Method OIV-MA-VI-08 : R2000
Type II method

Determination of the non-volatile reducing substances content in vinegars

(OENO 59/2000)

1. Definition

Non-volatile reducing substances are all the non-volatile substances having a reducing power in a copper-alkali solution and those that also have this power after hydrolysis.

2. Principles

Evaporation of volatile substances, hydrochloric hydrolysis, oxidization by a copper-alkali solution in excess with titling by iodometry of copper ions.

3. Reagents

3.1. Hydrochloric acid □20 = 1.19 g/ml

3.2. Sulfuric acid (□20 = 1.84 g/ml).

3.3. Solution of sodium hydroxide 12 M

3.4. Copper-alkali solution

Dissolve separately 25 g of copper sulfate ($CuSO4, 5H20$) in 100 ml of water, 50 g of citric acid ($C6H8O7, H2O$) in 300 ml of water and 388 g of crystallized sodium carbonate ($Na3CO3, 10H2O$) in approximately 400 ml of hot water. In a calibrated flask, successively add 1000 ml of the citric acid solution and the sodium carbonate solution, then add the copper sulfate solution. Cool, mix and bring up to the gauge line with water.

3.5. Solution of potassium iodine at 30% (m/v). Keep in a tinted glass flask.

3.6. Solution of sulfuric acid at 25% (v/v).

3.7. Starch additive solution at 5 g/l.

Thin out 0.5 g of soluble starch in a small amount of cold water so as to obtain a fluid paste. To 100 ml of boiling water, add, then maintain at the boil for 10 min. Allow to cool.

3.8. Solution of sodium thiosulfate 0.1 M.

3.9. Boiling regulator (glass balls, pumice stone).

4. Equipment and utensils

Laboratory equipment including:

4.1. Reflux apparatus consisting of a 500 ml conical flask connected by a ground seal to a condenser, or any other equivalent apparatus.

4.2. Cooling device consisting of a flow of cold water.

4.3. Water bath at 100 °C.

5. Preparation of sample

Homogenize the sample by stirring and filter if necessary.

6. Technique

6.1. Evaporation of volatile substances

In a 100 ml porcelain capsule, add 50 ml of vinegar and evaporate in a water bath at 100 °C to approximately 1 to 2 ml.

Add 10 ml of water and evaporate again. Repeat the operation with 10 ml of water.

Transfer the evaporation residue into a 100 ml calibrated flask using approximately 50 ml of hot water.

Allow to cool and bring up to the gauge line.

6.2. Hydrolysis

To the reflux apparatus flask, add 10 ml of the solution obtained in 6.1 and 0.3 ml of hydrochloric acid. Heat in a water bath at 100 °C for 2 min.

Cool to room temperature for 15 min. and neutralize with 0.3 ml of the sodium hydroxide solution.

6.3. Oxidization and titling

In the same flask, add 15 ml of water and 25 ml of the copper alkali solution (3.4). Add the boiling regulator and bring to the boil, which should take 2 min. Adapt the condenser to the flask and maintain at the boil for exactly 10 min.

Cool immediately in a flow of cold water without stirring. Successively add 2 ml of the starch solution (3.7), 10 ml of the potassium iodine solution (3.5) and 25 ml of the sulfuric acid solution (3.6). Title with the sodium thiosulfate solution (3.8).

Produce a specimen dose with 25 ml of water and 25 ml of copper-alkali solution.

7. Results

7.1. Calculation

Considering:

- V_1 the volume in ml of the sodium disulfate solution used in titling.
- V_2 the volume in ml of the sodium disulfate solution used in specimen dosing.
- n the quantity of test sample reducing substances in mg, given by the table and as a function of the difference

$$V_2 - V_1$$

The content in non-volatile reducing substances expressed in grams per l of vinegar is given by:

0.2 n

Table

V_2-V_1 (ml)	sub.red (mg)	V_2-V_1 (ml)	sub.red (mg)	V_2-V_1 (ml)	sub.red (mg)
1	2.4	9	22.4	17	44.2
2	4.8	10	25.0	18	47.1
3	7.2	11	27.6	19	50.0
4	9.7	12	30.3	20	53.0
5	12.2	13	33.0	21	56.0
6	14.7	14	35.7	22	59.1
7	17.2	15	38.5	23	62.2

8	19.8	16	41.3		

7.2. Presentation

Round off the results expressed in grams per liter to the nearest decimal.

8. Inter-laboratory validation (Hitos et al, 2000)

Units: % (m/V)

Sample	**r**	**S_r**	**RSD_r**	**R**	**S_R**	**RSD_R**	**RSD_R (Horwitz)**	**Horrat Index**
1 – 2.93 g/L	0.0892	0.032	1.09	1.0267	0.367	12.52	4.81	2.60
2 – 2.82 g/L	0.2635	0.094	3.34	1.4950	0.534	18.94	4.84	3.91
3 – 6.27 g/L	0.1214	0.043	0.69	1.6346	0.584	9.32	4.29	2.17
4 – 8.77 g/L	0.1591	0.057	0.65	1.5054	0.538	6.13	4.08	1.50
5 – 6.22 g/L	0.1019	0.036	0.58	1.2130	0.433	6.96	4.30	1.62

9. Bibliography

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