OIV-MA-AS5-01 Differentiation of fortified musts and sweet fortified wines

Type IV method

1. Principle of the method

1.1. Method of screening

The product definitions given by the O.I.V. (International Code of Enological Practices) imposes for fortified wines, a minimum of 4% acquired alcohol derived naturally by fermentation; and allows, for fortified musts, a maximum of 1% acquired alcohol. Consequently, these products may be differentiated by identifying their fermentation by-products via gas chromatography.

This method is applicable only if, as the definition anticipates, the alcohol used for production of the fortified musts is neutral.

1.2. Scientific investigation of citramalic acid by thin layer chromatography.

The presence of citramalic acid characterizes sweet fortified wines. Its identification is carried out by thin layer chromatography after separation of the sugars with the use of an ion exchange column.

2. Method of screening

2.1. Apparatus

Gas chromatograph with:

- Flame ionization detector,
- 3 m stainless steel column, 2 mm interior diameter,

Stationary phase: Carbowax 20 M 20%,

Support: Chromosorb W 60/80 mesh.

Chromatography conditions:

temperatures:

- injector: 210°C
- detector: 250°C

oven: isothermal at 70°C for 6 minutes; then programmed at 6°C/minute; upper temperature limit: 170°C

Other types of columns can be used.

The procedure described below is given as an example.

2.2. Procedure

2.2.1. Sample preparation

Carry out a separation according to the following conditions: To 25 mL of sample (fortified must or sweet fortified wine) are added to 7 mL ethanol and 15 g of ammonium sulfate, $(NH_4)_2SO_4$ agitate. Allow to settle to obtain separation of the phases.

2.2.2. Chromatography

Inject 2 µL of the organic phase and carry out the chromatography in accordance with the conditions indicated above.

The chromatogram of the fortified wine is differentiated by the presence of the peaks of the secondary products of alcoholic fermentation.

3. **Investigation of citramalic acid by thin layer chromatography.**

3.1. Apparatus

3.1.1. Glass column about 300 mm in length and 10-11 mm interior diameter supplied with a flow regulator (stopcock)

3.1.2. Rotary vacuum evaporator

3.1.3. Oven at 100 °C

3.1.4. Chromatography developing chamber

3.1.5. Micrometric syringe or micropipette

3.2. Reagents

3.2.1. Formic acid solution, 4 M, containing 150.9 mL formic acid (ρ_{20} = 1.227 g/mL) per liter.

3.2.2. Plates for chromatography ready to use with a layer of cellulose powder (for example MN 300) (20 x 20 cm).

3.2.3. Solvent:

iso-Propyl alcohol containing 1 g/L bromophenol blue 5 vol.

Eucalyptol 5 vol.

Formic acid (ρ_{20}= 1.227 g/mL) 2 vol.

Saturate the solvent with water and allow to stand for 24 hours before use.

3.2.4. Standard solutions.

Prepare an aqueous solution of:

citramalic acid 0.25 g/L

Lactic acid 0.5 g/L

citric acid 0.5 g/L

Tartaric acid 1.0 g/L

malic acid 1.0 g/L

3.3. Procedure

3.3.1. Preparation of the ion exchange column.

See chapter on *Tartaric acid*, usual method in 3.3.1.

3.3.2. Isolation of the organic acid of citramalic acid

Proceed as indicated in the chapter *Tartaric acid*, usual method in 3.3.2. for the fixation of organic acids on the ion exchanger.

Then elute the fixed acids using the 4 M formic acid solution (100 mL), collecting the eluate in a 100 mL volumetric flask.

Concentrate the eluate dry in a rotary evaporator at 40°C recovering the residue with 1 mL of distilled water.

3.3.3. Chromatography

The cellulose plate must be activated by placing it in the oven at 100°C for 2 hours.

Deposit on the starting line of the cellulose plate in a band 2 cm wide, 10 µL of this solution as well as 10 µL of the standard solutions of citramalic acid and the other organic acids.

Place the plate in the chromatography bath, above the solvent, for 45 minutes.

Proceed with the development and let the solvent migrate to a height of 15 cm.

3.3.4. Development of the chromatogram

Maintain the plate at ambient temperature under an air current, until the formic acid of the solvent is eliminated. Yellow spots appear on a blue background, indicating the presence of the acids.

Detect the presence or absence of citramalic acid in the product analyzed by comparing the spots of this chromatogram to the spots of standard solutions of citramalic acid and the other organic acids.

Bibliography

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