

## OIV-MA-AS311-01A Reducing substances

### Type IV method

#### 1. Definition

Reducing substances comprise all the sugars exhibiting ketonic and aldehydic functions and are determined by their reducing action on an alkaline solution of a copper salt.

#### 2. Principle of the method

The wine is treated with one of the following reagents:

- neutral lead acetate,
- zinc ferrocyanide (II).

#### 3. Clarification

The sugar content of the liquid in which sugar is to be determined must lie between 0.5 and 5 g/L.

Dry wines should not be diluted during clarification; sweet wines should be diluted during clarification in order to bring the sugar level to within the limits prescribed in the following table.

Description	Sugar content (g/L)	Density	Dilution (%)
Musts and mistelles	> 125	> 1.038	1
Sweet wines, whether fortified or not	25 to 125	1.005 to 1.038	4
Semisweet wines	5 to 25	0.997 to 1.005	20
Dry wines	< 5	< 0.997	No dilution

3.1. Clarification by neutral lead acetate.

3.1.1. Reagents

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- Neutral lead acetate solution (approximately saturated)
- Neutral lead acetate, Pb  $(CH_3COO)_2 \cdot 3H_2O$ : 250 g
- Very hot water to 500 mL
- Stir until dissolved.
- Sodium hydroxide solution, 1 M
- Calcium carbonate.

### 2. Procedure

- Dry wines.

Place 50 mL of the wine in a 100 mL volumetric flask; add 0.5 (n - 0.5) mL sodium hydroxide solution, 1 M, (where n is the volume of sodium hydroxide solution, 0.1 M, used to determine the total acidity in 10 mL of wine). Add, while stirring, 2.5 mL of saturated lead acetate solution and 0.5 g calcium carbonate. Shake several times and allow to stand for at least 15 minutes. Make up to the mark with water. Filter.

1 mL of the filtrate corresponds to 0.5 mL of the wine.

- Musts, mistelles, sweet and semi-sweet wines

Into a 100 mL volumetric flask, place the following volumes of wine (or must or mistelle), the dilutions being given for guidance:

- Case 1 - Musts and mistelles: prepare a 10% (v/v) solution of the liquid to be analyzed and take 10 mL of the diluted sample.
- Case 2 - Sweet wines, whether fortified or not, having a density between 1.005 and 1.038: prepare a 20% (v/v) solution of the liquid to be analyzed and take 20 mL of the diluted sample.
- Case 3 - Semi-sweet wines having a density between 0.997 and 1.005: take 20 mL of the undiluted wine.

Add 0.5 g calcium carbonate, about 60 mL water and 0.5, 1 or 2 mL of saturated lead acetate solution. Stir and leave to stand for at least 15 minutes, stirring occasionally. Make up to the mark with water. Filter.

*Note:*

- Case 1: 1 mL of filtrate contains 0.01 mL of must or mistelle.

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- Case 2: 1 mL of filtrate contains 0.04 mL of sweet wine.
- Case 3: 1 mL of filtrate contains 0.20 mL of semi-sweet wine.

### 3.2. Clarification by zinc ferrocyanide (II)

This clarification process should be used only for white wines, lightly colored sweet wines and musts.

#### 3.2.1. Reagents

Solution I: potassium ferrocyanide (II):

- Potassium ferrocyanide (II),  $K_4Fe(CN)_6 \cdot 3H_2O$ : 150 g
- Water to: 1000 mL

Solution II: zinc sulfate:

- Zinc sulfate,  $ZnSO_4 \cdot 7H_2O$ : 300 g
- Water to 1000 mL

#### 2. Procedure

Into a 100 mL volumetric flask, place the following volumes of wine (or must or mistelle), the dilutions being given for guidance:

- Case 1 - Musts and mistelles. Prepare a 10% (v/v) solution of the liquid to be analyzed and take 10 mL of the diluted sample.
- Case 2 - Sweet wines, whether fortified or not, having a density between 1.005 and 1.038: prepare a 20% (v/v) solution of the liquid to be analyzed and take 20 mL of the diluted sample.
- Case 3 - Semi-sweet wines having a density at 20°C between 0.997 and 1.005: take 20 mL of the undiluted wine.
- Case 4 - Dry wines: take 50 mL of undiluted wine.

Add 5 mL of solution I and 5 mL of solution II. Stir. Make up to the mark with water. Filter.

*Note:*

Case 1: 1 mL of filtrate contains 0.01 mL of must or mistelle.

