	4.6	—
5	5	5.0	7.6 ^a	—
0	12	8.4 ^a	—	—
5	12	7.9 ^a	—	—

^a Time at which off-odors were first detected.

From McMeekin, T. A., Pennington, P. I., and Thomas, C. J., *J. Food Saf.*, 6, 261, 1984. With permission.

these and other microorganisms, however, appears to be more pronounced at lower temperatures.^{145,146} Tuncan and Martin⁷² reported that L-serine and L-histidine uptake by *S. typhimurium* was inhibited more by sorbate at lower temperatures. Production of histamine by *Proteus morganii* and *Klebsiella pneumoniae* in presence of sorbate was greater at 10°C compared to 32°C.¹⁴⁷ Inhibition of growth (Figure 4) and patulin production by *B. nivea* in apple and grape juice was also slower at reduced temperature (21, 30, 37°C).^{142,143}

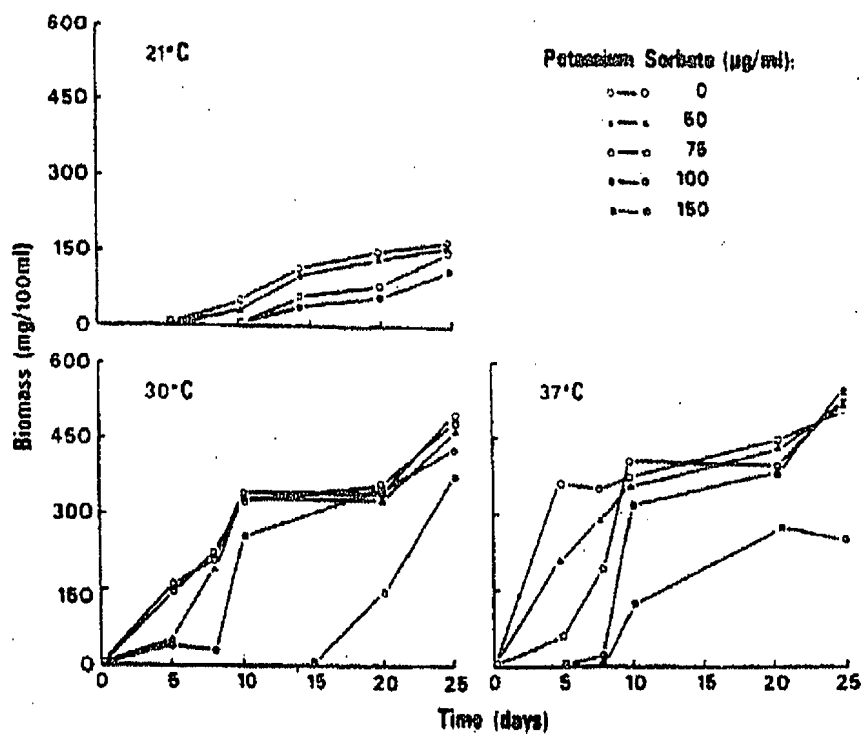


FIGURE 4. Influence of potassium sorbate and storage temperature on production by biomass by *Byssoschlamys nivea* in apple juice. (Reprinted from *J. Food Sci.*, 1984. Vol. 49, 402. © by Institute of Food Technologists.)

B. GAS ATMOSPHERE AND PACKAGING

The antimicrobial activity of sorbate is influenced by the type of packaging and gas atmosphere surrounding the product. Vacuum packaging (i.e., reduction or elimination of oxygen) and modified gas atmosphere (e.g., increased level of carbon dioxide) usually enhance the inhibitory effect of sorbate treatments (Figure 5). Reduced inhibitory activity of sorbate against *Candida albicans* under aerobic compared to anaerobic conditions was attributed to detoxification of sorbate by the yeast culture.¹⁴⁸ Aerobic detoxification varied with the composition of the substrate. Cysteine and glutathione reduced detoxification of sorbate, and they increased antimicrobial activity.

Decreased rates of microbial growth and increased shelf life under vacuum-packaged conditions and treatment with sorbate have been demonstrated with refrigerated (2°C) poultry meat,¹⁴⁴ pork loins (5°C),¹⁴⁹ scallops (4°C),¹⁵⁰ Morwong fillets (4°C),¹⁵¹ and other fish products (Table 5). McMeekin et al.¹⁴⁴ reported that microbial growth was completely inhibited in fresh poultry for up to 35 d at 2°C under vacuum-packaged conditions and treatment with sorbate. Myers et al.¹⁴⁹ also indicated that sorbate improved the keeping quality of vacuum-packaged and refrigerated pork loins and reduced numbers of psychrotrophic and pectinolytic bacteria (mostly *Yersinia* spp. and some *Klebsiella oxytoca*) by 97 to 99%. It was thus concluded that sorbate is effective in inhibiting growth of *Yersinia* spp. in vacuum-packaged, refrigerated, fresh pork. Increased evacuation also improved the shelf-life of sorbate-treated refrigerated bacon.¹⁵² Permeability of the packaging film, however, did not affect the influence of sorbate on growth of *Clostridium sporogenes* in sliced bologna.¹⁵³

Inhibition of microbial growth and delay of spoilage in refrigerated and vacuum-packaged fish^{150,151,154} is desirable from a marketing standpoint. The potential of growth and toxin production by nonproteolytic strains of *C. botulinum* under these conditions, however, should be considered, especially if toxin production will precede development of objectionable spoilage defects. Since sorbate is an effective antibotulinal agent (Chapter 9), its use may

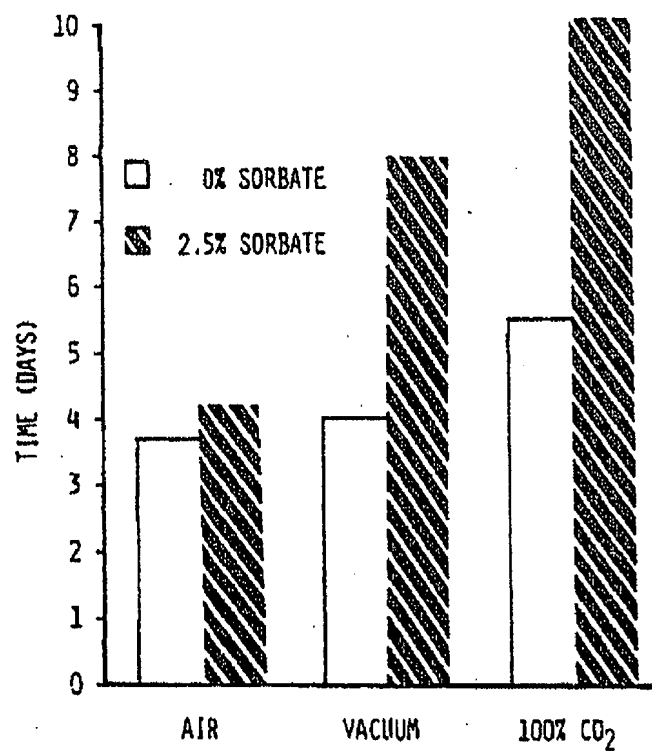


FIGURE 5. Influence of storage atmosphere and potassium sorbate on the shelf-life (d to reach 10⁷ organisms/cm²) of fresh poultry samples stored at 10°C. (Reprinted from *J. Food Sci.*, 1985. Vol. 50, 1360. © by Institute of Food Technologists.)

TABLE 5
Effect of Sorbate, Packaging and Storage Temperature on Trimethylamine Production (mg/100 g) in Blue Grenadier Fish Fillets²⁸⁹

Potassium sorbate (%)	Packaging	Storage temperature (°C)	Days of storage		
			0	11	18
0	Vacuum	4	0.3	2.9	78
0.1	Vacuum	4	0.4	0.7	1
0	Aerobic	4	0.3	14.6	83
0.1	Aerobic	4	0.2	1.1	3.6
0	Vacuum	-18	0.3	0.4	0.2

studies are needed, however, to verify its potential antimicrobial activity in the products and specified conditions.

