The Australian Wine Research Institute

Fact Sheet
CULTURE COLLECTION

Vinegar production – inoculation of a base wine with live acetic acid bacteria

Vinegar production

This fact sheet describes procedures for the inoculation of base wine with acetic acid bacteria to make vinegar.

For further background and historical information on vinegar production, William T. Brannt's book *A practical treatise on the manufacture of vinegar and acetates, cider and fruit wines, and the preservation of fruits and vegetables, meat, fish and eggs* (Brannt 1914) contains descriptions of equipment and techniques used in vinegar manufacturing.

Cultures

The AWRI has many cultures of acetic acid bacteria in its Wine Microorganism Culture Collection, which can be provided live on an agar slope. These cultures should be stored sealed at temperatures between 2 and 4°C but not frozen. If a slope is more than three months old, please discard it and obtain a replacement culture, or revitalise it by subculturing on GYC agar.

Base wine characteristics

Base wines for vinegar production should be analysed for alcohol, titratable acidity (TA) and sulfur dioxide (SO_2) and be adjusted where necessary to ensure quality. Ideal base wine parameters for vinegar production are approximately 8-10% (v/v) alcohol, pH above 3 and free SO_2 20 mg/L or less.

Starter culture preparation

A. Prepare the starter culture

Prepare 1 L of a wine/chlorine-free water mixture at 5% (v/v) final alcohol, in a 2 L bottle. Wash the contents from the agar slope with preservative-free grape juice (approximately 5 mL) using a sterile Pasteur pipette (or similar) and pour this into the wine mixture. Cover the bottle with a clean open-weave cloth to allow air ingress. Keep the wine mixture at approximately 25-30°C until it has grown a film; this usually takes 1-2 weeks.

B. Scale up the starter culture

Prepare a further 10 L of a wine/chlorine-free water mixture (8% v/v final alcohol) in a 20 L container. Add the 1 L of starter culture to this wine mixture and keep at approximately 25-30°C until it has grown a film; this usually takes 2-3 weeks.

This produces approximately 11 L of scaled-up starter culture. If a greater starter culture volume is required, additional scale-up steps can be performed. Note each subsequent scale-up stage requires a 20-25% volume of the culture to be added to a larger wine/water mixture at each stage. This usually takes an additional 1-2 weeks. For best results the cultures should be used while still young, preferably during the first three weeks after complete films have been formed.

C. Addition of the culture to wine

Pour the scaled-up culture volume carefully into a tank or barrel containing a wine/chlorine-free water mixture (8% v/v final alcohol). For barrels, only fill the barrel no more than two thirds full (150 L for a 225 L barrique or 200 L for a 300 L hogshead) to allow for sufficient oxygen contact. A 25% culture inoculation volume should be added to 75% wine/chlorine-free water mixture (8% v/v final alcohol) to achieve a final alcohol concentration of approximately 6% (v/v) in barrel. Thus between 40 and 50 L of culture inoculation volume is required for an ullaged barrique or hogshead.

The temperature of the wine should be held constant between 20 and 30°C where possible.

Equipment

Preparation of starter cultures should be performed in clean glass or clear plastic containers. This allows the culture to be easily monitored. Larger volumes can be transferred to barrel and sampled to determine activity.

The main methods of vinegar production in the wine industry are in stainless steel tanks or via the Orleans method in oak barrel. The main constraints with these systems are temperature control and oxygen availability.

In stainless steel tanks oxygen can be obtained via air pumped into the tank and dispersed with a sinter, and, if feasible, temperature control can be applied year-round to achieve a constant temperature between 20 and 30°C. Air lines should ideally contain an activated carbon filter to remove environmental odours and a coarse air filter to remove dust. Microbial sterility is not essential for vinegar fermentation once an active culture is growing in the final wine.

Oak barrel fermentation can be more difficult to handle but may give a different desirable flavour to the vinegar. Barrels must have air holes drilled into the barrel heads (both ends) to allow enough air to enter the barrel (Figure 1). Covering the bung hole and air holes with an open weave cloth or mesh will allow enough air to complete acetification and prevent pests entering the barrel. With limited oxygen there is potential for high ethyl acetate levels which are unpleasant and hard to remove; hence, it is important not to overfill the barrel. Fill barrels no more than two-thirds full. The temperature of the wine should be held constant between 20°C and 30°C; however, if temperature

control is not possible, the vinegar culture will become dormant and slow in cooler months. In this situation, it is best to have inoculated all vessels before spring, to take advantage of the warmer ambient temperatures, where the majority of the vinegar fermentation will occur.

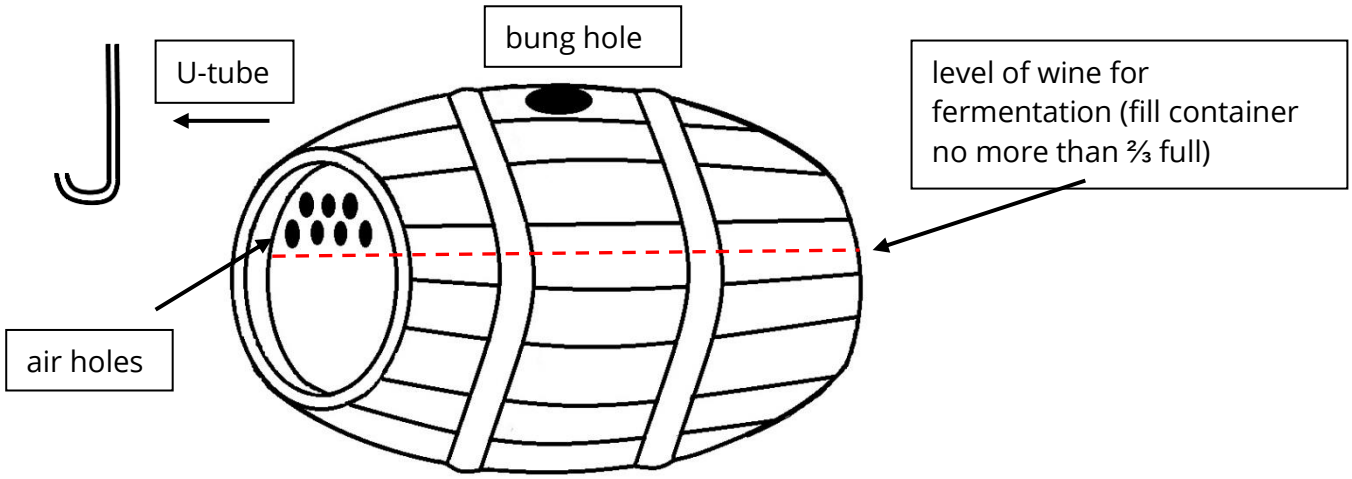

Figure 1. Wine barrel with air holes drilled into the heads. All movement of wines into a barrel should be via pumping through a U-tube placed below the surface of the wine. This is to prevent disturbance of the mother culture that forms on the surface of the wine.

Acknowledgement

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Reference and further reading

Brannt, W.T. (ed). 1914. A practical treatise on the manufacture of vinegar and acetates, cider and fruit wines, and the preservation of fruits and vegetables, meat, fish and eggs. Philadelphia: Henry Carey Baird & Co.

LeFevbe, E. 1924. Making vinegar in the home and the farm. *Agriculture Farmers Bulletin* no. 1424.

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