Influence of Wine Composition on the Heat Resistance of Potential Spoilage Organisms

D. F. SPLITSTOESSER,* LAURA LEE LIENK, MARTHA WILKISON, AND J. R. STAMER

New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456

Received for publication 23 May 1975

Pasteurization studies were conducted on 29 yeasts and five lactic acid bacteria. In general, the yeasts were more heat resistant in wine than were the bacteria. The one exception was a strain of Lactobacillus fructivorans that gave an average D-value of 1.7 min at 60 C. Alcohol was the wine constituent that had the greatest effect on resistance; D-values for all test species were inversely related to the ethanol concentration. The response of organisms to other factors such as pH, sugar, and sulfur dioxide varied with the species.

Bottled wines that have been sweetened or which contain some residual fermentable carbohydrate are susceptible to spoilage by yeasts and lactic acid bacteria (7, 8, 12). Multiplication of these organisms often results in off-flavors as well as objectionable turbidity and sediment (1).

One of the methods used to prevent spoilage is pasteurization. Although a very old procedure, surprisingly few quantitative data are available regarding the conditions necessary to impart biological stability to wine. As a result, the heat processes that are presently used differ markedly in severity. Some wineries, for example, flash pasteurize at 80 C or higher; others use hot bottling procedures in which the wine is filled into bottles at temperatures ranging from 45 to over 70 C.

In addition to the need for minimum process data, impetus for this research was provided by the so-called "mod" or "pop" wines that have been introduced in recent years. It seemed that the different composition of these wines might have a significant effect on their pasteurization requirements.

MATERIALS AND METHODS

Media. Yeasts were propagated in 10° Brix Thompson seedless grape juice. Potato dextrose agar, pH 5.6, was the plating medium used for viable counts.

The lactic acid bacteria were grown in tryptone-glucose-yeast extract-salts broth (11) supplemented with 10% tomato juice serum and 0.5% fructose. (The latter ingredients were needed for growth of Leuconostoc oenos and Lactobacillus fructivorans.) For plating, 2% agar was added to the medium.

Heat resistance. Heating was conducted in flame-sealed capillary tubes (4, 10). In a typical trial, stationary-phase cells were centrifuged from the culture medium, then suspended in the test solution, usually a wine. A Hamilton syringe with a 500-μl capacity was used to transfer 20-μl volumes of the cell suspension to capillary tubes (1.6 by 50 mm); a Hamilton repeating dispenser facilitated this operation. The tubes, held in Thomas pinch clamps, were submerged in a constant-temperature water bath. After heating for a given time period, the capillaries were cooled in 95% ethanol. (A dual function of the alcohol was to destroy many of the microorganisms that might be present on the surface of the tubes. Ethanol-resistant organisms apparently were eliminated by subsequent dilutions; contamination was not a problem.) Five tubes from a given treatment were pooled in a dilution bottle containing 5 ml of 0.1% peptone solution. After crushing the tubes with a sterile glass rod, appropriate decimal dilutions for plating were made in 0.1% peptone solution. The yeasts were incubated at 28 C, and the lactic acid bacteria were incubated at 32 C. The incubation period ranged from 2 to 7 days, depending upon the culture.

An experimental wine prepared in our pilot plant from Niagara grapes served as our model in most experiments. This 1971 vintage wine contained 12.1% ethanol, total acid of 0.82% (expressed as tartaric), 20 mg of tannin per 100 ml, less than 5 mg of free SO2 per liter, and less than 0.1% reducing sugar. Its pH was 3.2. The methods for these analyses are described by Amerine and Ough (2).

Modifications of the wine were made in such a way as to minimize changes in the concentration of the various constituents. Thus solutions used to adjust pH and the concentration of glucose were prepared by dissolving the reagents directly into samples of the wine.

RESULTS AND DISCUSSION

Species resistance. During the course of this study, the resistance of 29 yeasts heated in the Niagara wine was determined. Many of the cultures were strains of Saccharomyces cerevisiae used in commercial wine making; others
were flor yeasts, species of *Saccharomyces* involved in the sherry fermentation. The average survival figures are summarized in a histogram (Fig. 1). It can be seen that most of the yeasts yielded D-values of under 0.6 min when heated at 49 C. The most heat-resistant yeast, a strain of *S. cerevisiae*, gave an average D-value in five experiments of 1.6 min. The single nonwine yeast examined, *Candida utilis* NRRL Y-900, gave a D-value of under 0.1 min at 49 C.