Several studies have also shown that combinations of carbon dioxide and sorbate can be effective inhibitors of microbial growth.¹ Sorbate and carbon dioxide can inhibit mold spoilage of grains.¹⁵⁵ Modified atmosphere (CO₂) and sorbate are inhibitory, not only to spoilage organisms, but also to bacterial pathogens, such as *Salmonella enteriditis* and *Staphylococcus aureus* (Figure 6). Synergistic effects have been demonstrated in model systems^{68,71} and in poultry meat.^{156,157} The synergistic relationship between carbon dioxide and sorbate is demonstrated by the fact that by increasing carbon dioxide levels, the concentration of sorbate necessary for equivalent inhibition is reduced. The synergistic antimicrobial effect is more pronounced with increasing concentration of carbon dioxide and sorbate and with decreasing pH.^{68,71,156,157}

Concentrations of 1.5 to 5% sorbate and 100% carbon dioxide at pH 5.5 can be com-

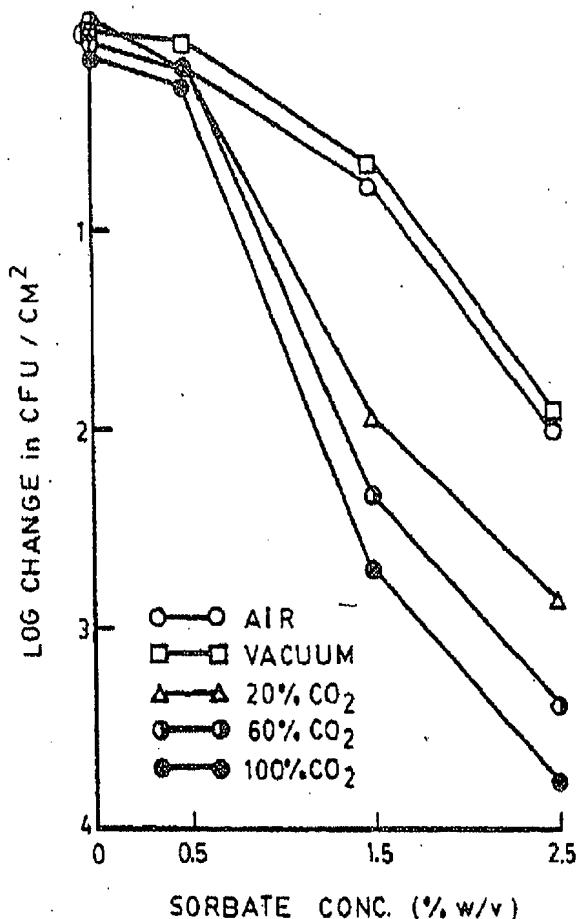


FIGURE 6. Growth of *Staphylococcus aureus* in trypticase soy agar of pH 5.5, containing potassium sorbate, after 60 h of exposure to different gas atmospheres. (From Elliot, P. H., Tomilins, R. L., and Gray, R. J. H., *J. Food Prot.*, 45, 1112, 1982. With permission.)

pletely bacteriostatic.¹⁵⁶ The inhibitory effect of sorbate in combination with a carbon dioxide atmosphere is more pronounced compared to that in vacuum environments.⁶⁸ No synergistic effect is observed at pH 6.5, but it increases as the pH is reduced and it is maximized at pH 5.5.¹⁵⁷

Sensitivity of microorganisms to carbon dioxide and sorbate combinations is variable with species. Elliot et al.⁶⁸ found that in a nutrient medium a combination treatment (i.e., carbon dioxide and sorbate) was up to 100 times more effective against *S. aureus* than *Salmonella enteritidis*. Although combinations of carbon dioxide and sorbate in poultry meat were effective against both *S. enteritidis* and *Staphylococcus aureus*, the former was more sensitive to sorbate, and the latter, to carbon dioxide, when tested individually.¹⁵⁶ Spoilage microorganisms in fresh poultry meat were less sensitive to sorbate than *Salmonella enteritidis* and *Staphylococcus aureus* under similar conditions.¹⁵⁷ The combination of carbon dioxide and sorbate provided the most inhibitory system against poultry spoilage organisms, especially *Pseudomonas* spp., without creating a selective environment for lactic-acid-producing microorganisms.¹⁵⁷

Although the lactic-acid-producing bacteria are believed to be resistant to inhibition by modified atmospheres and sorbate, the combination of carbon dioxide and sorbate does not appear to create conditions for selection of these species that would result in early souring of the product.¹⁵⁷ Thus, combining sorbate and carbon dioxide atmospheres has a good potential for improving the shelf life of fresh poultry. This application would be more

applicable to poultry meat intended for further processing into luncheon meat and frankfurter.

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type items. Combinations of carbon dioxide and sorbate are also effective in extending the shelf life of fish.^{158,159}

The antimicrobial activity of carbon dioxide-sorbate combinations may be even more pronounced if lactic or acetic acid are introduced to adjust the pH of low acid systems (i.e., fish, poultry), because these acids potentiate the action of sorbate more than hydrochloric acid.⁵⁷

V. ADDITIVES

A. ACIDS

The type of acid naturally present or added to the food may influence the antimicrobial activity of sorbate. It is not only the activity of the acid as an acidulant, but also the specific acid anion itself may be influential.¹⁶⁰ Several studies have demonstrated that individual acids affect differently the antimicrobial activity of sorbate. In general, however, preservation of foods with sorbate is more effective in presence of acids which lower the pH and increase the amount of the more effective undissociated form of sorbate.

Publications and patents have presented information indicating preservation of products, such as sweet alcoholic beverages, with combinations of sorbic and lactic acids;¹⁶¹ fish paste (kamaboko) with potassium sorbate and glucono-delta-lactone;¹⁶² and cooked noodles with several carboxylic acids (e.g., lactic, glutamic, caproic) and sorbate.¹⁶³ Earlier (1964) Perry et al.¹⁶⁴ reported that the shelf life of eviscerated, refrigerated, cut-up, "dry-pack", poultry meat was extended by the synergistic action of a two-step process consisting of acidic hydration and sorbic acid application. Poultry parts were hydrated in an ice slush solution of sodium dihydrogen phosphate for 2 h, which lowered the surface pH to below 5.5. After draining, the parts were sprayed with a hot (i.e., 71°C) solution of 7.5% sorbic acid. The solvent consisted of 70% propylene glycol, 20% water, and 10% glycerol. These ingredients increase the solubility of sorbic acid at 71°C to 5 to 10%.

The specific type of acid present will also affect the extent of antimicrobial action by sorbate. Inactivation of *Salmonella typhimurium* by sorbate was greater in the presence of acetic than lactic acid, which were used to adjust the pH.²³ Acidification in general permitted the use of lower sorbate levels for similar extent of antimicrobial activity in poultry frankfurters, bacon, and chopped ham.¹⁶⁵⁻¹⁶⁷ The type of substrate may also influence the potentiating effect of the acid on antimicrobial activity, since the effect was higher in ham than in bacon. Citric acid increased the antimicrobial activity of sorbate in turkey but not in chicken frankfurters.¹⁶⁵

Acetic and citric acids potentiated the inhibitory effect of sorbate on mold in raw, cured, Italian dry salami, while lactic and succinic acids showed little or no effect.¹⁶⁸ In general, inorganic acids and lactic acid appear to be less effective potentiators of antimicrobial activity than other acids, such as acetic and citric. Gilliland and Ewell,¹⁶⁹ however, reported that cells of *Lactobacillus lactis* added to refrigerated raw milk in combination with sorbate were more inhibitory to psychrotrophs than either lactobacilli or sorbate tested alone.

The relative influence of specific acids on antimicrobial activity may depend on specific microorganisms under consideration. Restaino et al.⁵⁷ examined the effect of sorbate in combination with citric, lactic, phosphoric, and hydrochloric acids on the growth of six food-related bacteria in culture media of constant pH (5.5). Sorbate (0.1 to 0.2%) with any of the acids tested was bacteriostatic to *Y. enterocolitica* (Figure 7) and *Salmonella* group D and extended the lag phase of *P. fluorescens* by 24 h. The combination of sorbate with the acids also reduced the growth rate of *Lactobacillus plantarum* and an unidentified *Lactobacillus* isolated from frankfurters. *Pseudomonas aeruginosa*, however, was unaffected by any sorbate-acid combination. Overall, citric and lactic potentiated the antimicrobial action of sorbate more than other acids.

