Sorbic Acid as a Wine Preservative—Its Efficacy and Organoleptic Threshold

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Sorbic acid was added to wines in different concentrations to determine its effect on the inhibition of yeasts in semi sweet wines. Sorbic acid proved to be an effective inhibitor of yeast growth when used at a concentration of 200 mg/ℓ in conjunction with a concentration of 100 mg SO_2/ℓ. As the sorbic acid does not kill the yeast cells but only inhibits them it is imperative that the wine should still be filtered as sterile as possible.

Sweet must should not be preserved with sorbic acid because of the danger of bacterial spoilage and the subsequent development of the so-called "geranium" odour in the wines sweetened with infected sweet must.

The flavour threshold of sorbic acid itself in dry wines was determined to be between 300 and 400 mg sorbic acid/ℓ. This is virtually double the amount recommended for inhibition, at which concentration no effect should be (nor was) encountered on the quality of the wine.

It is generally known that very high doses of sulphur dioxide are required to protect must or semi-sweet wine from infection by yeasts. Apart from the fact that high SO_2 doses are organoleptically unacceptable, the perpetual intake of large amounts of SO_2 is regarded by some to be potentially harmful to human health. Research is currently aimed at finding a yeast-inhibitor complying with the standards set for food preservatives, and having no effect on the delicate organoleptic properties of wine.

Sorbic acid (2,4-hexadïenoic acid) has possibilities in this respect but controversy surrounds its effectiveness in controlling yeast spoilage as well as its effect on wine quality. Sorbic acid is an unsaturated fatty acid which inhibits yeast growth by suppressing the activity of the oxydative phosphorilase enzyme system. No inhibition will result when the concentration of yeast cells is too high. Under such conditions fermentation will continue, and sorbic acid will even be metabolized as a fatty acid, thus stimulating in stead of inhibiting fermentation (Bouthilet, 1962; O'Rourke & Weaver, 1962). This might also explain why high concentrations of sorbic acid without SO_2 seem less effective than lower concentrations when used in conjunction with SO_2 (Auerbach, 1959; Ough & Ingraham, 1960).

The pH of the substrate is critical to the inhibitory effect of sorbic acid. Bell, Etchells & Borg (1959) found that the undissociated molecule is the most potent form of sorbic acid. At the pH of white wine (3,2–3,4) very little dissociation takes place, and sorbic acid inhibits virtually optimally. A disadvantage of sorbic acid is that it does not inhibit bacteria (Auerbach, 1959; Postel & Lück, 1970), necessitating the use of SO_2 for this purpose.

Sorbic acid can be detected organoleptically. Ough & Ingraham (1960) and van Zyl (1962), for instance, pointed out that sorbic acid can be smelled at relative low concentrations. Auerbach (1959), Tercelj & Adamic (1965) and Postel & Drawert (1970) determined the threshold of sorbic acid to be between 240 and 500 mg/ℓ, while the so-called "untrained" panels of Lück & Nue (1965) and Jakob (1973) could not significantly distinguish between sorbic acid treated and untreated samples. Ough & Ingraham (1960) and Amerine & Joslyn (1967) also questioned the desirability of sorbic acid additions to wines of good quality. It is, however, not only sorbic acid which has an organoleptic effect but also its metabolites. Peynaud (1963) stated that the "off"-character could be attributed to crotonaldehyde ($CH_3 - CH = CH - CHO$), chemical compounds of the type $CH - CO - C = O$ or acyclic terpenes such as geraniol. Recent research by Crowell & Guymon (1975) indicated that the geranium odour could in fact be attributed to the substance 2-ethoxyhexa-3,5-dien which is formed by the bacterial metabolism of sorbic acid.

Lück & Nue (1965) and Lück (1970) summarized the limits for sorbic acid allowed in different countries. These limits range from 200 to 2 000 mg sorbic acid/ℓ, indicating that it has not yet been ascertained what concentration sorbic acid inhibits yeast growth. In this respect the amount of yeasts contaminating the wine is of great importance. Effective filtration is, therefore, important if sorbic acid is to be used as a preservative (O'Rourke & Weaver, 1962; Würdig & Kullman, 1971).