The initial studies with lactic acid bacteria were conducted with the first four species listed in Table 1. Because they were rapidly killed at 45 C, a temperature 4° lower than that used for the yeasts, it first was concluded that the lactic acid bacteria as a group possessed considerably less resistance than yeasts. *L. oenos* ML 34, an important malo-lactic strain (6), was especially sensitive; 43 C was a lethal temperature for it. These results are in agreement with those of Murdock et al. (5) whose studies with orange juice also indicated leuconostocs to be most heat sensitive, followed by the lactobacili and then the yeasts. Our hypothesis about the lactics had to be revised, however, when we later isolated a strain of *L. fructivorans* from a spoilage outbreak involving a premixed Bloody Mary cocktail. This organism was considerably more heat resistant than any of the yeasts: it was difficult to detect destruction of it at temperatures below 58 C.

*L. fructivorans* is relatively alcohol tolerant and our isolate grew rapidly in broth containing over 12% by volume. This raised the question as to whether the low heat resistance of the other bacteria was related to an alcohol sensitivity. Perhaps the shock of being exposed to alcohol for the first time was partly responsible for their death. To test this hypothesis, an attempt was made to select for more heat-resistant strains by growing the lactic acid bacteria in broth containing ethanol. As the results with *Lactobacillus plantarum* illustrate (Table 2), we were not successful. Ethanol-grown cells did not exhibit significantly greater heat resistance than those propagated in the standard medium.

Ethanol. Of the different wine constituents that were studied, alcohol had the greatest effect on heat resistance (Table 3). As illustrated here, the resistance of both lactics and yeasts was reduced. The flor yeast was affected to a greater extent than *L. fructivorans*: the D-value for it was over 50-fold greater in the absence of ethanol than when heated in the presence of 12%; the lactic exhibited a difference of less than fourfold.

Thermal death time curves have been plotted

**TABLE 1.** Heat resistance of lactic acid bacteria in Niagara wine (12.1% ethanol)

| Organism          | Temp (°C) | D-value (min) |
|-------------------|-----------|---------------|
| *Leuconostoc oenos* ML-34 | 43        | 0.33          |
| *L. mesenteroides* C-33       | 45        | 0.12          |
| *Pediococcus cerevisiae* E-66  | 45        | 0.26          |
| *Lactobacillus plantarum* B-246 | 45     | 0.35          |
| *L. fructivorans* WBM            | 60        | 1.7           |

**TABLE 2. Influence of growth medium on the heat resistance of *L. plantarum***

| Expt* | Growth medium                   | D-value (min at 45 C) |
|-------|----------------------------------|-----------------------|
| 182   | Standard                         | 11                    |
|       | Standard with 8.4% ethanol       | 9.1                   |
| 194   | Standard                         | 0.26                  |
|       | Standard with 13.8% ethanol      | 0.42                  |

* Experiment 182, heated in 8% ethanol wine; and experiment 194, heated in 12.1% ethanol.

**TABLE 3. Heat resistance in 5 C Brix Concord grape juice containing varying concentrations of ethanol***

| Ethanol (vol %) | D-value (min) |
|-----------------|---------------|
| Flor yeast Y-2  | *L. fructivorans* |
| 0               | 16            | 10          |
| 4               | 9.5           | 8           |
| 6               | 4.6           | 8           |
| 8               | 2.0           |             |
| 10              | 0.82          |             |
| 12              | 0.30          | 2.6         |
for our most resistant yeast and lactobacillus strains when heated in wine containing two levels of ethanol, 8 and 12% (Fig. 2). The parallel slopes show that these differences in concentration did not affect the organism’s response to a change in temperature. In other words, the z-values for these organisms were not affected by alcohol.