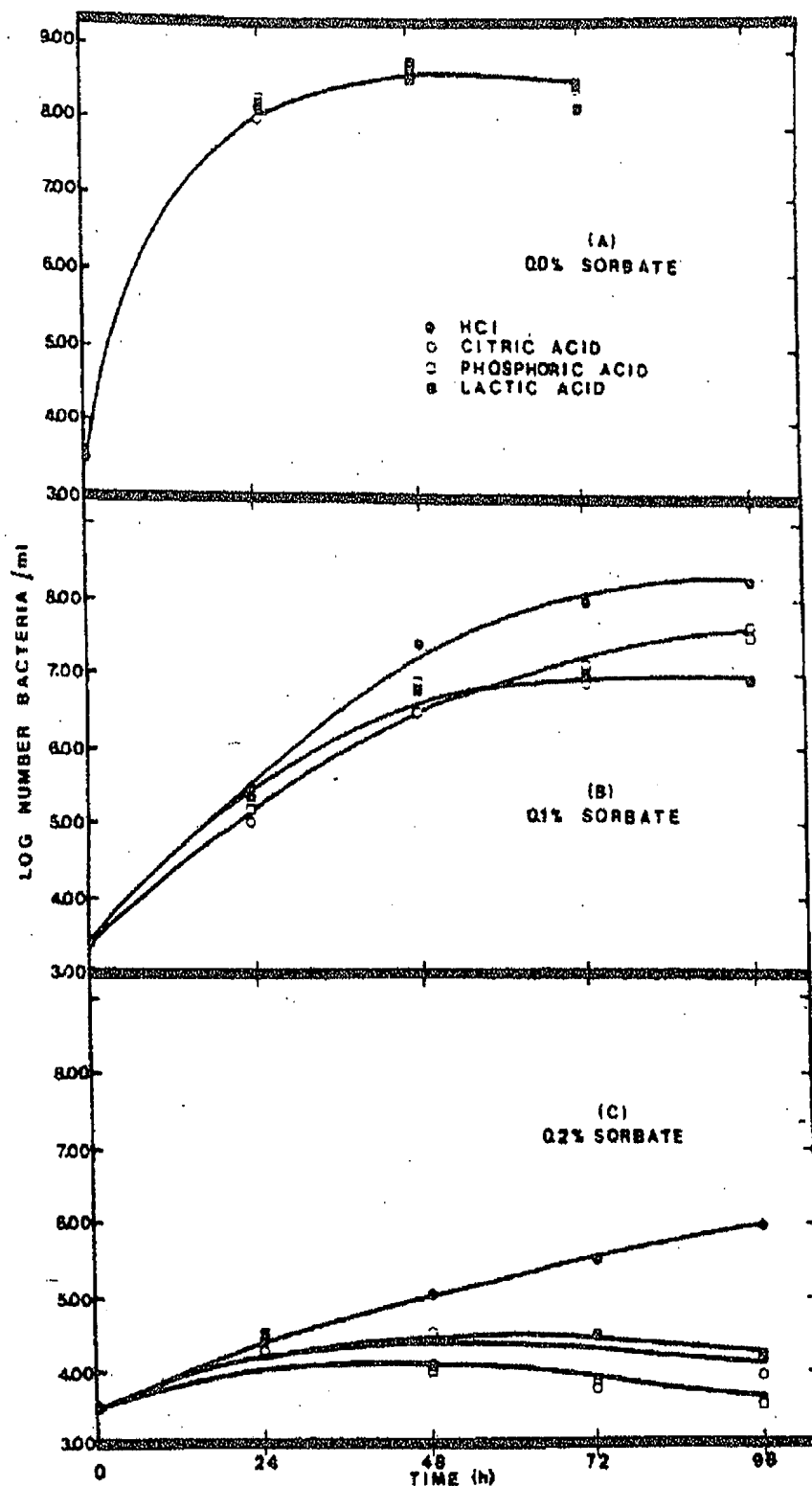


FIGURE 7. Influence of sorbate and acids on growth of *Yersinia enterocolitica* 0:17 in trypticase soy broth of pH 6.0 at 25°C. (Reprinted from *J. Food Sci.*, 1981, Vol. 46, 134. © by Institute of Food Technologists.)

Restaino et al.²² tested citric, lactic, malic, phosphoric, and hydrochloric acids in combination with sorbate on growth of osmophilic yeasts, including *Saccharomyces bailii*, *S. acidifaciens* (*S. bailii* var. *osmophilus*), *S. rouxii*, and *S. bisporous* in nutrient agar at pH 5.0 and water activity 0.88 to 0.89. Citric acid potentiated the antimicrobial activity of

as a longer lag phase or a reduction of the rate of growth. The lack of activity by lactic acid was attributed to either the acid being antagonistic to sorbate or the specific yeast strains tested having been adapted to the acid, if it was a natural component in their source of isolation. Thus, use of acids, other than those naturally present in a food, may be advisable when potentiation of antimicrobial activity is the target. Additional studies are needed, however, to develop combinations of sorbate with acids and other additives.

B. ANTIOXIDANTS

Lipid oxidation and rancidity development in food products is inhibited by addition of several chemical compounds which act as antioxidants. The most commonly used antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroxyquinone (TBHQ), and propyl gallate (PG). Several of these compounds have shown inhibitory activity against various microorganisms.¹⁷⁰ Thus, they could be valuable by serving the dual function of antioxidant and antimicrobial.

Several studies have shown that certain combinations of sorbate with these antioxidants inhibit microorganisms, such as *Staphylococcus aureus* and *Salmonella typhimurium*, under certain conditions.¹ Combinations of sorbate with BHA or TBHQ delayed growth of *Staphylococcus aureus* in culture media, while the sorbate-BHA combination did not delay initiation of growth of *Salmonella typhimurium*.¹⁷¹ Overall, however, all combinations with sorbate increased the antimicrobial activity. Synergistic effects of sorbic acid and BHA against *Clostridium perfringens* have also been reported.¹⁷² Sorbate (1 to 5%) in combination with BHA, BHT, and PG (50 to 100 ppm) exerted greater bactericidal and bacteriostatic effect at pH 5 and 7 against *Staphylococcus aureus*, while TBHQ was highly inhibitory both in the presence and absence of sorbate. BHA in combination with sorbate was more effective than BHT and PG.⁶⁷ Combinations of sorbate and TBHQ were synergistic in inhibiting one of two strains (12600) of *S. aureus*, while certain combinations of BHA and sorbate were synergistic against both strains (S-6 and 12600) (Figure 8).¹⁹

Certain combinations of sorbate with antioxidants (i.e., BHA, TBHQ, and PG) resulted in synergistic inhibition of *Salmonella seftenberg* in culture media of pH 5.5.¹⁷³ The most effective combination consisted of 0.2% potassium sorbate and 200 ppm BHA. All sorbate/antioxidant combinations also significantly inhibited the test organism in ground beef compared to control samples. The antimicrobial effectiveness of sorbate/antioxidant combinations, however, was markedly diminished in ground beef compared to culture media. The diminished effect can probably be attributed to the antioxidant being used up to prevent autooxidation of the lipids.

Sorbate (0.1%) was tested alone and in combination with BHA (150 ppm) against growth of the natural flora in raw turkey meat and against *S. typhimurium* inoculated into cooked turkey meat.¹⁷⁴ There was no inhibition of the natural flora by BHA or sorbate tested alone, but there was a slight inhibition when the two compounds were combined. Sorbate and sorbate-BHA combinations did not differ in their effectiveness and were more effective than BHA alone in reducing numbers of *S. typhimurium* in cooked turkey. Overall, there was no synergistic effect evident, but a combination of sorbate and BHA was more effective than sorbate alone in inhibiting Gram-negative organisms and in reducing survival of *Salmonella*.

Earlier studies, however, had indicated that combinations of sorbate with antioxidants were successful in preserving foods. Nickerson and Starr¹⁷⁵ were awarded a patent for preserving fresh pork and beef sausage, hamburger, fresh fish fillets, eviscerated cut up poultry, etc. Combinations of fat soluble (e.g., BHA, BHT, catechol, quercetin, and 2,6-dimethoxyphenol) and water soluble (e.g., ascorbic, citric, and phosphoric acids) compounds with sorbate inhibited rancidity and bacteria, respectively.

In addition to bacteria, sorbate/BHA combinations have inhibited growth of *Aspergillus*

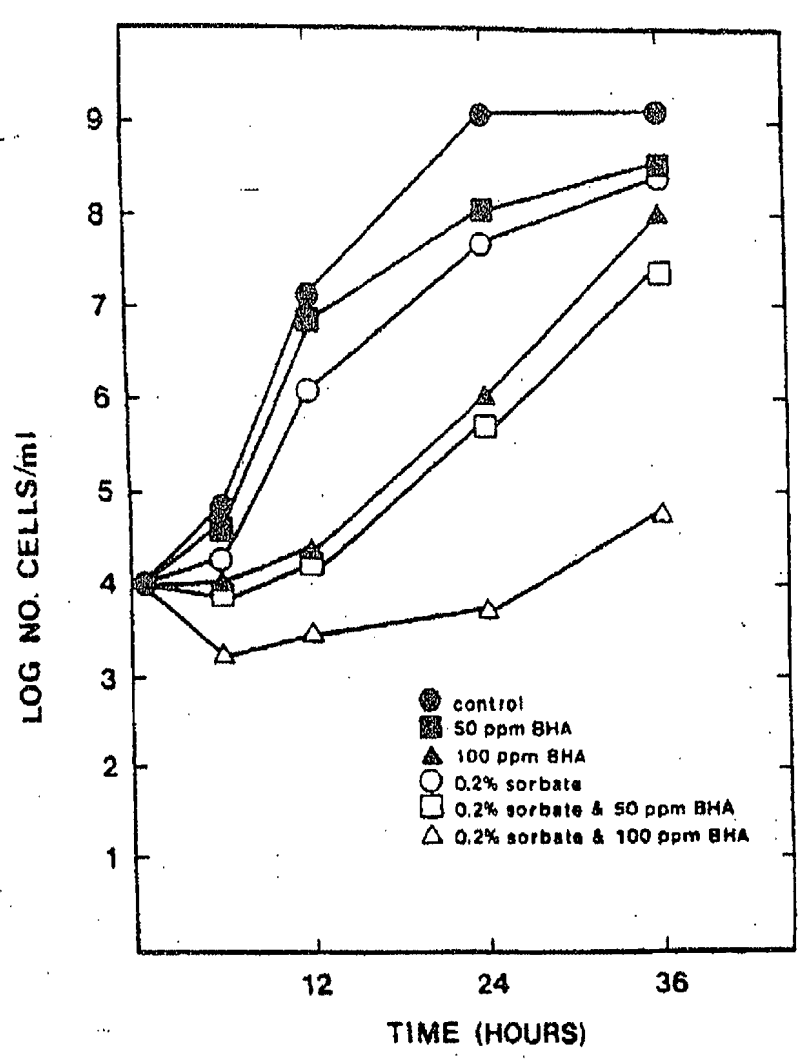


FIGURE 8. Effect of butylated hydroxyanisole (BHA) and potassium sorbate on growth of *Staphylococcus aureus* S-6 in trypticase soy broth of pH 6.0 at 37°C. (From Robach, M. C. and Stateler, C. L., *J. Food Prot.*, 43, 208, 1980. With permission.)

