OIV-MA-AS4-02A Detection of preservatives and fermentation inhibitors

Type IV method

1. Fermentability Test

1.1. Objective

To show without specifying their nature, the possible presence of one or several substances which act as fermentation inhibitors in wine.

1.2. Principle

The wine, whose free sulfur dioxide has been bound by addition of an aqueous solution of acetaldehyde, is brought to 10% (*v/v*) alcohol. Glucose is added in order for the sugar concentration to be between 20 and 50 g/L in the nutrient solutions.

After inoculation with a yeast strain resistant to alcohol, the fermentation is followed by weighing the quantity of carbon dioxide released.

The fermentation rate is compared to that of an authentic natural wine similar in make up to the wine analyzed, and also to that of the test wine whose pH has been adjusted to 6 (the majority of the mineral and organic acids are not active in fermentation at this pH). These two reference wines are inoculated in the same manner as the test wine.

1.3. Apparatus

90 mL flask sealed with a rubber stopper with a hole into which is placed a narrow tube tapered at the uppermost portion.

1.4. Reagents and media

1.4.1. Aqueous acetaldehyde solution:

Solution prepared from acetaldehyde obtained by distillation of metaldehyde or paraldehyde, in the presence of sulfuric acid, and standardized by the method using sodium sulfite. Adjust the concentration of the solution to 6.9 g/L.

1 mL of this solution fixes 10 mg of sulfur dioxide.

1.4.2. Nutrient Solutions:

Ammonium Sulfate, $NH_4)_2SO_4$:25 g/L

Asparagine 20 g/L

These solutions must be stored in the refrigerator.

1.4.3. Culture Medium:

Solid medium: malt agar.

Powdered malt 3 g
Glucose 10 g
Pancreatic peptone 5 g
Powdered yeast extract 3 g
Agar 20 g
Water 1 L
pH 6
Sterilize for 20 min. at 118 °C.
This mixture exists in a commercial prepared form.
Liquid medium (an option):

- Divide the grape juice containing 170 to 200 g/L of sugar, in tubes stoppered with cotton, at a rate of 10 mL per tube; sterilize in a water bath at 100 °C for 15 min.
- Liquid malt: same medium as the solid medium, but without agar.

4. Culture and maintenance of the *Saccharomyces bayanus* strain and preparation of the yeast.

a) Culture and maintenance of the strain on solid medium: From a collection strain, inoculate in lines (streak) onto tubes of solid medium. These tubes are put in an incubator at 25°C until the culture is very visible (about 3 days); the tubes can be stored in the refrigerator. This is sufficient for 6 months.

b) Preparation of the yeast:

One of the tubes of the liquid medium is inoculated in accordance with proper microbiological techniques from the strain cultivated on solid medium; after growth (24 to 48 h), repeat 2 times successively into the same medium enriched with 10% alcohol (*v/v*), to acclimate the strain.

The second culture when actively fermenting will contain about 50 million yeast per milliliter. This culture will serve to inoculate the wine to be studied. Perform a count and inoculate at a rate of 10^5 yeast/mL.

1.5. *Procedure*

Preparation of the wine:

100 mL of wine is treated with the necessary quantity of acetaldehyde calculated in accordance with the amount of free sulfur dioxide (44 mg of aldehyde binds 64 mg of sulfur dioxide). Wait 24 hours and check that the wine contains less 20 mg free sulfur

dioxide per liter.

If the alcoholic strength is greater than 10% (*v/v*), the wine should be diluted with one of the solutions of glucose and water in amounts calculated to result in a sugar concentration between 20 and 50 g/L, and to reduce the strength to about 10% (*v/v*). For wines containing less than 10% vol., add solid glucose to bring without dilution the amount of sugar between these values, so the fermentation rate is not altered by the amount of sugar.

Fermentability test:

In a 90 mL flask, place 60 mL of wine prepared as above, 2.4 mL of ammonium sulfate solution and 2.4 mL of asparagine solution. Inoculate with 3 drops of a 3 day old culture of *Saccharomyces bayanus*, to obtain an initial population close to 10^5 yeast/mL. Install the stopper with the pointed tube, weigh the assembly to the nearest 10 mg and place in an oven at 25°C.

Weigh daily for at least 8 days.

Run each time concurrently, a wine of comparable make up and origin which does not contain any preservative along with the test wine which has been adjusted to pH 6.

A flask of non-inoculated wine indicates loss by evaporation.

1.6. *Interpretation*

In most cases, the fermentation begins within 48 hours and the daily liberation of gas is greatest between the 3rd and the 5th day.

One can confirm the presence of a fermentation inhibitor only in the following conditions:

a) If the fermentation does not begin or is delayed at least 2 days compared to one of the 2 controls. When the delay is brief, it is difficult to ascertain the presence because there may be "false positive" results, since certain natural sweet wines sometimes behave as if they contained traces of inhibitors (in particular sweet wines made from grapes having noble rot).

b) If the maximum daily release has not taken place between the 3rd and 5th day, but after the 7th day, this release must be greater than or equal to 50 mg for 60 mL of wine.

Plotting the fermentation curve and the curve of daily release of CO_2 as a function of time can facilitate the interpretation in a difficult case.