Antimicrobial Agents*

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Antimicrobial Agents

Food is essential for survival. Since the beginning, people have been interested in preserving food for later consumption. Overtime, many methods of food preservation have been tried. These include heating, freezing, drying, fermenting, and adding chemical preservatives. In recent years, the use of chemical preservatives has increased. This is due to the developments in marketing and distribution of the food we consume and also because of the large variety of foods offered for consumption. At one time, food was produced and consumed locally and required short preservation time. Nowadays, food is produced at one location, may be processed in another, and later distributed to many other locations throughout the country. This means that the interval between food production and consumption has become substantially longer and preservation of food over a long period has become essential.

We are also consuming more variety of food items and often these items are made available all year round. To support this new trend of food processing, distribution, and consumption, the use of chemical preservatives has become more important. We now have a deeper and better understanding of chemical preservatives and their role in the preservation of food. It is now less expensive and safer to enjoy a wide variety of food all year round.

Chemical preservatives are used to prevent or retard both chemical and biological deterioration of food. Chemical deterioration includes oxidation and browning of food. Biological deterioration involves degradation of food by microorganisms.

In wine, $S0_2$ acts as an antioxidant and antimicrobial agent. Sorbic acid is used as an antimicrobial agent.

Selection of Antimicrobial Agents

The selection of a proper antimicrobial agent is essential to obtain the best results in preserving food. Many factors need to be considered in choosing the right antimicrobial agent.

Range of microorganisms controlled and the mode of action

It is desirable to use an antimicrobial agent that can inhibit a wide range of spoilage-causing microorganisms, but this is hard to achieve. Also, a good understanding of the chemicals' mode of action is useful in selecting a preservative.

Chemical and physical properties

Properties such as solubility, boiling point, and dissociation are important in choosing an antimicrobial agent. Water solubility is important since microbial growth requires water. Lipid solubility is desired where the mode of action requires a reaction between the chemical and the cell membrane. If a volatile compound is used, it can be lost if the food is heated during processing. Dissociation of sorbic acid has an important bearing on its effectiveness in inhibiting harmful microbes. For example, certain compounds, such as SO_2 and sorbic acid, are more effective in undissociated form.

Stability

Sometimes the preservative can react with certain constituents in the food and this can reduce the effectiveness of the chemical. Constituents such as proteins and fiber can bind with antimicrobial agents and lower their activity. In some cases, the chemical can be altered by oxidation or hydrolysis, and thus can lose its activity. For this reason, the stability of antimicrobial agents during storage is very important.

Microbial load

Food should always be processed under the most rigorous sanitary conditions to minimize contamination. A high microbial population would require higher doses of antimicrobial agents. Use of antimicrobial agents should not be viewed as a substitute for good sanitation.

Legality and safety

It is obvious that antimicrobial agents must not be toxic to humans. The use of antimicrobial agents is often strictly regulated. As a processor, it is essential to use chemicals as prescribed by the regulations.

Antimicrobial Agents Used in Wine

Pathogenic yeasts and bacteria do not seem to survive in wine. This is due to high alcohol (10-12%) and low pH conditions. However, some bacteria and yeast can spoil a wine so that it is no longer pleasant to drink. One way to prevent spoilage and prolong shelf life is to use certain preservatives (chemical compounds).

The most commonly used preservatives in the wine industry include sulfur dioxide, and sorbic acid/potassium sorbate. Sulfur dioxide is an antioxidant, and also an antimicrobial agent. Sorbic acid (and its potassium salt) is a fungistatic agent.

Diethyl dicarbonate is an antimicrobial agent no longer permitted in the United States, but used elsewhere. Use of dimethyl dicarbonate is under review.
Diethyl dicarbonate (DEDC). This compound is toxic to fungi; it reacts irreversibly with the amino groups on the active sites of the enzymes. At 40 to 50 mg/L, it is effective in controlling yeast in sweet table wines. In contact with water, the compound breaks down into ethanol and $C0_{2.}$

$$DEDC + H_2O \text{ -----------» } \underset{\text{ethanol}}{2C_2H_5OH} + \underset{\text{carbon dioxide}}{2CO_2}$$

It also can react with ammonia and form ethyl carbamate. Many factors determine the amount ethyl carbamate produced. Under normal winery bottling conditions, about 1 to 5 1l9/L of ethyl carbamate may be produced. This amount is small and relatively harmless. But since ethyl carbamate is a known carcinogen, the use of DEDC in wine is prohibited in the United States.