flavus in a liquid culture medium.¹⁷⁶ One advantage of such combinations is that sorbate levels needed for inhibition may be reduced. In addition, rancidity of the fat can also be inhibited.

C. PHOSPHATES

Phosphates used in foods have several functions, including their effects on water and fat binding, emulsification, antioxidant activity, and overall improvement of cooking yields, texture, flavor, and color.¹⁷⁷ Phosphates are especially useful in low-salt meat products, where they improve the technological and quality properties and allow reductions in the sodium content. Their effect as antimicrobials, however, depends on the specific type of phosphate used, the composition of the product, its pH, the type of microorganisms, and presence of other chemicals.^{54,177-179}

Certain studies have indicated interaction of phosphates with sorbate and improvement of antimicrobial activity when both compounds are used in a formulation. A patent was issued for sausage preparation with a mixture of alkaline phosphates with potassium sorbate or sorbic acid.¹⁸⁰ Ivey and Robach¹⁸¹ reported that either sodium acid pyrophosphate (SAPP) or sodium hexametaphosphate (SHMP) displayed statistically significant interactions with

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found that only SAPP, not SHMP or sodium tripolyphosphate (STPP), showed anticlostridial activity in combination with nitrite and sorbic acid in a similar product.

Increased inhibition of *C. botulinum* by SAPP/sorbate combinations in beef-pork frankfurters was also reported.¹⁸² SAPP was more effective than other phosphates (SHMP, and especially STPP) in inhibiting *C. botulinum* in frankfurters made with mechanically deboned chicken meat.¹⁸³ Effectiveness, however, was present only in combinations of the phosphate with sorbic acid or potassium sorbate and sodium nitrite and not in treatments without nitrite. A polyphosphate, consisting of 53% STPP and 47% sodium pyrophosphate, had no apparent additional effect on preservation of fish fillets treated with or without sorbate.¹⁵¹ Bacteria isolated from spoiled kamaboko were inhibited by combinations of sorbic acid, with SHMP and sodium ultrapolyphosphate, but not by STPP or sodium pyrophosphate.

Interactions of phosphates with sorbate have also been observed in studies with culture media. Seward et al.⁶⁶ observed interactions of STPP (0.5%) and potassium sorbate (0.5%) at pH 7.1 in the inhibition of *C. botulinum* type E. Treatments containing SAPP and potassium sorbate in culture broth were also more effective than other formulations in delaying growth of *C. botulinum*.⁵¹ SAPP/sorbate combinations in culture media at pH 5.85 did not prevent *C. botulinum* spore germination and outgrowth but may prevent cell division. The same treatment at pH 5.5, however, inhibited spore germination.⁵³ This demonstrates the influence of pH on the inhibitory activity of phosphate/sorbate combinations. Sorbate/SAPP combinations were also inhibitory against *S. aureus*.¹⁸⁴ Thus, alkaline phosphates (e.g., STPP), which raise the pH of the substrate, are usually ineffective as antimicrobials,¹⁷⁷ with or without sorbate in the formulation. However, acidic phosphates (e.g., SAPP) are usually more effective as antimicrobial agents either alone or in combination with sorbate.

D. ANTIMICROBIALS

1. Benzoate and Propionate

Combinations of sorbate with benzoate can be useful in the preservation of food products because they can expand the range of microorganisms inhibited at lower concentrations of each preservative.^{9,10,185} Use of lower concentrations also reduces any potential undesirable effects on product flavor. Graham and Chaparro,¹⁸⁶ however, indicated that a combination of 0.05% sodium benzoate and 0.05% potassium sorbate was less effective than 0.07 to 0.1% sodium benzoate in preserving "mabi", a fermented drink, that needs refrigeration, and is derived from the bark of the mabi tree (*Colubrina elliptica*). In contrast, rubber and cork gaskets used in car and tractor engines and in food industry equipment under warm weather conditions were made fungus resistant by treating with a combination of 1% sorbic acid and 1% benzoic acid.¹⁸⁷ Such materials can be used in manufacturing containers for citrus fruit, which are susceptible to *Penicillium* and *Aspergillus* rot during refrigerated storage. Sodium benzoate (0.5%) was most effective in preserving hake fish in ice than EDTA or sorbate tested individually; all, however, increased shelf life. Combinations of two preservatives failed to result in better shelf life than sodium benzoate used alone.¹⁸⁸ Mixtures of sorbate with propionate are also useful in the preservation of bread and other yeast-leavened bakery products. The interactions of sorbate with nitrite are discussed in Chapters 9 and 11.

2. Antibiotics

Sorbate has been found to be synergistic, additive, antagonistic or has shown no interaction with antibiotics in inhibiting microbial growth. The specific effect depends on individual microorganisms, antibiotics, and substrates.¹⁸⁹

Rehm et al.⁶ tested the interaction of sorbic acid with various antibiotics for inhibition of bacteria. The preservative generally had an additive effect with tetracycline, but in some cases, especially with *S. aureus*, there was an antagonistic effect. Antagonistic interactions

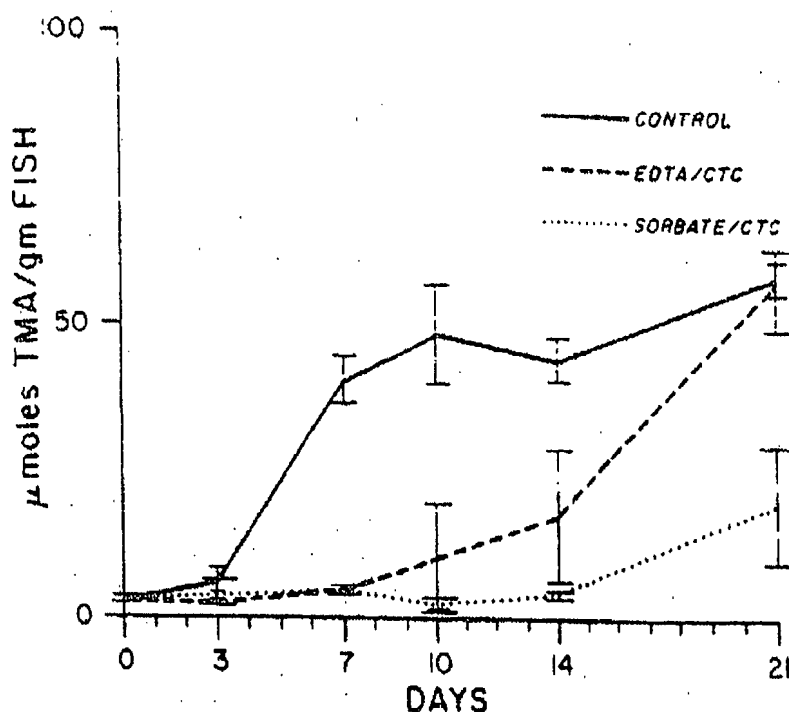


FIGURE 9. Influence of a potassium sorbate (1%), ethylene diamine tetraacetic acid (EDTA, 1%) and chlortetracycline (CTC, 5ppm) immersion (1 min.) solution on trimethylamine production in vacuum packaged rockfish fillets stored at 2°C. (Reprinted from *J. Food Sci.*, 1984. Vol. 49, 188. © by Institute of Food Technologists.)

were also found with novobiocin and with polymyxin, bacitracin, and colistin against *S. aureus*. There was no interaction with penicillin, and the interaction was synergistic with chloramphenicol, streptomycin, and neomycin.

