Method OIV-MA-VI-10 : R2000
Type IV method

Determination of total ascorbic acid content in vinegars

(OENO 61/2000)

1. Introduction

In the wine vinegar industry, the technological use of ascorbic acid is regulated in the various producing and consuming countries. The application of this practice must be controlled and its presence quantified if possible.

2. Principle

Oxidization of ascorbic acid by iodine with transformation into dehydro ascorbic acid, precipitation of this acid by 2.4 - dinitrophenylhydrazine in the form of bis (2.4-dinitrophenylhydrazine). Separation by thin film chromatography, solubilization in acetic medium and colorimetric determination at 500 nm.

3. Reagents

3.1. Solution of metaphosphoric acid at approximately 30 g per 100 ml.

Weigh 30 g of vitreous previously titled metaphosphoric acid.

Wash rapidly while covering in water, then stir. Discard the washing water. Add to a 100 ml flask, dissolve with water, stir and bring up to the mark.

The solution will be kept for 1 week at most in the refrigerator.

3.2. Solution of metaphosphoric acid at 3% (v/v).

At the time of use, prepare a dilution of 3.1.

3.3. Metaphosphoric solution at 1% (v/v).

At the time of use, prepare a dilution of 3.1.

3.4. Polyamide suspension

Weigh 120 g of powdered polyamide for chromatography and add to a 250 ml conical flask. Add 60 ml of water, stir and leave in contact for 2 h (this quantity is enough for 4 determinations).

3.5. Thiourate.

3.6. Iodine solution 0.05 M.

3.7. Glacially cold acetic acid

3.8. Sulfuric acid (p20 = 1.84 g/ml).

3.9. Aceto sulfuric solution of 2.4 dinitrophenylhydrazine

Add to a 6 g flask, 2.4 dinitrophenylhydrazine and 50 ml of glacial acetic acid. A suspension will form. Add 50 ml of sulfuric acid to dissolve the 2.4 dinitrophenylhydrazine.

3.10. Ethyl acetate to which glacial acetic acid has been added (98+2 by volume).

3.11. Chloroform

3.12. Silicagel for chromatography

3.13. Soluble starch solution at 0.5 g/100 ml.

3.14. L-ascorbic acid standard solution

In a 100 ml calibrated flask, add 100 mg of L-ascorbic acid weighed to the nearest 0.1 mg. Dissolve with solution 3.3 and bring up to the gauge line.

3.15. Eluant

Ethyl acetate-chloroform-glacial acetic acid (50 : 60 : 5, v/v/v). Use only 12 h after preparation.

4. Equipment and utensils

Standard laboratory equipment including:

4.1. Development chamber for chromatography

4.2. Equipment appropriate for the preparation of slides

4.3. Glass slides for thin film chromatography, 20 x 20 cm, prepared as follows:

- In a 250 ml conical flask, add 30 g of silicagel, 70 ml of starch solution (3.13) and stir for 1 min. Spread the suspension on the plates to obtain a uniform film 0.3 mm in thickness. Dry the plates in air, then keep them in a dryer containing silicagel. Activate them before use, keeping them in an oven at 105°C for 1 h 30 min. The indicated quantities are sufficient for the preparation of 5 slides.

These sides are also available over the counter.

4.4. Spectrophotometer allowing readings at 500 nm with

dishes having a 1 cm optical path.

4.5. 1200 rpm centrifuge, at the least, with 50 ml tubes and screw stoppers.

5. Preparation of sample

Homogenize the sample by stirring, then filter if necessary.

6. Technique

6.1. Oxidization of ascorbic acid

In a 100 ml calibrated phial, add a test sample of 50 ml and 15 ml of polyamide suspension and bring up to the mark with solution 3.2. Leave in contact for 1 h while stirring frequently. Filter with a pleated paper filter.

Add 20 ml of filtrate to a centrifugal tube. Add 1 ml of iodine solution (3.6) and shake after 1 min. Reduce the excess by adding approximately 25 mg of thiurate (3.5).

6.2. Forming an extraction of bis (2.4-dinitrophenylhydrazine)

Place the tube in a bath of water at a temperature included 5 and 10°C inclusive. Add 4 ml of the solution of 2.4 dinitrophenylhydrazine (3.9). Stop the tube and shake with care, taking care not to wet the stopper. Keep the tube thoroughly stopped in a bath at 20°C for approximately 16 h.

Add 15 ml of solution 3.9. Stop the tube and shake thoroughly for 30 s. Then centrifugate for 5 min. at 1000 - 1200 rpm. Remove 10 ml of the surface solution and add to a conical flask with an emery stopper. Add to the tube 5 ml of solution 3.10, stir again for 30 s then centrifugate for 5 min. at 1000 - 1200 rpm. Remove 5 ml of the surface solution and add to a conical flask to the 10 ml of the initial extraction. Stir.

6.3. Separation of bis (2.4-dinitrophenylhydrazine) by thin film chromatography

Apply chromatographic separation for the 2 h following extractions on a

chromatography slide, along a line 2 cm from the lower and side edges; apply 0.2 ml of the extraction solution obtained in 6.2. In a development chamber previously saturated with eluant (3.15), taking up approximately 1 cm of the height, insert the slide and allow the eluant to migrate to the upper edge. Remove the slide and dry for 1 h in a ventilated place.

Maintain the slide in a vertical position on a sheet of gloss non-porous paper, scrape with a spatula perpendicularly to the direction of migration in the reddish coloring zone characteristic of bis (2.4-dinitrophenylhydrazine), away from any drafts.

Transfer all of the powdered products obtained in a small weighing flask with an emery lid and add 4 ml of glacial acetic acid (3.7). Leave in contact for 20 min. while stirring frequently. Filter through a small filter paper folded directly into the bowl of the spectrophotometer and allow to pass the first 25 to 30 droplets of the filtrate through the filter, once again, to obtain total limpidity.

6.4. Reading of absorbants

Using the spectrophotometer, read the absorbance of the filtrate at a wavelength of 500 nm while using glacial acetic acid (3.7) as reference.

6.5. Calibration curve

Into 100 ml calibrated flasks, add respectively 5, 10 and 15 ml of solution 3.14 and bring each flask up to the line with solution 3.3. These solutions contain respectively, 50, 100 and 150 mg of L-ascorbic acid per L. Take a 50 ml sample of each solution instead of the specimen and, for each and successively, perform the operations described from 6.1 to 6.4.

Establish a calibration curve with the concentrations on the abscissas and the absorbances on the ordinates. The graph should be a straight line passing through the origin.

7. Results

7.1. Calculation

Determine the L-ascorbic acid content expressed in milligrams per L of vinegar on the calibration curve and as a function of the absorbance obtained in 6.4.

7.2. Presentation

Round off the results expressed in milligrams per L to the nearest unit.

8. Bibliography

1. AOF / WHO - Commission of Codex Alimentarius, Doc. OX/EURO 82/3, Part II, Rome (1982).