Dimethyl dicarbonate (DMDC).
Dimethyl dicarbonate is used in some countries in place of sorbic acid as a yeast inhibitor. Its antimicrobial action is similar to DMDC, but it does not produce a carcinogen. Its decomposition products include methanol and carbon dioxide. The methanol content resulting from DMDC treatment of wine is usually small and does not pose any significant risk to the health of the consumer. Its use in the United States is being reviewed by the regulatory agencies. It is also known as velcorin. Since it is slightly soluble in water, a special pump is used to add velcorin to the beverages.

Microorganisms Associated with Wine
Since we are talking about antimicrobial agents, it may be helpful to briefly discuss the microorganisms involved in winemaking, and particularly, wine spoilage. The following table shows the major microorganisms associated with winemaking.

Table 1. Microorganisms associated with wine.

Microorganism	**Significance**
Yeast	alcoholic fermentation, deacidification, spoilage
Acetic acid bacteria	spoilage, stuck fermentation
Lactic acid bacteria	malolactic fermentation, spoilage
Fungi (mold)	*Bottytisized*wine, spoilage, corky tant

The microorganisms listed in Table 1 participate in winemaking to various degrees. The important point to note here is that they all can cause spoilage.

Table 2. Summary of spoilage yeast activity.

Yeasts causing spoilage	**Spoilage**	**Control**	**Remarks**
KJoeckera apicuJata, Hanseniaspora uvurum	Produces relatively large amounts of acetic acid, ethyl acetate, mannitol, sorbitol and	Clean fruit, settle must, inoculate with strong fermenting commercial yeast	Dominates in initial stage of fermentation but can also occur in bottled wine

	ribitol		
Pichia, Candida (film forming yeasts)	Oxidized character	Store wine, away from air exposure and at cooler cellar temperatures	Tolerates $S0_2$ (2 to 3 mg/L molecular)
Brettanomyces/ Dekkera	Unpleasant taste and aroma	Follow stringent sanitary measures	$S0_2$ is effective in controlling it
Zygosaccharomyces bailli	Haze, sediment, succininc and acetic acid	Avoid contaminated concentrate	Hard to control.Tolerates high concentrations of sugar and preservatives, such as $S0_2$, sorbic acid and others

Note that $S0_2$ at concentrations used commonly in winemaking do not effectively control spoilage causing yeasts. Spoilage by the yeasts mentioned above can occur in bottled wine.

Sorbic Acid

Historical Background

A German chemist, A. W. Van Hoffman, was the first to isolate sorbic acid from the oil of unripened rowan berries in 1859. The antimicrobial properties of this compound were recognized in the 1940s. In 1945, a United States patent was awarded to C. M. Gooding and Best Food, Inc. for recognizing the role of sorbic acid as a fungistatic agent for foods and food wrappers. Soon after this, the chemical was commercially produced and tested in many foods for its preservative properties. In wine, its use was legalized in France in 1959, and in Germany, in the year 1971. According to recent BATF regulations (June, 1990), the use of sorbic acid and potassium salt of sorbic acid is authorized for the treatment of wine in the United States. The maximum concentration permitted in finished wine is not more than 300 mg/L. This limit seems reasonable, since yeast growth is inhibited by sorbic acid in the range of 180 to 200 mg/L in wine.

Chemical Properties

Sorbic acid (2,4-hexadienoic acid) is a straight chain unsaturated fatty acid, with a molecularweight of 112.12 and the formula CH3-CH =CH -CH =CH - COOH. The carboxyl group (COOH) is reactive and can form salts and esters. The potassium salt of sorbic acid is commercially available as a powder or granules. Its molecular weight is 150.22 and it is very soluble in water.

Solubility is an important factor in determining its application. Table 3 contains the data regarding the solubility of sorbic acid and potassium sorbate in a water, ethanol, and sucrose solution. Notice that in water, sorbic acid solubility is very low compared to potassium sorbate, and it increases with an increase in temperature.

In ethanol, sorbic acid is more soluble than potassium sorbate. With an increase in ethanol concentration, the solubility of sorbic acid increases while that of potassium sorbate decreases considerably. The presence of other solids in solution decreases the solubnity of both the sorbic acid and potassium sorbate. This point is important since potassium sorbate is used in the production of wines containing residual sugar.

Table 3. Sorbic acid and potassium sorbate solubilities.