Interactions of sorbate with antibiotics in food systems are also variable. The approved-for-use-in-foods antibiotic nisin when used in combination with sodium sorbate (0.06%) retarded mold growth on the surface of process cheese and improved its storage stability.¹⁹⁰ Nisin (150 units/g) and sorbic acid (0.075%) also decreased growth of butyric acid bacteria and fungi, and increased shelf life of process cheese at 20 to 35°C by 2 to 3 times.¹⁹¹ Sorbic acid (0.07%) in combination with 0.02% nisin may also be used for preserving apple pectin carboxymethyl cellulose.¹⁹²

Combinations of other antibiotics with sorbate have also been tested in food systems. Oxytetracycline (4 ppm) and 0.04% sorbic acid had no additional effect on the shelf life of herring compared to either compound tested by itself.¹⁹³ Antimicrobial activity was also not improved with a combination of sorbic acid and aureomycin against *E. coli*, *B. subtilis*, *Proteus vulgaris*, and *Staphylococcus candidus*.¹⁹⁴ Improved antimicrobial activity was found, however, with a combination of sorbate and chlorotetracycline in rockfish fillets (Figure 9);¹⁹⁵ with kasugamycin in cucumbers;¹⁹⁶ and with tylosin in fish sausage.¹⁹⁷ Use of most antibiotics in food products, however, is not permitted.

E. OTHER INTERACTIONS

1. Ascorbate

Fruit products may be preserved more effectively with combinations of sorbate and ascorbate. A patent has described preservation of strawberry jam with combinations of sorbic acid (0.09 g/kg) and ascorbic acid (0.5 g/kg).¹⁹⁸ This treatment allows storage of the product at temperatures of up to 25°C, instead of the necessary storage at less than 10°C in the

for inhibition of microbial spoilage in raw or partially cooked foods (e.g., marmalade) stored at room temperature.

Combinations of sorbic and ascorbic acids were also more effective than sorbic acid by itself in inhibiting microbial spoilage of carbonated fruit drinks. The optimum combination consisted of 50 to 100 mg/l sorbic acid and 100 to 200 mg/l ascorbic acid. Svorcova²⁰⁰ also reported that potassium sorbate and ascorbic acid were more effective than either one used singly in preserving mixtures of fruit juices with sugar and mineral water. Fresh unpasteurized apple juice with 0.35% sorbic acid and 50 mg per 100 ml ascorbic acid may be preserved without refrigeration for a minimum of 2 weeks. Sorbic acid inhibits yeast growth, and ascorbic acid prevents oxidation of sorbic acid and inhibits *Acetobacter* growth. Oxygen, however, must be excluded from the product.²⁰¹

2. Sulfur Dioxide

Combinations of sorbate and sulfur dioxide have proven useful in the preservation of wines where they control undesirable yeasts and bacteria and maintain its quality (Chapter 8). Several studies have reported on their interaction in expanding the range of microorganisms being inhibited in wine environments at reduced concentrations. Although sorbic acid by itself may give the wine an unfavorable taste, a reduced level (≤ 200 ppm) in combination with sulfur dioxide (100 ppm) gives an increased shelf-life by suppressing yeasts such as *Candida*, *Pichia*, and *Hansenula*, as well as bacteria and chemical deterioration.²⁰² The wine is preserved for long periods of time with no adverse effects on its taste. Other combinations of sorbate and sulfur dioxide that preserve wines have used 0.013 to 0.026% potassium sorbate and 30 mg/l sulfur dioxide, or 100 to 250 mg/l sorbic acid and "small" amounts of free sulfur dioxide.²⁰³ Combinations of sorbic acid (25 to 75 mg/l) and sulfur dioxide (100 to 125 mg/l) prevent chemical, microbiological, and sensory changes even in sweetened red or white table wines,²⁰⁴ while each compound used individually is usually ineffective. A combination of sorbic acid and sulfur dioxide is also effective in preserving other fruit products, such as strawberry pulp.

3. Emulsifiers

Sucrose esters of fatty acids are compounds derived from sucrose esterified with a mixture of palmitic and stearic acids and are effective as emulsifiers. These sucrose fatty acid esters also possess antimicrobial properties.²⁰⁵⁻²⁰⁷ A patent indicates that a composition containing sorbic acid, water, and emulsifiers (i.e., sucrose fatty acid ester, sorbitan fatty acid ester, lactate fatty acid ester, and lecithin) was a preservative for noodles.²⁰⁸ The antimicrobial activity of sorbate or propionate with sucrose esters was also investigated by Marshall and Bullerman²⁰⁹ against growth and aflatoxin production by *Aspergillus parasiticus*. A synergistic effect on inhibition of growth was observed at high levels of propionate or sorbate in combination with the esters (Figure 10), while aflatoxin production remained relatively unaffected. Thus, sucrose esters in combination with sorbate or propionate may provide added antimycotic activity at pH values higher than the optimum for either inhibitor alone. Thus, various combinations may be useful in low acid food commodities.

Other emulsifiers (e.g., Tween® 20, 40, 60, 80) neither suppressed nor potentiated the action of sorbic acid in meat-peptone broth inoculated with *S. aureus*.²¹⁰ In contrast, both sodium dodecyl sulfate and sucrose monopalmitate showed antibacterial activities by themselves, which were higher than that of sorbic acid.

4. Propylene Glycol

The interaction of sorbate and propylene glycol on antimicrobial activity appears to be variable. Propylene glycol caused partial antagonism of 0.1% sorbate in creams, as well as an increase in the minimum concentration of sorbate needed to inhibit strains of *Pseudomonas aeruginosa* in broth of pH 6.5 to 7.0.²¹¹

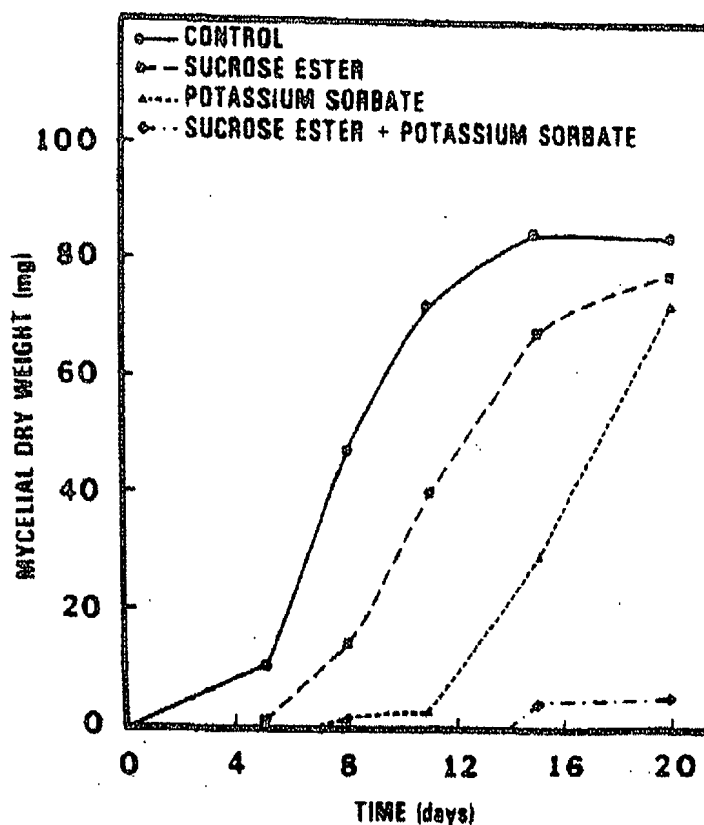


FIGURE 10. Influence of potassium sorbate (0.3%) and sucrose fatty acid ester (1%) on growth of *Aspergillus parasiticus* in a culture broth of pH 6.0 at 25°C. (From Marshall, D. L. and Bullerman, L. B., *J. Food Prot.*, 49, 378, 1986. With permission.)

In foods, however, sorbate-propylene glycol combinations appear to be effective antimicrobials. Sorbic acid and propylene glycol prevented growth of fungi on dried seasoned saury for 1 month at 30°C.²¹² A patent was also awarded for preservation of cooked noodles by soaking in a solution of sorbic acid and propylene glycol, draining, and packaging.²⁰⁸

5. Fatty Acids

Mixtures of sorbate with other fatty acids have the potential of improving the shelf life of various foods, as indicated by several patents. The preservative activity of sorbates in raw meat and fish has been enhanced by mixing the compound with an unsaturated fatty acid of chain length $\leq C_{18}$.^{213,214} A composition containing sorbate and C_{1-10} and C_{16-20} saturated fatty acids was a preservative for processed meats.²¹⁵

Derivatives of fatty acids have also enhanced the antimicrobial activity of sorbate. A composition of sorbate, caprylic acid monoglyceride, and sodium nitrite was effective in inhibiting growth and toxin production by *Clostridium botulinum* in meat.²¹⁶ Sorbic acid and several derivatives of fatty acids (i.e., amides, aminimides, and monoglycerides) were inhibitory to toxigenic *A. flavus* and *A. parasiticus*.²¹⁷ Monolaurin, a fatty acid derivative emulsifier, inhibited *S. aureus* to a greater extent when used in combination with sorbic acid.²¹⁸ Robach et al.,²¹⁹ however, found no synergistic effect of these compounds against *Salmonella*.

