OIV-MA-F1-13 Specific methods for the analysis of grape sugar-Folin-Ciocalteu Index

Type IV method

1. Definition

The Folin-Ciocalteu Index is (IFC) the result obtained from the application of the method described below.

This method applies to rectified concentrated must (RCM).

2. Principle of the method

All phenolic compounds contained in RCM are oxidized by the Folin-Ciocalteu reagent. In RCM other reducing substances can interfere such as, for example, sugars and sulfur dioxide. This interference can be evaluated by measuring a "blank" devoid of phenolic substances, prepared by treating the corresponding sample with activated charcoal.

The Folin-Ciocalteu reagent is formed from a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) which, after oxidation of the reducing substances present in the RCM, is reduced to a mixture of the blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). The blue coloration produced has a maximum absorption in the region of 750 nm.

3. Reagents

These must be of analytical reagent quality.

The water used must be distilled or water of equivalent purity.

3.1. Chemicals

3.1.1. Sodium tungstate dihydrate, $Na_2WO_4.2H_2O$ (CAS Number: 10213-10-2);

3.1.2. 3.1.2 Sodium molybdate dihydrate, $Na_2WO_4.2H_2O$ (CAS Number: 10102-40-6);

3.1.3. Phosphoric acid solution 85 wt. % in H_2O,H_3PO_4 , □ $_{20}$ = 1.71 g/ml (CAS Number 7664-38-2);

3.1.4. Hydrochloric acid, HCl, □ $_{20}$ = 1.2 g/ml (CAS Number 7647-01-0);

3.1.5. Lithium sulfate monohydrate, $Li_2SO_4.H_2O$ (CAS Number: 10102-25-7);

3.1.6. Bromine, Br_2 (CAS Number: 7726-95-6);

3.1.7. Sodium carbonate anhydrous,Na_2CO_3 (CAS Number: 497-19-8);

3.1.8. Activated charcoal decolorizing (CAS Number 7440-44-0).

3.2. Folin-Ciocalteu reagent

This reagent is available commercially in a form ready for use.

It may be prepared as follows: dissolve 100 g of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) and 25 g of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) in 700 ml of distilled water. Add 50 ml of 85 % phosphoric acid (□ $_{20}$ = 1.71 g/ml) and 100 ml of concentrated hydrochloric acid (□ $_{20}$ = 1.19 g/ml). Bring to the boil and boil for 10 hours under reflux conditions. Then add 150 g of lithium sulphate ($Li_2SO_4 . H_2O$) and a few drops of bromine and boil once more for 15 minutes. Allow to cool and make up to one liter with distilled water.

3.3. Anhydrous sodium carbonate, Na_2CO_3, made up into a 20 % w/v solution.

4. Apparatus

Normal laboratory apparatus, particularly:

4.1. 100 ml volumetric flasks.

4.2. Spectrophotometer capable of operating at 750 nm.

4.3. Technical balance ($\pm$ 0.01 g)

4.4. 50 ml graduated cylinder;

4.5. 50 ml beaker;

4.6. Magnetic stirrer;

4.7. Rapid-filtration filter papers;

4.8. 5 ml calibrated pipette;

4.9. Optical-glass cuvette;

4.10. Ultrasonic bath

5. Procedure

Good repeatability of results is achieved by using scrupulously clean apparatus (volumetric flasks and spectrophotometer cells).

5.1. Preparation of sample

Let P = percentage (m/m) of total sugars in the rectified concentrated must.

Dilute the RCM with distilled water until a total sugar concentration of the solution equal to 25 $\pm$ 0.5 % (m/m) (25° Brix): weigh a mass in g equal to 2500/P into a volumetric flask (4.1) and make up to 100 g with water.

5.2. Preparation of a blank sample

Take 10 ml from solution 5.1 using the graduated cylinder (4.4), put into a 50 ml beaker (4.5) and add 0,5g of activated carbon (3.1.8).

Leave in contact for 15 minutes with the aid of a magnetic stirrer (4.6), and then filter

the solution thought a rapid-filtration paper (4.7).

5.3. Color reaction

5.3.1. Color development in the sample

Introduce the following reagents or solutions into a 100 ml volumetric flask (4.1) strictly in the order given below:

- 5 ml of the sample (5.1) using a pipette (4.8),
- 50 ml of distilled water by means of a graduated cylinder (4.4) ,
- 5 ml of Folin-Ciocalteu reagent (3.2) using a pipette (4.8),
- 20 ml of sodium carbonate solution (3.2) by means of a graduated cylinder (4.4).

Make up to 100 ml with distilled water. Stir to homogenize. Wait 30 minutes for the reaction to stabilize.

During this period the flasks should be put in an ultrasonic bath (4.10) for 15 minutes to remove any bubbles that may interfere in the spectrophotometric measurement.

5.3.2. Color development in blank

Proceed as described in (5.3.1) by replacing the sample (5.1) with a blank (5.2).

5.4. Measurement

Determine the absorbance at 750 nm through an optical-glass cuvette with a path length of 1 cm, with respect to a blank (5.2).

If the absorbance is not around 0.3 an appropriate dilution should be made until the measured absorbance falls to around 0.3.

6. Expression of results

6.1. Method of calculation

The result is expressed in the form of an index obtained by multiplying the absorbance by 16, with one decimal point.

The FCI value calculated should be corrected for the blank according to the following formula:

FCI measured at 25°Brix = 16 d (A − A_{blank})

where:

- A = absorbance at 750 nm measured on the sample at 25 ± 0.5 % (m/m) (5.1)
- A_{blank} = absorbance at 750 nm measured on the blank (5.2)

- d = an appropriate factor if a dilution has been done (5.4)

7. Method performance

The method was checked in the ICQRF laboratory in Conegliano (Italy) analyzing two RCM samples. Each sample was further enriched in SO_2 at a concentration 5 mg/Kg TS (mg per Kg of total sugar). The samples were analyzed three times:

Samples	FC index at 25°Brix			Media	SD	r=2.8xSD
RCM 1	5.4	4.9	5.3	5.2	0.3	0.8
RCM 2	2.2	2.4	1.9	2.2	0.2	0.7

Further data was provided from the ASAE laboratory (Portugal):

Number of samples	Concentration range	Sr	r	SR	R
21	From 1 to 5.3	0.25	0.7		
21	From 1 to 5.0			0.91	2.5