Studies by other workers also have shown ethanol to reduce the heat resistance of yeasts (3, 4, 9). Schanderl (9) observed that differences in alcohol concentration of only 3% had a marked effect on survival, which is in agreement with our findings. Carroll and Lopez (3), on the other hand, obtained little difference in thermal destruction of S. cerevisiae when heated in buffers containing 6 and 10% ethanol.

pH. The effect of pH on heat resistance was determined by adjusting the reaction of Niagara wine with NaOH or tartaric acid. The study was limited to the pH range of 3 to 4 since these are common values for wine. The results (Table 4) indicated that individual species respond differently to hydrogen ion concentration. Thus the flor yeast gave comparable survivals over this range while L. fructivorans showed an increase in D-value as the pH was raised. Data obtained with L. plantarum B246 (not shown) indicated comparable survivals between pH 3.4 to 4.0 and significantly lower figures at pH 3.0 and 3.2.

Sugar. Glucose is another common wine constituent to which individual species responded differently. Thus the addition of 10% to Niagara wine increased the D-value of L. fructivorans almost threefold but afforded only slight protection to L. plantarum and the flor yeast (Table 5). These results suggest, of course, that the pasteurization requirements for a dessert or a pop wine might be somewhat greater than for a dry, table wine.

Sulfur dioxide. In the studies with SO2, freshly prepared solutions of potassium metabisulfite were mixed with the wine just prior to filling the capillary tubes. It is assumed, however, that the actual amount of free SO2 was lower than the calculated value since some would react with aldehydes and other wine constituents. The results (Table 6) again showed a variable response depending upon the organism: SO2 appreciably reduced the resistance of L. fructivorans, whereas an effect on the yeasts could not be detected. We were not too sur-

**Table 4. Effect of pH on thermal resistance in Niagara wine (12.1% ethanol)**

| pH   | D-value (min) |
|------|---------------|
|      | Flor yeast Y-2 | L. fructivorans |
|      | (49 C)         | (60 C)          |
| 3.0  | 0.75           | 0.68            |
| 3.2  | 0.70           | 1.7             |
| 3.4  | 0.88           | 2.9             |
| 3.6  | 0.95           | 3.2             |
| 3.8  | 0.74           | 6.4             |
| 4.0  | 0.84           | 7.2             |

**Table 5. Influence of glucose on heat resistance in Niagara wine (12.1% ethanol)**

| % Glucose | D-value (min) |
|-----------|---------------|
|           | Flor yeast Y-2 | L. fructivorans |
|           | (49 C)         | (60 C)          |
| 10        | 0.83           | 0.39            | 4.8 |
| 5         | 0.83           | 0.31            | 3.1 |
| <1        | 0.79           | 0.10            | 1.8 |

**Table 6. Influence of SO2 on heat resistance in Niagara wine (11% ethanol)**

| Added SO2 (mg/liter) | S. cerevisiae | Flor yeast Y-2 | L. fructivorans |
|----------------------|---------------|----------------|----------------|
| 0                    | 1.2           | 0.89           | 4.4            |
| 25                   | 1.2           | 1.1            | 1.2            |
| 50                   | 1.3           | 0.81           | 0.70           |

**Fig. 2. Thermal death time curves of S. cerevisiae GS1 and L. fructivorans WBM when heated in Niagara wine containing 8 and 12% ethanol by volume.**
prised by these results since yeasts generally are more resistant to this germicide than are bacteria. The unheated *L. fructivorans* controls showed no decrease in viable counts when suspended in wine containing 50 mg of SO₂ per liter.

**Other constituents.** In addition to the more obvious variables already described, it seemed that wines might contain other substances that would influence heat resistance of potential spoilage organisms. For example, certain tannins or other polyphenolic compounds, natural constituents of the grape, might either protect organisms or cause them to be more heat sensitive.

One approach in the search for other factors was to compare heat resistance in various concentrations of the Niagara wine. In these studies the wine was diluted with a solution of 12.1% aqueous ethanol so that the concentration of all constituents except alcohol would be decreased. The results (Table 7) suggest that a small amount of protection was provided by some ingredient(s) of the wine. Thus the survivals of *S. cerevisiae* and *L. fructivorans*, averages of two experiments, were slightly lower when heated in the straight aqueous ethanol solution.

In other trials resistance was compared in wines fermented from different grape varieties. To eliminate the influence of ethanol, after determining the amount present, small quantities of water were added so that all of the wines ended up with the same concentration of ethanol, 11%. Again the results failed to reveal the presence of other wine constituents that had more than a modest effect on heat resistance (Table 8). It is suspected that the Delaware wine actually permitted somewhat higher survivals since the averages of two trials agreed very well. So far, however, it has not been possible to relate increased resistance to some intrinsic property of the Delaware wine such as pH, total acid, or free SO₂.