6. Amino Acids

Foods can also be preserved by mixing sorbate with amino acids and other compounds. A patent was awarded for preserving fruits and vegetables by addition of cysteine in com-

indicates that fish and meat products can be preserved by treatment with an acidic amino acid coated with an edible fat or oil and sorbate, and/or 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide. Glutamic and aspartic acid were more effective in the mixture than other amino acids.

7. Glucose Oxidase

Glucose oxidase can also enhance preservation of foods with sorbate. A combination of sorbic acid with glucose oxidase inhibited grape juice fermentation more than sorbic acid alone.²²² The natural properties of apple and cherry juices were also preserved for more than 6 months by the synergistic antimicrobial action of sorbic acid (0.02 to 0.04%) and glucose oxidase (50 to 100 units/l).²²³ Glucose oxidase (5 units) and sorbate (0.03%) also suppressed growth of microflora in sweetened condensed milk.²²⁴

8. Other

Several patents and publications indicate interactions which have enhanced the antimicrobial activity by sorbate when used in combination with several compounds in foods, culture media, or other materials. Several of these interactions are mentioned here.

Sorbic acid (0.03%) and diethyl ester of polycarbonic acid (0.04%) inhibited molds in grape juice.²²⁵ Mixtures of diethyl ester of polycarbonic acid and sorbic acid inhibited yeast cells in a fermentation broth with 12.4% alcohol, but when used singly the ester was more inhibitory than sorbic acid.²²⁶ A food preservative was formulated with cinnamic acid and one or more acids, including sorbic acid.²²⁷

Compositions containing sorbic acid or its salts and 2-(4-thiazolyl) benzimidazole were microbiocides and were effective in controlling *Spherothea fuliginosa* on cucumbers.²²⁸ Potassium sorbate in combination with benzimidazole reduced *Penicillium* decay in citrus fruits.²²⁹

A patent was issued on the use of acyloxyalkanoic acid together with sorbic acid to preserve foods and feeds.²³⁰ A combination of sorbic acid (0.02%) and vitamin K₃ (0.03%) completely inhibited growth of wine yeasts and acid-forming bacteria for 30 d.²³¹

Sulfhydryl-containing compounds (e.g., cysteine-HCl, glutathione) in combination with sorbic acid inhibited molds for up to 220 d at room temperature. Such compositions were suggested as preservatives for a variety of food products, including meats, fish, dairy, fruits, vegetables, oils, and confectionary.²³²

Carboxymethylcellulose was ineffective in preserving apple jam which spoiled in 8 to 13 d at 30 to 37°C and 90% relative humidity. Sorbic acid alone preserved the product for 4 months, while a combination of carboxymethylcellulose (1%) and sorbic acid (0.05%) inhibited spoilage for 5 months at 30°C.²³³

Dihydroxyacetone has also shown increased antimicrobial activity in combination with sorbate or other carboxylic acids in foods and feeds.²³⁴ Mashed potatoes with 5% dihydroxyacetone and 0.3% sorbate did not spoil for 40 months at room temperature, while controls spoiled in less than 1 month, and with dihydroxyacetone alone they were preserved for less than 3 months.

Sorbic acid in combination with 5-hydroxy-1-methyl-2-isopropyl-benzene or 5-nitro-2-furylacrylic amide were bacteriostatic in sausages.²³⁵ Sodium bicarbonate (0.01%) has also increased the effectiveness of sodium sorbate (0.1%) in preserving apple cider.²³⁶

Sorbic acid has promoted the preservative effect of gelatin (1%) and egg white lysozyme (0.05%) on fish paste (kamaboko).²³⁷ Kamaboko is also preserved with a combination of sorbate and furylfuramide.²³⁸ Furylfuramide (5 ppm) and sorbic acid (0.2%) used together were also more effective against several organisms isolated from Vienna sausage.²³⁹ The combination, however, was ineffective against *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Comamonas* spp.

TABLE 6
Effect of Sorbate, Sodium Chloride, pH, Heating Treatment, Inoculum Level and Storage Temperature on Probability of Toxin Production by *Clostridium botulinum* in Pork Slurries⁴⁴

Potassium sorbate (%)	Sodium chloride (%)	pH	Inoculum (spores/bottle)	Unheated		Heated (80°C)	
				Storage temp (°C)		Storage temp (°C)	
				15	35	15	35
0	2.5	5.7	10	21	38	34	53
0.26	2.5	5.7	10	6	13	11	22
0	2.5	5.7	1000	54	90	68	94
0.26	2.5	5.7	1000	11	50	19	65
0	2.5	6.6	10	87	100	53	97
0.26	2.5	6.6	10	62	98	22	33
0	4.5	5.7	10	2	26	4	40
0.26	4.5	5.7	10	1	8	1	14
0	4.5	6.6	10	34	99	8	95
0.26	4.5	6.6	10	12	97	2	83

Sorbic acid (0.15%) and *o*-phenylphenol (0.05%) were also synergistic as antimicrobials in wheat-starch paste.²⁴⁰ When the combination was used as an antiseptic for starch adhesives, there was no decay at 30°C for 240 d, compared to a shelf life of less than 15 d for the control.²⁴¹ Extremely low (0.00001%) concentrations of 1,5-pentanedial and 0.1% sorbic acid in isotonic buffered saline were destructive to *Pseudomonas aeruginosa* and can be useful as preservatives for contact lenses.

Meat-based animal feeds with 15 to 30% moisture can be stabilized by incorporation of a combination of 0.1% sorbate and 0.1% levulinic acid to prevent mold growth. Inhibition by the combination is better than with the individual compounds.²⁴² Sorbate in combination with smoke has also inhibited mold spoilage in fish.²⁴³ Treating peas with a 1:5 to 1:3 dilution of an emulsion of paraffin, wax, and sorbic acid in water decreased spoilage and weight losses. The same solution also preserved cucumbers and peppers but not onions and radishes.^{224,225}

VI. MULTIFACTOR INTERACTIONS

In most practical applications of food preservation, the action of sorbate and other chemical food preservatives is to interact with various other factors and together to extend the shelf life of the product (Table 6). These factors have been discussed in other parts of this chapter and include processing variables such as heat, drying, irradiation; parameters associated with the raw ingredients, such as pH and composition; chemical additives used in the formulation; and environmental parameters, such as gas atmosphere, temperature of storage, and packaging. The interaction of such factors constitutes the "total preservation system", which maintains product quality and/or safety. Each one of these factors, including sorbate, is important and, if changed, may compromise product preservation.

In addition to interactions of sorbate with one or more factors commonly used in food processing, the literature contains numerous publications and patents of interactions of sorbate with multiple other factors. These multifactor interactions may include both commonly used and approved variables and treatments, as well as compounds that are not approved for common use or may have undesirable effects on product quality and safety. It is interesting, however, to present some of these multifactor interactions that result in inhibition of microbial

Combinations of tetracycline antibiotics, irradiation, and sorbic acid were found effective in delaying microbial spoilage of prepackaged meats.²⁴⁶ Before the widespread testing of sorbate as an antibotulinal agent in meat products (Chapter 9), a patent was issued,²⁴⁷ indicating that sausages could be preserved by incorporation of mixtures of sodium metaphosphate, glucono-delta-lactone, sodium chloride, and sorbic acid. Application of a solution combining 2% gelatin, 2% liquid smoke, 0.2% corn starch, and 1% potassium sorbate on the inside of regenerated cellulose casings inhibited mold growth during curing of sausages.²⁴⁸ A composition containing sorbate, C₁₆₋₂₀ fatty acid monoglycerides, nitrite and/or hexameta-phosphate could preserve meat products, especially against *Clostridium botulinum*.

Wieners and fish paste have been preserved with a combination of sorbic acid and one or more substances selected from organic and inorganic or metallic ions. Propylene glycol caproic acid ester (5 parts), sorbitan caproic acid ester (10 parts), and glycerol stearate (15 parts) were liquefied by heating and to the mixture was added sorbic acid (20 parts), citric acid (10 parts), sodium polyphosphate (35 parts), and exsiccated alum (5 parts). Wieners containing this mixture (1%) and a conventional sorbic acid preservative (1%) were preserved for 16 d, while controls spoiled within 3 d.^{249,250} Wieners were also well preserved by adding mainly sorbic acid and sodium polyphosphate, and if necessary alum, gluconolactone, arabolactone, and antifungal organic acids.²⁵¹

Preservatives for meat products containing 15 to 50% moisture were formulated with compounds selected from sodium chloride, potassium chloride, alkali metal salts of hydroxycarboxylic acids, orthophosphates, metaphosphates, ultraphosphates, and polyphosphates.²⁵² Diced ham, boiled in a solution of sodium chloride (5%), potassium citrate (5%), potassium sorbate (0.8%), and water (89.2%) for 7 min, then cooled to 30 to 40°C, left in the salt solution for 1 h and dried at 38 to 43°C to 39% water, was preserved. Meat products were also preserved by dipping in or spraying with a 5 to 18% solution of sodium, potassium, calcium, or ammonium sorbate in water or in an aqueous solution of gelatin, starch, or cellulose derivatives. The treated product resisted microbial growth for 5 weeks at 10°C and 90% relative humidity.²⁵³

Ground meat and fish products were preserved at pH < 6.7 with 0.1 to 1.0% long-chain ($\geq C_{18}$) unsaturated fatty acids, optionally containing a hydroxyl group or their alkali metal salts together with sorbate.²¹⁴ Addition of unsaturated fatty acids markedly increased the effectiveness of sorbates as preservatives.

