Gas-Volume Measurement System for Evaluating Effectiveness of Antimicrobial Compounds

A. D. KING, JR., W. L. STANLEY, AND H. R. BOLIN

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710

Received for publication 3 April 1972

Yeast spoilage was followed by measuring gas volume produced inside a sealed bag of inoculated fruit. Volume of gas produced correlates with plate counts.

Osmophilic fermentative yeasts, the major cause of spoilage in fruit juices and dried fruits, produce gas during the logarithmic growth phase. This phenomenon has been used in the rapid, simple, nondestructive method described below in evaluating the effectiveness of chemical antimicrobial agents for fruit products. Plate-counting the number of organisms present was too cumbersome and was not convenient for analyzing large numbers of samples. Gross examination of the samples for visible signs of spoilage was not satisfactory because it was too insensitive. Our method, though indirect, was found to correlate reasonably well with actual plate counts and to be reliable in determining onset of spoilage. The procedure used is to put the treated, inoculated fruit product (juice or solid) into a polyester bag and heat-seal it after collapsing the bag to remove as much air as possible. In some applications, the inoculated fruit is sealed in the bag and the treatment is added later. For example, a gaseous preservative is added after the bag is sealed by puncturing the bag near the corner with a syringe needle and then resealing it below the puncture. Obviously, more elaborate gas-volume measuring techniques (such as the Warburg respirometer) are available. The advantage of this method is its simplicity.

An initial volume measurement is made by submerging the bag in water and measuring the displaced volume (Fig. 1). Further volume measurements are made at appropriate intervals during the period of incubation. The volume data are then plotted (Fig. 2). The logarithmic portion of the curve is determined by extrapolation to the base volume to establish the onset of logarithmic growth. The length of time elapsed until the inflection point was used to gauge the amount of preserving effect of the treatment. This system greatly reduces the necessity of frequent measurements.

The important element used in this technique is the bag. We employ Scotchpak heat-sealable polyester film bags. These bags have a low permeability to gases and water vapor so that any volume measured is equivalent to the gas produced within the bag. Appropriate controls indicate that the gas is a result of microbial action. Commonly we used 6- by 11-inch (ca. 15.2 by 27.9 cm) bags which have a maximum volume of about 1 liter and contain 100 g or 100 ml of product. For smaller samples we used smaller bags.

Figure 2 shows the influence of 200 µg and 500 µg of potassium sorbate per ml, each added to 100 ml of reconstituted white grape juice inoculated with Saccharomyces cerevisiae, as compared with an untreated control. The potassium sorbate delays the onset of logarithmic growth and thus increases the storage life. Increasing levels of potassium sorbate provide longer preservation (Fig. 2).

The viable yeast count of the grape juice in the bags is also shown (Fig. 2). The viable count correlates best with bag volume at the longer incubation times. For the 500 µg of sorbate/ml treatment the correlation coefficient is 0.99 for seven observations, and for 200 µg it is 0.92 for six observations. The correlation between bag volume and viable count for all the data in Figure 2 is 0.87. All these correlations are significant statistically (P < 0.01). The correlation for the four observations in the control is 0.75, which is not significant.
FIG. 1. Gas-measuring apparatus consisting of the flexible plastic bag containing the inoculated material and the antimicrobial treatment, the container holding water, and the measuring cylinder for collecting displaced water equivalent to bag volume.

The lag between viable count and bag volume is due, in part, to the amount of carbon dioxide required to saturate the product. With 100 ml of juice, this calculated volume is about 70 ml of carbon dioxide (1). Another factor is the lag between growth and gas production in microbial growth.

The reproducibility of the measurements we have observed is also shown in Figure 2. The first volume measurements of nine bags for the sorbate-treated juice (500 μg/ml), before the volume inflection, ranged from 123 to 130 ml over a period of 73 hr. This is less than 6% variation in measurement when no control was made over pressure or temperature. At higher bag volumes, the repeatability of bag measurements is the same, so the percentage of variation is less.

This technique has also been used to examine the spoilage of inoculated and rehydrated dried fruit. In some cases where spoilage is very slow and gas volume is not noticeable for a month or longer, it is possible to observe yeast growth before a definite increase in bag volume is detected. With prolonged incubation, the slow transfer of gases through the plastic bag wall may influence the volume of gas and thus would make this test inappropriate.

LITERATURE CITED
1. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1957. Manometric & biochemical techniques. 3rd ed., Burgess, Minneapolis, Minn.