Case 2: 1 mL of filtrate contains 0.04 mL of sweet wine.

Case 3: 1 mL of filtrate contains 0.20 mL of semi-sweet wine.

Case 4: 1 mL of filtrate contains 0.50 mL of dry wine.

#### 4. Determination of sugars

##### 4.1. Reagents

Alkaline copper salt solution:

- Copper sulfate, pure,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ : 25 g
- Citric acid monohydrate: 50 g
- Crystalline sodium carbonate,  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ : 388 g
- Water to: 1000 mL

Dissolve the copper sulfate in 100 mL of water, the citric acid in 300 mL of water and the sodium carbonate in 300 to 400 mL of hot water. Mix the citric acid and sodium carbonate solutions. Add the copper sulfate solution and make up to one liter.

Potassium iodide solution, 30% (*m/v*):

- Potassium iodide, KI: 30 g
- Water to : 100 mL

Store in a colored glass bottle.

Sulfuric acid, 25% (*m/v*):

- Concentrated sulfuric acid,  $\text{H}_2\text{SO}_4$ ,  $\rho_{20} = 1.84$  g/ml 25 g
- Water to 100 mL

Add the acid slowly to the water, allow to cool and make up to 100 mL with water.

Starch solution, 5 g/L:

- Mix 5 g of starch in with about 500 mL of water. Bring to boil, stirring all the time, and boil for 10 minutes. Add 200 g of sodium chloride, NaCl. Allow to cool and then make up to one liter with water.
- Sodium thiosulfate solution, 0.1 M.

Invert sugar solution, 5 g/L, to be used for checking the method of determination.

Place the following into a 200 mL volumetric flask:

- Pure dry sucrose: 4.75 g
- Water, approximately: 100 mL
- Conc. hydrochloric acid ( $\rho_{20} = 1.16 - 1.19$  g/mL): 5 mL

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Heat the flask in a waterbath maintained at 60°C until the temperature of the solution reaches 50°C; then keep the flask and solution at 50°C for 15 minutes. Allow the flask to cool naturally for 30 minutes and then immerse it in a cold waterbath. Transfer the solution to a one-liter volumetric flask and make up to one liter. This solution keeps satisfactorily for a month. Immediately before use, neutralize the test sample (the solution being approximately 0.06 M acid) with sodium hydroxide solution.

### 4.2. Procedure

Mix 25 mL of the alkaline copper salt solution, 15 mL water and 10 mL of the clarified solution in a 300 mL conical flask. This volume of sugar solution must not contain more than 60 mg of invert sugar.

Add a few small pieces of pumice stone. Fit a reflux condenser to the flask and bring the mixture to the boil within two minutes. Keep the mixture boiling for exactly 10 minutes.

Cool the flask immediately in cold running water. When completely cool, add 10 mL potassium iodide solution, 30% (*m/v*); 25 mL sulfuric acid, 25% (*m/v*), and 2 mL starch solution.

Titrate with sodium thiosulfate solution, 0.1 M. Let *n* be the number of mL used. Also carry out a blank titration in which the 25 mL of sugar solution is replaced by 25 mL of distilled water. Let *n'* be the number of mL of sodium thiosulfate used.

### 4.3. Expression of results

#### 4.3.1. Calculations

The quantity of sugar, expressed as invert sugar, contained in the test sample is given in the table below as a function of the number (*n' ÷ n*) of mL of sodium thiosulfate used.

The sugar content of the wine is to be expressed in grams of invert sugar per liter to one decimal place, account being taken of the dilution made during clarification and of the volume of the test sample.

Table giving the relationship between the volume of sodium thiosulfate solution: ( <i>n' ÷ n</i> ) mL, and the quantity of reducing sugar in mg.					
$Na_2S_2O_3$ (ml 0.1 M)	Reducing sugars (mg)	Diff.	$Na_2S_2O_3$ (ml 0.1 M)	Reducing sugars (mg)	Diff.
1	2.4	2.4	13	33.0	2.7

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2	4.8	2.4	14	35.7	2.8
3	7.2	2.5	15	38.5	2.8
4	9.7	2.5	16	41.3	2.9
5	12.2	2.5	17	44.2	2.9
6	14.7	2.6	18	47.2	2.9
7	17.2	2.6	19	50.0	3.0
8	19.8	2.6	20	53.0	3.0
9	22.4	2.6	21	56.0	3.1
10	25.0	2.6	22	59.1	3.1
11	27.6	2.7	23	62.2	
12	30.3	2.7			

### Bibliography

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