Solvent	**% Solubility Sorbic acid**	**% Solubility Potassium sorbate**
Water		
20ºC (68ºF)	0.16	58.20
50ºC (112ºF)	0.55	61.00
100ºC (212ºF)	4.00	64.00
Ethanol		
5%	0.16	57.40
100%	12.90	2.00
Sucrose		
10%	0.15	58.00
40%	0.10	45.00

60%	0.08	28.00

Source: Gooding et al. (1955), Pfizer (1974), Monsanto (1978), and Sofos and Busta (1981). Cited by Sofas and Busta (1983).

Antimicrobial Activity

The antimicrobial action of sorbic acid is primarily against yeast and molds. Its action against bacteria appears to be selective. At concentrations used in wine it does not seem to prevent spoilage from either acetic or lactic acid bacteria. Must and wine related yeast inhibited by sorbic acid include species of the genera Brettanomyces, Candida, Hansenula, Pichia, Saccharomyces, Torulaspora, and Zygosaccharomyces.

The inhibitory effect of sorbic acid on yeast strains is not uniform. Certain species are more tolerant than other. For example, according to one report Zygosaccharomyces bailiiwas not inhibited by sorbic acid at 0.06% in 10% glucose. It should be noted that yeast Zygosaccharomyces bailii is also resistant to sulfur dioxide and diethyl pyrocarbonic acid (DEPC) and it can ferment high sugar must such as grape juice concentrate at 55 to 72 % sugar. If contaminated concentrate is used for sweetening, it is likely to cause fermentation even if sorbic acid is present in normal concentrations.
The minimum inhibitory concentration of sorbic acid for various strains of yeast is shown in Table 4.

Table 4. Inhibitory action of sorbic acid on yeasts.

Name of test organism	**Minimum pH value**	**Inhibitory concentration of ppm**
Saccharomyces cerevisiae Saccharomyces ellipsoideus Saccharomyces spp.	3.0 3.5 3.2 - 5.7	25 50 - 200 30 - 100
Hansenula anomala Brettanomyces versatilis Byssochlamys fulva Rhodotorula spp.	5.0 4.6 3.5 4.0 - 5.0	500 200 50 - 250 100 - 200
Torulopsis holmii Torula lipolytica	4.6 5.0	400 100 - 200
Kloeckera apiculata 3.5 - 4.0 1 00 - 200	3.5 - 4.0	100 - 200
Candida krusei 3.4 100 Candida lipolytica 5.0 100	3.4 5.0	100 100

Source: Rehm (1961), Lueck (1972). Cited by Erick Lueck (1980).

The inhibitory influence of sorbic acid is greatest when it is in the undissociated form. The pKa of sorbic acid is 4.75. The antimicrobial action increases as the pH value decreases below 4.75. In other words, the proportion of the undissociated form of sorbic acid increases (above 50%) as the pH drops below 4.75 and this leads to increased antimicrobial action. The effect of pH on the dissociation of sorbic acid is shown in Table 5.

Table 5. Sorbic acid dissociation at various pH values.

pH	% undissociated acid
7.00 6.00 5.80 5.00 4.75 4.40 4.00 3.70 3.00	0.6 6.0 7.0 37.0 50.0 70.0 86.0 93.0 98.0

Source: Sauer (1977), Sofas and Busta (1981).
Cited bv Sofos and Busta (1983)

Besides pH, the ethanol content of the wine also influences the antimicrobial action of sorbic acid. For this reason, with a relatively high amount of alcohol in the wine, lower levels of sorbic acid would be needed. Peynaud (1980) recommended following doses of sorbic acid in clarified wine based on alcohol content. This is shown in Table 6.

Table 6. Recommended doses of sorbic acid based on alcohol content.

% Alcohol in wine (clarified wine)	Sorbic acid mg/L
10	150
11	125
12	100
13	75
14	50

It should be emphasized that the wine must be clarified to reduce yeast population below 100/ml for sorbic acid to be effective.

Sorbic acid also inhibits mold growth. Some of the important species that are suppressed by sorbic ing doses of sorbic acid belong to the genera Alternaria, Botrytis, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, and Trichoderma. Molds can be a problem in wine cellars. To control mold in the wine cellar, sorbic acid should be included in the antimicrobial compounds used for sanitizing.

Several microorganisms can metabolize sorbic acid particularly when it is present in small concentrations. For this reason, it is not a suitable preservative in foods with high microbial count. To derive the maximum benefit from the antimicrobial action of sorbic acid, it is important to clean the wine well and keep the microbial count low in bottled wine. It should be emphasized that sorbic acid inhibits yeast and mold, but not the acetic and lactic acid bacteria. In fact, lactic acid bacteria can metabolize sorbic acid and produce off flavored compounds.