It is concluded from these studies that potential spoilage organisms often possess little resistance when heated in a traditional table wine and that many wines may receive a more severe pasteurization than is actually required. The data also show that some formula modifications would increase the process requirements, in particular, the lowering of the alcohol content, the raising of the pH, and the addition of sugar.

The single organism encountered in this study that possessed relatively high heat resistance, *L. fructivorans*, would not present a spoilage problem for many wines because we have found that it will not initiate growth in the tryptone-glucose-yeast extract salts broth when acidified to pH 3.8. Most wines have a pH lower than this. It is also possible that *L. fructivorans* would be inhibited by the levels of free SO₂ that generally are present in commercial wines.

**Table 8. Heat resistance in different red and white wines (11% ethanol)**

| Variety | pH | *L. fructivorans* (60 °C) | *Flor yeast* Y-2 (49 °C) |
|---------|----|--------------------------|--------------------------|
| Red     |    |                          |                          |
| Foch    | 3.7| 2.0                      | 0.87                     |
| Chelois | 3.5| 1.8                      | 1.5                      |
| Concord | 3.3| 1.0                      | 0.97                     |
| White   |    |                          |                          |
| Aurora  | 3.4| 1.0                      | 1.1                      |
| Niagara | 3.2| 1.6                      | 1.0                      |
| Catawba | 3.0| 1.0                      | 1.3                      |
| Delaware| 3.3| 2.3                      | 2.2                      |

**Table 7. Heat resistance in Niagara wine diluted with 12.1% ethanol**

| % Wine | **D-value (min)** | **S. cerevisiae** GSI (49 °C) | **L. fructivorans** (60 °C) |
|--------|-------------------|-------------------------------|-----------------------------|
|        | **Flor yeast Y-2** (49 °C) |                               |                             |
| 100    | 0.83              | 1.9                           | 2.9                         |
| 50     | 0.60              | 2.2                           | 2.1                         |
| 25     | 0.60              | 2.0                           | 1.3                         |
| 0      | 0.81              | 1.4                           | 1.2                         |

**LITERATURE CITED**

1. Amerine, M. A., and M. A. Joselyn. 1970. Table wines: the technology of their production. University of California Press, Berkeley.
2. Amerine, M. A., and C. S. Ough. 1974. Wine and must analysis. John Wiley & Sons, New York.
3. Carroll, D. E., and A. Lopez. 1969. Lethality in radiofrequency energy upon microorganisms in liquid, buffered, and alcoholic food systems. J. Food Sci. 34:320-324.
4. Jacob, F. C., T. E. Archer, and J. G. B. Castor. 1964. Thermal death time of yeast. Am. J. Enol. Viticult. 15:69-74.
5. Murdock, D. I., V. S. Troy, and J. F. Folinazoo. 1953. Thermal resistance of lactic acid bacteria and yeast in orange juice and concentrate. Food Res. 18:55-59.
6. Pilone, G. J., and R. E. Kunkee. 1972. Characterization and energetics of *Leuconostoc oenos* ML 34. Am. J. Enol. Viticult. 23:81-70.
7. Rankine, B. C., and D. A. Bridson. 1971. Bacterial spoilage in dry red wine and its relationship to malo-lactic fermentation. Australian Wine, Brewing Spirit Rev. 98:44-50.
8. Rankine, B. C., and D. A. Pilone. 1973. *Saccharomyces* buizli, a resistant yeast causing spoilage of bottled table wine. Am. J. Enol. Viticult. 24:55-58.
9. Schanderl, H. 1959. Mikrobiologie des mostes und
weins, 2nd ed. Eugen Ulmer, Stuttgart, Germany.
10. Stern, J. A., and B. E. Proctor. 1954. A micro-method and apparatus for the multiple determination of the rates of destruction of bacteria and bacterial spores subjected to heat. Food Technol. 8:139–143.
11. Stamer, J. R., M. N. Albury, and C. S. Pederson. 1964. Substitution of manganese for tomato juice in the cultivation of lactic acid bacteria. Appl. Microbiol. 12:165–168.
12. Vaughn, R. H. 1955. Bacterial spoilage of wines with special reference to California conditions. Adv. Food Res. 6:67–108.