MATERIALS AND METHODS

Effect of sorbic acid on yeasts: Sorbic acid was added to semi-sweet wine (20 g reducing sugar/ℓ) with low alcohol (5% by volume) and to the same wine with a high alcohol (10% by volume) concentration to obtain different concentrations of sorbic acid. The wines were subsequently inoculated with different concentrations of a few yeast strains in the following manner:

Low alcohol wine: Fourteen wines were prepared to contain 0, 50, 100, 150, 200, 300 and 400 mg sorbic acid/ℓ at two SO_2 levels of 0 and 100 mg/ℓ. Each of these 14 wines was subsequently divided into four lots. Two of these were inoculated with *Saccharomyces cerevisiae*, and the other two with *Saccharomyces bayanus* at inoculum concentrations of respectively 5×10^3 and 5×10^4 yeast

cells/mℓ for each yeast strain. The wines were then stored at 25 °C, and the development of turbidity noted as a function of time.

High alcohol wine: The same technique as stated above was employed with the following amendments: *Saccharomyces beticus* was also used with the other two strains, and the inoculum concentrations changed from 5×10^3 and 5×10^4 to respectively 3×10^3 and 3×10^4 cells/mℓ.

Bottled wine: A natural semi-sweet wine (11,4% by volume alcohol; 27 g reducing sugar/ℓ) was adjusted to two levels of SO_2, namely 80 and 150 mg/ℓ, and treated with respectively 0, 100, 150, 200, 300 and 400 mg sorbic acid/ℓ. The wines were then inoculated with 5×10^5 yeast cells (*S. cerevisiae*)/mℓ, bottled and sampled regularly for the counting of yeast cells.

Effect of sorbic acid on wine quality: *"Geranium" odour:* Recent research showed that bacteria can metabolize sorbic acid, giving rise to the so-called "geranium" odour in wines. Organoleptic tests were therefore carried out to determine whether a panel of judges could recognize this odour, and what its effect was on wine quality.

A Chenin blanc wine was sweetened with sweet must which was preserved with sorbic acid and had undergone bacterial infection. As a control a duplicate sample of the same Chenin blanc wine was also sweetened with sweet must that was preserved with sorbic acid but without any bacterial infection. The wines were presented to a panel of judges in a "paired sample" test, and they were asked for their preference. A triangle test was also utilized to determine whether the panel could significantly discern between the two wines as well as correctly identify the geranium odour.

Sorbic acid: A dry white wine containing the following levels of sorbic acid: 0, 50, 150, 200, 250, 300, 400, 600 and 1 000 mg/ℓ was prepared. A triangle test as prescribed by Amerine, Pangborn & Roessler (1965) was employed, and the Chi-square calculated to determine whether the tasters could significantly distinguish between the treated wines and the control.

RESULTS AND DISCUSSION

Effect of sorbic acid on yeasts: The results obtained in the two experimental series where the low and high alcohol wines were utilized are summarized in Tables 1 and 2 respectively.

TABLE 1
Effect of sorbic acid on yeast growth in white wine containing 20 g sugar/ℓ and having an alcohol concentration of 5 vol. %

Sorbic acid treatment (mg/ℓ)	Number of days from inoculation until start of growth							
	S. bayanus				*S. cerevisiae*			
	5×10^3 cells/mℓ		5×10^4 cells/mℓ		5×10^3 cells/mℓ		5×10^4 cells/mℓ	
	No SO_2	100 mg SO_2/ℓ	No SO_2	100 mg SO_2/ℓ	No SO_2	100 mg SO_2/ℓ	No SO_2	100 mg SO_2/ℓ
0	2	5	1	1	5	7	5	5
50	5	5	2	5	5	8	5	6
100	5	5	2	5	6	9	6	7
150	5	5	2	5	12	12	9	9
200	5	7	5	7	*	23	18	22
300	12	13	6	12	*	*	25	*
400	*	*	*	*	*	*	*	*

*No growth apparent after 45 days

TABLE 2
Effect of sorbic acid on yeast growth in white wine containing 20 g sugar/ℓ and having an alcohol concentration of 10 vol. %

Sorbic acid treatment (mg/ℓ)	Number of days from inoculation until start of growth											
	S. bayanus				*S. cerevisiae*				*S. beticus*			
	3×10^3 cells/mℓ		3×10^4 cells/mℓ		3×10^3 cells/mℓ		3×10^4 cells/mℓ		3×10^3 cells/mℓ		3×10^4 cells/mℓ	
	No SO_2	100 mg SO_2/ℓ	No SO_2	100 mg SO_2/ℓ	No SO_2	100 mg SO_2/ℓ	No SO_2	100 mg SO_2/ℓ	No SO_2	100 mg SO_2/ℓ	No SO_2	100 mg SO_2/ℓ
0	6	8	6	7	13	13	7	8	6	16	4	16
50	7	13	6	8	16	16	13	13	8	29	6	23
100	16	20	8	13	34	*	*	*	11	37	8	34
150	20	26	16	17	30	*	34	*	16	*	11	*
200	26	*	20	*	*	*	*	*	42	*	16	*
300	*	*	*	*	*	*	*	*	*	*	*	*
400	*	*	*	*	*	*	*	*	*	*	*	*