Addition of sorbic acid often results in the formation of ethylsorbate, which is said to import an unpleasant odor when present at a significant level.

As mentioned earlier, lactic acid bacteria can decompose sorbic acid and produce 2-ethoxyhexa-3,5 diene, which gives a geranium-like off odor. Crowell and Guyman (1975) explained the reaction as follows:

Sorbic acid + EtOH, H+
(2, 4-hexadienoic acid) -------» Ethyl sorbate

Lactic acid bacteria

(EtOH)

Sorbyl alcohol ---------» Ethyl sorbyl ether
(2, 4 - hexadien - 1 - 01)

rearrangement, H+

(EtOH)

3,5- Hexadien -2-01 ~ 2, ethoxyhexa-3,5 diene

They suggested that in addition to 2 - ethoxyhexa -3,5 diene (main compound with geranium-like odor) other compounds such as 2, 4 - hexadien - 1 - 01, 3,5- Hexadien -2- 01, and 1 - ethoxyhexa - 2, 4 diene probably contribute to overall off odor in wine (containing sorbic acid) and spoiled by lactic acid bacteria.

To prevent bacterial spoilage in sweet wines it is important to add a sufficient amount of sulfur dioxide in addition to sorbic acid.

The antimicrobial action of sorbic acid is due to its inhibitory influence on various enzymes in the microbial cell. The enzymes inhibited by sorbic acid include the following:

1. Enzymes involved in carbohydrate metabolism such as enolase and lactate dehydrogenase.
2. Enzymes of the citric acid cycles such as malate dehydrogenase, isocitrate dehydrogenase, ketoglutarate dehydrogenase, succinate dehydrogenase, and fumerase.
3. Several enzymes containing SH group, and other enzymes such as catalase and peroxidase.

Application In Winery

Potassium sorbate is used in the production of sweet white table wines. Although BATF permits its use in wine, up to 300 ppm, it is important to remember that its taste threshold is well below the legal limit. The taste threshold for experienced tasters has been reported to be about 130 ppm.

The key points in sorbic acid application in wine are summarized below.

1. Potassium sorbate (most soluble form of sorbic acid) should be used. However, this can cause bitartrate precipitation problems.
2. The solubility of potassium sorbate is influenced by temperature, therefore, it should not be added to cold wine.
3. Wine should be mixed well after sorbate addition.
4. Sorbate should be used in conjunction with sulfur dioxide.
5. Certain yeast and bacteria are not inhibited by sorbic acid.
6. Conditions such as low microbial count (properly clarified wine), low pH, and high ethanol are desirable in reducing the amount of sorbic acid needed to control yeast.
7. Sorbic acid addition should never be considered as a substitute for good sanitation.

Calculating Potassium Sorbate Addition

Sorbic acid is added to a wine in the form of potassium salt called potassium sorbate. Potassium sorbate contains 73.97% sorbic acid. In order to calculate the amount of potassium sorbate, the following formula should be used.

ppm = mg 1/liter
mg/liter of sorbic acid = mg/liter of
x 1.35 potassium sorbate

Example: To obtain 200 ppm sorbic acid concentration in wine, the following steps may be used.

I. 200 ppm = 200 mg/liter
II. 200 mg/liter x 1.35 = 270 mg/liter of potassium sorbate
III. 270 mg/liter x 3.785 = 1021.95 mg/gallon or 1.022 gram/gallon

Table 7, shows the amount of potassium sorbate needed (1 to 1000 gallons) to obtain various levels of sorbic acid concentration in wine.

Table 7.

*ppm of sorbic acid desired	Grams of Potassium Sorbate Required			
	gm/ gal	gm/ 10 gal	gm/ 100 gal	gm/ 1000 gal
50	0.255	2.55	25.5	255
75	0.383	3.83	38.3	383
100	0.511	5.11	51.1	511
125	0.639	6.39	63.9	639
150	0.767	7.67	76.7	767
175	0.894	8.94	89.4	894

200	1.022	10.22	102.2	1022
225	1.150	11.50	115.0	1150
250	1.278	12.78	127.8	1278
275	1.405	14.05	140.5	1405
300	1.533	15.33	153.3	1553
325	1.661	16.61	166.1	1661
350	1.789	17.89	178.9	1789

*Values given in the left column are for sorbic acid, while the values given in other columns are for potassium sorbate. For example, to obtain 150 ppm sorbic acid level, you need to add 150 x 1.35 = 202 mg/L of potassium sorbate and not 150 mg/L of potassium sorbate. This is due to the fact that potassium sorbate on a molecular weight basis contains about 74% sorbic acid.

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