Three chemical dips consisting of combinations of potassium sorbate (10 to 20 g), sodium acetate (5 to 10 g), sodium chloride (5 g), water (100 ml), with or without propylene glycol (20 ml), glycerol (10 ml), sodium citrate (10 g), and 0.5 N sodium hydroxide (to pH 6), were tested against *E. coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Clostridium perfringens* at 20 and 30°C in unchilled fresh beef.²⁵⁴ All treatments inhibited bacterial growth to a similar extent, and shelf life was extended to 44 h at 30°C or 68 h at 20°C. Control samples spoiled in less than 32 h.

The shelf life of beef carcasses was doubled by spraying with a mixture of sorbate, acetate, and sodium chloride.²⁵⁵ Ice containing glycol diformate (0.02 to 0.04%) and sorbic acid (0.1%) was superior in preserving chickens and crawfish to plain ice or ice and tetracycline.²⁵⁶ A 5-cycle rapid-freeze/rapid-thaw process was used in conjunction with chemicals to reduce *Salmonella typhimurium* counts on poultry meat.²⁵⁷ Chemicals used included chlorine, sorbate, lactic acid, and propionate.

A proposed mold inhibitor for application in the dry form on meat and cheese products consists of a mixture of 10 to 90% sodium bicarbonate and 10 to 90% sodium chloride with which are mixed 0.5 to 3.5 g sodium diacetate and 1 to 4 g sorbic acid per pound of dry mix.²⁵⁸ Fish, such as salmon, were preserved by smoking and treatment with a solution containing sodium sorbate and sodium erythorbate to inhibit yeast and mold growth and enhance and stabilize the desirable pink-red color of the fish. The preservative solution could be applied either before or after smoking.²⁵⁹ Sturgeon soft caviar was preserved with sodium

benzoate, sorbic acid, and sodium hexametaphosphate, and it was rated equivalent to pasteurized caviar in microbial stability, nutrition, and flavor.²⁶⁰

Potassium sorbate (0.26%), ethyl alcohol (5%), glycine (2%), and/or serine (2%) were effective in preventing deterioration of packaged kamaboko for more than 6 d at 25°C.²⁶¹ The mixture was effective at pH values of 6.13 to 6.90, and the most effective combination consisted of 0.26% sorbate, 3% ethanol, and 1% glycine. Fish paste or kamaboko was also preserved without pH adjustment by a mixture of propylene glycol (1 to 1.5%), glycine (0.5%), and sorbate (0.26%).²⁶²

Mackerel fish was dry-cured with sodium chloride + sodium benzoate + sodium acid pyrophosphate + sodium bicarbonate + sodium hexametaphosphate + ascorbic acid and subsequently dipped in a solution of sorbate prior to drying and it was stable for 6 to 7 months at room temperature.²⁶³ Sodium or potassium phosphates, sorbate, and citric acid also improved the shelf life and quality of fish.²⁶⁴ Dipping of ice-stored herring in a mixture of oxytetracycline, sorbic acid, and propyl gallate, however, did not improve its shelf life.¹⁹³

Dehydro-irradiation has been described as useful for ambient storage of shell-on tropical shrimp.²⁶⁵ The process integrates treatments such as reduced water activity by drying to 40% moisture; incorporation of sodium chloride (3.5 to 4.0%) and sorbic acid (0.03 to 0.04%); packaging; and irradiation (0.25 Mrad). The product retained its color, texture, and flavor during storage.

A solution containing citric acid (3 to 5 parts), sorbate (0.5 to 1.5 parts) and optionally saccharin (0.1 to 0.5 parts), and sufficient water to give 500 to 1000 parts was found to be a preservative for prepackaged vegetables and had only a slight acid taste.²⁶⁶ Raw, cleaned potatoes could be stored for 50 h without browning after they were soaked for 5 min in any of the following preservative solutions: sodium bicarbonate; benzoic acid and 0.1% sodium bicarbonate; sodium bicarbonate + sorbic acid + ascorbic acid; or sorbic acid + nisin.²⁶⁷ Cut lettuce could be stored for up to 4 weeks under refrigeration by dipping for about 1 min in a 5% solution of $\text{Na}_2\text{S}_2\text{O}_5$ (0.9%), citric acid (0.6%), ascorbic acid (0.03%), and potassium sorbate (0.2%). It was packaged in film and closed tightly after draining.²⁶⁸ A preservative for pickles consisted of finely powdered sorbic acid suspended in an organic acid solution (e.g., malic), optionally in the presence of sucrose esters.²⁶⁹ Yeast growth on sauerkraut was inhibited with 0.05% sodium benzoate or 0.02% sorbic acid in the presence of 3% sodium chloride and 1.5% lactic acid.²⁷⁰ A mixture of glucose oxidase, sorbic acid, and ethanol ensured stabilization and preservation of the high quality of thermally sealed strawberry, raspberry, and black currant juices over a period of 6 to 8 months.²²³

The inhibitory effect of sorbic acid, sulfur dioxide, and formic acid on alcoholic fermentation with *Saccharomyces ellipsoideus* was enhanced when ultrasound was applied simultaneously.²⁷¹ A mixture containing tannin, sorbic acid, and ethanol was mixed with, sprayed onto, or impregnated into food products in an acidic pH. The preservative effect of tannin was increased synergistically with sorbic acid and ethanol.²⁷²

Rice was prepared by cooking in a stabilizing solution consisting of 3 to 7% polyhydric alcohol, sodium chloride, and sufficient antimycotic (e.g., sorbic acid) to inhibit mold growth.²⁷³ The same solution could be used to preserve pasta products.²⁷⁴

The stability of low-fat mayonnaise was improved by chemical stabilization with a mixture of sodium benzoate and potassium sorbate and 45 min of heating at 90°C. The product was suitable for storage at 15°C for 12 weeks, while without the treatment maximum storage was only 4 weeks.²⁷⁵ Incorporation of citric acid, sucrose, and sorbic acid increased the effectiveness of sorbic acid as an inhibitor of molds in chapaties.²⁷⁶

Best results for preserving milk samples in storage pending microbiological testing were obtained with a mixture consisting of boric acid (50 g), potassium sorbate (0.75 g), glycerol (10 g), and 1% methylene blue (0.5 ml). The mixture was dissolved in 1 liter of water,

boric acid, glycerol, potassium sorbate, and nystatin was also suitable for preventing the multiplication of microorganisms in milk and caused the least change in the direct epifluorescent filter technique count of bacteria and somatic cells.²⁷⁸

Concentrated (about 100 g solids/100 g water) sweet whey was stabilized against microbial spoilage by a combination of a slight reduction of water activity, lowered pH, and addition of potassium sorbate. This product could be stored for up to 3 months at 30°C without bacterial or mold deterioration and with no need for spray drying.²⁷⁹

An aqueous composition containing propionic acid, sorbic acid, and preferably dehydroacetic acid and methyl paraben preserved hides from bacterial growth for 30 d when stored in a 40% aqueous solution containing 0.4% of the mixture.²⁸⁰

Sorbic acid, *o*-phenylphenol, and sodium benzoate prevented microbial growth and physical deterioration (i.e., water separation and coagulation) of a starch paste (glue).²³⁸

A combination of starch, hydroxypropyl cellulose, sorbate, and an antioxidant gave water-dispersible, biodegradable products, which were resistant to fungal attack and useful for production of tampon applicators.²⁸¹

Several other patents also indicate synergistic antimicrobial effects of complex compositions containing sorbate. One of these includes sorbic acid, dehydroacetic acid, or their salts; 2-pyridinethiol-1-oxide or its salts; tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione; or tetracyldi (aminoethyl) glycine.²⁸² A series of five patents was based on compositions containing N-(fluorodichloro-methyl thio) phthalimide and one or more of dehydroacetic acid, sorbic acid, and their salts. In other patents, the former compound was replaced with methyl-1-(butyl carbomoyl)-2-benzimidazole carbamate, *o*-phenylphenols, *p*-chloro-*m*-xylene, or 2,4,5,6-tetrachloro isophthalonitrile, respectively.²⁸³⁻²⁸⁸

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1. APPLICATION MATERIALS REQUIRED FOR A CHANGE OF CONTROL OF A COLOMBIAN INSURANCE COMPANY

The application materials listed in this checklist must be prepared for transactions in which at least 10% of the outstanding shares of a Colombian entity subject to CFS supervision are being transferred. According to informal conversations with the CFS, substantially the same documents will be required for transactions resulting in a change of the ultimate beneficiary owner of at least 10% of the outstanding shares of a Colombian entity subject to CFS supervision.