*No growth apparent after 45 days

In the case of the low alcohol wine as much as 400 mg sorbic acid/ℓ was necessary to preserve the wine even at the relatively high SO_2 concentration of 100 mg/ℓ (Table 1). The difference in resistance against sorbic acid of the two strains used is evident: At 200 mg sorbic acid/ℓ *Saccharomyces bayanus* spoiled the wine much sooner than *Saccharomyces cerevisiae*.

The higher alcohol content of the 10 vol. % wine increases the effectivity of the sorbic acid to a large extent (Table 2). At this alcohol level the growth of all the yeast species was effectively controlled by a concentration of 200 mg sorbic acid/ℓ in conjunction with 100 mg SO_2/ℓ. The wine was, however, not preserved by 200 mg sorbic acid/ℓ alone. This is in accordance with the findings of Ough & Ingraham (1960) that SO_2 has a synergistic effect on the effectiveness of sorbic acid.

The results in Table 2 further show that *S. cerevisiae* (Epernay strain) is less resistant against sorbic acid than the other strains, as yeast growth of the former was effectively inhibited by 100 mg sorbic acid/ℓ in the presence of 100 mg SO_2/ℓ. From Table 1 and 2 it is also evident that at low concentrations of sorbic acid (50 to 150 mg/ℓ) a relatively low cell concentration of 3×10^3 to 5×10^3 cells/mℓ was already sufficient to spoil the wine in most cases.

The above results were verified by an experiment conducted with bottled wine. A concentration of 150 mg sorbic acid/ℓ effectively inhibited inoculated yeasts at a concentration of 80 mg SO_2/ℓ (Fig. 1). At this SO_2 concentration the 100 mg sorbic acid/ℓ treatment was, however, insufficient for inhibition of yeast growth. In the case of the control in which only 80 mg SO_2/ℓ was present the bottles popped their corks because of CO_2 pressure buildup as a result of yeast growth. In the case of the 150 mg SO_2/ℓ control the same happened, whilst a concentration of 100 mg sorbic acid/ℓ at this SO_2 concentration was sufficient to inhibit yeast growth and even to diminish their numbers.

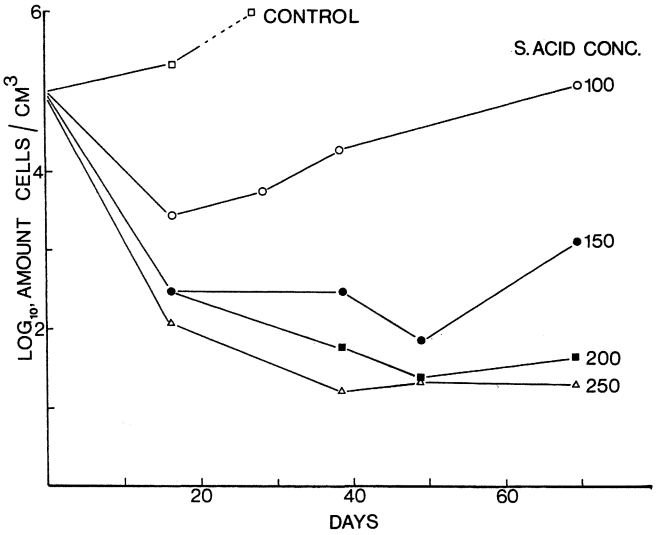

FIG. 1

The effect of different concentrations of sorbic acid in a wine containing 80 mg SO_2/ℓ on the number of yeast cells with time.

Effect of sorbic acid on wine quality: *"Geranium":* Organoleptic evaluation results for the Chenin blanc wines which were sweetened by either the healthy sweet reserve (slightly fermented sweet must used for sweetening wines) or the sweet reserve that underwent bacterial spoilage, are shown in Table 3. The paired sample test demonstrates that 53 out of a possible 54 evaluations preferred the control without the geranium odour. This panel of nine judges thus decided that the wine with the presumably "off" character was significantly inferior to the control. The triangle test further demonstrates that the panel was able to significantly identify the correct duplicates as well as correctly pointing out the geranium odour.