In the context of Project Andorra, we understand that Mapfre Caución y Crédito, Compañía Internacional de Seguros y Reaseguros, S.A. (“MCC”) will become a joint venture entity (the “JV”), of which the ultimate beneficiary owners¹ will be Euler Hermes S.A. and Mapfre, S.A. Accordingly, Euler Hermes S.A. as new UBO of the JV (the “UBO”) will have to deliver the documents described below, as applicable².

Please note that the CFS usually requests additional documents or information upon filing of the information required. Once the documents are complete, to the satisfaction of the CFS, a decision will usually take 4 months.

	Required Document	Document description	Execution requirements	Notes
1.1	Request Letter	The letter must include the following information: (a) the complete name of the JV, nationality, identification type and number; (b) the quantity of shares to be indirectly transferred and the stake (percentage) that they represent with respect to the outstanding shares of the local target entity; (c) the number of shares of which the JV is already	The letter must be in Spanish, notarized and duly legalized or apostilled and executed by the JV’s legal representative or an attorney-in-fact. If a POA is granted, it must be notarized and duly legalized or apostilled.	Item 1.1(h) is complied with by presenting the financial statements described in section 1.5, and other documents such as income tax returns, income tax withholding certificates, etc. It also requires disclosure of the

¹ Pursuant to Article 2.31.3.1.12 of Decree 2555, “Ultimate Beneficiary Owner” means any person or group of persons whom, directly or indirectly, by themselves or through an intermediary, by virtue of contract, agreement or otherwise, have in respect to a share of a corporation, or may have, by owning bonds mandatorily convertible into shares, decision making capacity, meaning (i) the capacity or the power to vote in the election of directors or representatives, or directing, guiding and controlling such vote, and (ii) the capacity or power to transfer and order the transfer of, or to create encumbrances over, such shares. For purposes of this decree, parent companies and their subordinates constitute the same beneficiary owner.

² Please note that the CFS has the discretionary authority to request the same documents from each entity in the chain of control between MCC and the UBO, which requirement will depend on the level of decision making to take place within each one of those intermediate entities.

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	beneficiary in the local target entity, if any, and the price at which they were acquired; (d) complete name of the transferring entity; (e) the consideration to be paid for the shares and their market price; (f) terms of payment of the shares to be indirectly transferred; (g) if the shares to be directly acquired at the holding level are subject to preference rights, the document that supports compliance with any preference rights procedures must be attached; (h) detailed explanation of the source of the funds to be used in the acquisition; (i) the investments held by the JV in other companies (financial or non-financial) either directly or through a parent or affiliate; (j) whether the JV has outstanding indebtedness to Colombian financial entities, the names of any such lenders, the amounts of outstanding indebtedness and whether the payments are being timely made (including an explanation for any delays); (k) if the JV has any type of common interest, either directly or indirectly, with the other shareholders of the local target entity, as applicable, explaining the type of such interest.	All the documents attached to the letter must be consecutively numbered, and a reference to "procedure 6" must be included in the letter. All documents which are in English must be duly translated into Spanish by an official translator.	amount of the acquisition that will be financed with debt and the financial and economic relationships held with any such lender. The UBO must demonstrate (i) a patrimony of at least 1.3 times the amounts invested, and (ii) that at least one third of the consideration to be paid comes from its own funds and not from borrowing.
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1.2	Certificate of existence and good standing or equivalent document	Document that certifies the existence of the JV and the UBO, its corporate purpose, and the names of their legal representatives and of the members of its Board of Directors or similar corporate body.	The document must be a recently issued and apostilled original, and include updated information.	
1.3	Information on management personnel	(a) The curriculum vitae (" CV ") of each director, legal representative and shareholder holding at least 5% of the outstanding shares of the JV or the UBO; and (b) Copies of the passports of each of the abovementioned persons.	Each CV must be signed and transmitted using the M-PI-AUT-001 format established by the CFS. Each passport copy must be notarized, and legalized or apostilled.	If the CFS already has a CV for a relevant individual, an update is required. A form of CV is attached for reference.
1.4	Authorization to investigate background information or records	Authorization by the JV and the UBO to the CFS, to investigate background information and records with the corresponding authorities and risk data bases.	The authorization must be signed by the legal representative of each entity that is required to transmit information to the CFS. The authorization must be in Spanish, notarized, and legalized or apostilled.	
1.5	Financial Statements	Yearly financial statements with notes of the JV for the three previous years; and financial statements with notes of the UBO for the previous year.	Financial statements must be audited and certified.	
1.6	Evidence of legal capacity to invest	Letter to the CFS indicating that the JV has the legal capacity to complete the transactions resulting in the change of control of the shares. The letter must also indicate whether the JV requires any authorization from any internal corporate body to complete the transaction, and include copies thereof.	The letter must be signed by the legal representative of the JV. The letter may be in English (notarized and legalized or apostilled) with an official	

			translation into Spanish.	
1.7	Documents relating to conglomerates	The following documents or information must be attached to or included in the request letter described in Section 1.1: (a) list of shareholders of the JV, its parent and affiliates up until the UBO, indicating for each entity: nationality, domicile, type of activity or business, and their participation percentages within the conglomerate; (b) document identifying the UBO of the JV and their share participation, including the title for holding such shares; (c) document indicating the rules and regulations under which the JV and the UBO are monitored, showing evidence that those entities are or will be subject to consolidated regulatory surveillance from the competent regulatory authorities of their domicile. In regard to the UBO, the type and scope of the supervision by the financial regulatory authorities must be indicated; (d) the JV and the UBO must attach a copy of any authorizations issued by the foreign regulatory authorities to consummate the transaction, if required pursuant to applicable laws; otherwise, the letter must include the local rule or regulation waiving the necessity for such authorization; (e) in regard to the UBO, it must be indicated their responsibilities and obligations with respect to the acquisition of the local target entity, together with the applicable rules in their domicile regarding the constitution of affiliates abroad; (f) if the acquisition is to be made through vehicles incorporated in jurisdictions with no financial regulatory authorities, or in jurisdictions which do not have an	The document indicated in item 1.7(c) should be presented as a notarized and legalized or apostilled certification issued by a lawyer. The authorization described in item 1.7(d) must be notarized and legalized or apostilled.	In regard to item 1.7(g), the CFS may request any information deemed necessary for the analysis of the transaction, and require the signature of mandatory compromises for the acquiring entities.

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		agreement with the CFS for comprehensive and consolidated supervision purposes, a document explaining the reasons for such vehicle's involvement in the transaction; (g) in the case of international conglomerates (i) describe the composition of the conglomerate by countries, (ii) attach the UBO's consolidated financial information and the reports of the external auditors for the last year, (iii) identify and classify all the companies of the conglomerate by the type of business, and indicate if any of those companies will engage in any type of business with the local target entity, and (iv) disclosure of any future intra-group agreements between the companies of the conglomerate and the local target entity; (h) document identifying the corporate structure in charge of detecting, managing and controlling the group's global risks, explaining the way in which such controls will be activated in regard to the local target entity; and (i) report on the type of shares of the UBO (whether nominative or bearer shares), if such shares are listed in any stock exchange, or if there are restrictions or limitations on their negotiability of the shares.		
1.8	Information about the payment source	Provide information about the ultimate source of the funds to be used for the acquisition, including any information necessary regarding any indirect shareholders.		
1.9	By-laws of the JV and UBO	Provide a copy of the Articles of Incorporation and/or By-Laws of the JV and the UBO.	The Articles and By-laws must be notarized and legalized or apostilled.	

1.10	Corporate governance structure	Provide a document with the following information: (a) modifications, if any, planned for the local target entities' corporate governance structure, including (i) ownership structure; (ii) exercise of minority shareholders' rights; (iii) composition and responsibilities of the board of directors; (iv) situation of the independent members of such board of directors; (v) composition of any committees within the board of directors and any other committees within the local target entities; and (vi) the responsibility levels of, and control mechanisms over the senior management within the local target entities; (b) planned amendments, if any, to the local target entities' internal regulations, including by-laws, corporate governance codes, conduct code, internal controls and manuals on operations with affiliates; (c) statement of intention to register before the commercial registry a control or conglomerate situation resulting from the acquisition, if any; and (d) information on the way in which conflicts of interest will be managed following the acquisition.		If no modification to the corporate governance structure or amendments to the target's internal regulations are planned, then the documents referred to in items 1.10(a) and 1.10(b) need not be furnished.
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