TABLE 3

Judging of a semi-sweet white wine with the geranium odour against a control wine

Paired sample test*		Triangle test*			
Preferred		Indication of correct duplicates		Correct indication of geranium odour	
Control	Geranium odour	Correct	Wrong	Correct	Wrong
53***	1	46***	8	42***	12

*Nine judges, six repetitions
***($P \leq 0{,}001$)

TABLE 4

Triangle test data for the determination of the threshold of sorbic acid in white wine

Sorbic acid (mg/ℓ)	50	100	150	200	250	300	400	600	1 000
% correct judgings	41,7	38,9	58,3	50,0	58,3	59,4	80,6	78,1	75,0
% above chance	12,5	8,3	37,5	25,0	37,5	39,1	70,8	67,2	62,5
Chi square	0,78	0,28	9,03**	3,29	9,03**	8,63**	34,0**	26,9**	26,3***

**($P \leq 0{,}01$)
***($P \leq 0{,}001$)

It is clear, therefore, that the geranium odour is both prominent as well as detrimental to wine quality. Should semi-sweet wine be made by the addition of sweet reserve which was preserved with sorbic acid, great care must be taken to ensure that this sweet reserve did not undergo some bacterial spoilage.

Sorbic acid odour: The organoleptic threshold of sorbic acid was determined in an experiment, the results of which are summarized in Table 4. It is evident that the Chi-square, which was calculated to show whether the judges could significantly distinguish between the treated wines and the control, did not yield satisfactory results (150 mg sorbic acid/ℓ could, while 200 mg sorbic acid/ℓ could not be distinguished from the control by the judges).

A second calculation was therefore carried out as described by Salo (1970), where the percentage, above chance, of the correct evaluations were calculated. A graphical presentation in which the above values (converted to probit values) were plotted against the log of the sorbic acid concentrations is shown in Figure 2. A

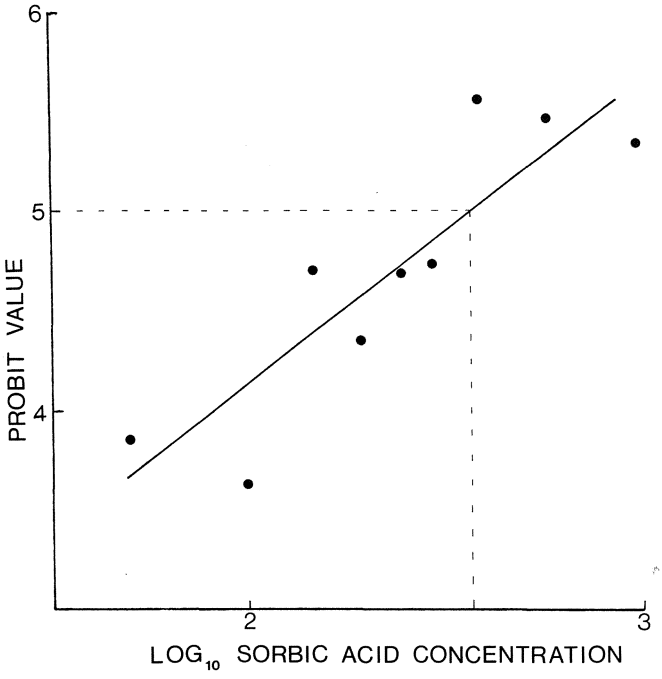

FIG. 2
Relationship between probit number and log of the sorbic acid concentration.

regression line was calculated, and the threshold value determined at a probit value of 5 (50% probability). The threshold thus obtained was 389 mg sorbic acid/ℓ. According to Salo (1970) judges were only able at this concentration to distinguish sorbic acid from the control at a level of 50% above chance. These results were further verified by the conditions stipulated by Blom (1955) for triangle tasting.

He maintained that if the number of evaluations are between 30 and 39 a minimum of 50% correct answers must be obtained to keep the first degree error (α) at 5% and the second degree error (β) at 5% to 10%. In this experiment either 32 or 36 replications per treatment were carried out, and according to Table 4 the threshold lies between 300 and 400 mg sorbic acid/ℓ.

CONCLUSIONS

Sorbic acid is an effective preservative against yeasts when applied at a concentration of 200 mg/ℓ in combination with 100 mg SO_2/ℓ. Special precautions must be taken when using *Saccharomyces bayanus* as a pure culture as this strain is resistant to sorbic acid. It should be kept in mind that sorbic acid is not lethal to yeast cells but only inhibits them. A large concentration of yeast cells is more liable to metabolize sorbic acid as a fatty acid than a small concentration. It is therefore imperative that semi-sweet wine be filtered as sterile as possible when employing sorbic acid as a preservative.

It would be preferable not to preserve sweet reserve with sorbic acid because of the danger of a bacterial infection causing the wine with which it was sweetened to develop the geranium odour. This has been shown to be easily detected by a panel of judges and also to be detrimental to wine quality.

The threshold of sorbic acid is between 300 and 400 mg/ℓ, which is 1,5 to 2 times the prescribed amount for inhibition. Sorbic acid is therefore capable of preserving wines against infection by yeast without necessarily sacrificing wine quality.

LITERATURE CITED

AMERINE, M. A. & JOSLYN, M. A., 1967. Table wines. 2nd. ed. Berkeley and Los Angeles: University of California Press. pp. 997.

AMERINE, M. A., PANGBORN, R. & ROESSLER, E. B., 1965. Principles of sensory evaluation of food. Food Science and Technology: A series of Monographs. New York: Academic Press.

AUERBACH, R. C., 1959. Sorbic acid as a preservative agent in wine. *Wines and Vines* **40** (8), 26–28.

BELL, T. A., ETCHELLS, J. L. & BORG, A. F., 1959. Influence of sorbic acid on the growth of certain species of bacteria, yeasts and filamentous fungi. *Journal of Bacteriology* **77**, 573–580.

BLOM, G., 1955. How many taste testers. *Wallerstein Laboratory Communications* **18**, 173–178.

BOUTHILET, R. J., 1962. The chemistry of sorbic acid with special reference to the wine industry. *Wines and Vines* **43** (10), 27–28.

CROWELL, E. A. & GUYMON, J. F., 1975. Wine constituents arising from sorbic acid addition, and identification of 2-ethoxyhexa—3,5-dien as source of geranium-like off odour. *Am. J. Enol. Vitic.* **26**, 97–102.

JAKOB, L., 1973. Überlegungen zur Anwendung von Sorbinsäure zur Haltbarmachung von Flaschenweinen. *Allg. Dtsch. Weinfachztg.* **109**, 360–361.

LÜCK, E., 1970. Technologie Band III: Sorbinsäure. Hamburg: B. Behr's Verlag GmbH.

LÜCK, E. & NUE, H., 1965. Verhütung des Nachgärens von restsüssen Weinen durch Sorbinsäure. *Z. Lebensmittelunters. u.-Forsch.* **126**, 325–335.

O'ROURKE, C. E. & WEAVER, E. A., 1962. Sorbic acid: One key to quality in wines. *Wines and Vines* **43** (5), 28–30.

OUGH, C. S. & INGRAHAM, J. L., 1960. Use of sorbic acid and sulphur dioxide in sweet table wines. *Am. J. Enol. Vitic.* **11**, 117–122.

PEYNAUD, E., 1963. Emploi de l'acide sorbique dans la conservation des vins. *Annls Technol. agric.* **12**, 99–114.

POSTEL, W. & DRAWERT, F., 1970. Über den Geschmacksschwellenwert der Sorbinsäure bei deutschen Weissweinen. *Z. Lebensmittelunters. u.-Forsch.* **144,** 245–252.

POSTEL, W. & LÜCK, E., 1970. Die verwendung der Sorbinsäure bei der Weinbereitung. *Wein-Wiss.* **25,** 20–32.

SALO, P., 1970. Determining the odour thresholds for some compounds in alcoholic beverages. *Journal of Food Science* **35,** 95–99.

TERCELJ, D. & ADAMIC, J., 1965. Biologische Stabilisierung süsser Weine mit Sorbinsäure und Pyrokohlensäurediäthylester (PKE). Mitteilungen. *Rebe und Wein* **15,** 279–290.

VAN ZYL, J. A., 1962. The microbiology of South African Winemaking. Part II. The preservation of musts and wines with pyrocarbonic acid diethyl ester. *S. Afr. J. Agric. Sci.* **5,** 293–304.

WÜRDIG, G. & KULLMAN, H., 1971. Über die Empfindlichkeit von in abgefüllen Weiner gefunden Hefen gegenüber Sorbinsäure. *Allg. Dtsch. Weinfachztg.* **107,** 1011–1012.