OIV-MA-AS315-22 Determination of carboxymethyl cellulose (cellulose gum, CMC) in white wines

Type IV method

1. Introduction

Carboxymethyl cellulose (CMC) is a polymer derived from natural cellulose that has been routinely used for many years now as a food additive (INS 466) in products such as ice creams and pre-cooked meals [1], to give them smoothness. The use of CMC in white wines and sparkling wines to contribute to their tartaric stabilisation [2] was recently accepted by the OIV in resolution OENO 2/2008 provided that the dose added to the wine is less than 100 mg/l. A specific method for determination of CMC in white wine has therefore been developed based on the method of H.D Graham published in 1971 [3].

2. Field of application

The method applies to white wines (still and sparkling).

3. Principle

Once the CMC has been isolated from the wine by dialysis, it is hydrolysed in an acid medium to form glycolic acid which is then degraded to form formaldehyde. 2,7-Dihydroxynaphthalene (DHN) is added to form 2,2,7,7-tetrahydroxydinaphthylmethane in the presence of formaldehyde. The complex formed develops a purple-blue colour under the action of concentrated sulphuric acid, at 100 °C, allowing colorimetric measurement at 540nm (Figure 1).

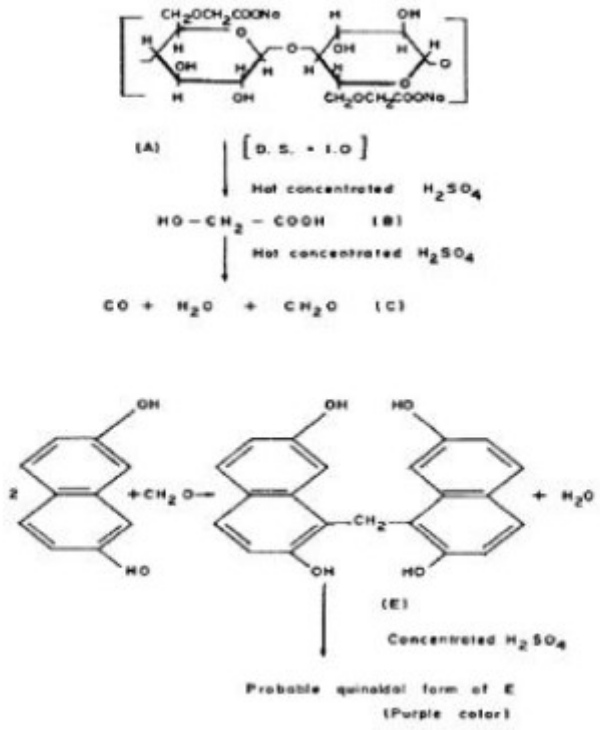

Figure 1: Mechanism of reaction of CMC with DHN in hot concentrated sulphuric acid (Feigl, 1966)

4. Reagents

- Sodium carboxymethylcellulose [N° CAS 9004-32-4] (21902 - average viscosity 400-1000 mPa·s, substitution degree 0.60-0.95)
- 2,7-Dihydroxynaphthalene [N° CAS 582-17-2] (purity > 98,0 % - HPLC
- 95 % concentrated sulphuric acid
- Purified water for laboratory use (example of quality: EN ISO 3696)

5. Equipment

- Laboratory glasswar
- Dialysis membrane (6000 to 8000 Da)
- Temperature-controlled bath

- Double-beam UV-visible spectrophotometer

6. Operating procedure

6.1. Preparation of the reagent

- Place 50 mg of DHN weighed to within 1 mg in a calibrated 100 mL phial.
- Add concentrated sulphuric acid up to the gauge line.
- Place the calibrated phial in a temperature-controlled bath at 28 °C for 4h (without stirring).
- After heating, decant the reagent into a brown flask and store it in a refrigerator at 4 °C.

6.2. Preparation of wine test specimens

- Insert 20 mL of wine, after degassing, into the dialysis membrane.
- Place the dialysis membrane containing the wine in a 6-litre flask filled with distilled water.
- Leave to dialyse for 24h, changing the dialysis water twice.

6.3. Colour reaction

- Place 1 mL of dialysed wine into a test tube.
- Add 9 mL of reagent.
- Place the test tube in a temperature-controlled bath at 100 °C for 2h.
- Analyse the coloured solution by UV-visible spectrophotometer at 540nm and read the absorbance value.

6.4. Calculation of the wine's CMC content

- Recording the absorbance value read in point 6.3 on the calibration curve obtained for a wine (see figure 2)

7. Characteristics of the method

Certain elements of the internal validation were determined but these do not constitute a formal validation according to the protocol governing the planning, the implementing and the interpretation of performance studies pertaining to analysis methods (OENO 6/2000)

7.1. Linearity of the response

A white wine has been added with incremental quantities of CMC ranging between 0 and 100 mg/L, then submitted to dialysis and treated in the conditions defined in the procedure described above. The response is linear for the concentrations under consideration (figure 2).

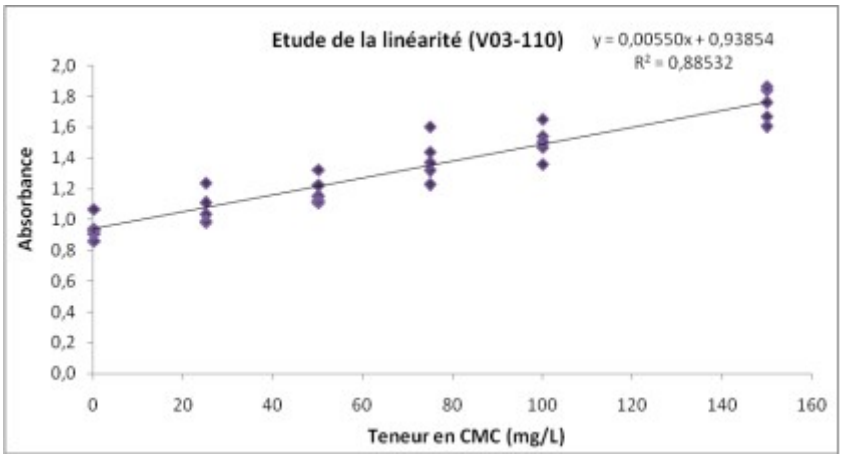

Figure 2: Linearity of CMC determination in white wine

7.2. Repeatability

The repeatability of the determination of CMC in white wines was defined on the basis of the results achieved on 22 samples of wine that underwent 2 successive analyses, so as to be analysed in identical conditions. The results are given in table 1.

		calculated values
Repeatability:		
	standard deviation	0,075
	CV in %	7,2 %
	r-limit	0,21
	r-limit in %	20 %

Table 1: Repeatability of CMC determination in white wine

7.3. Reproducibility

The reproducibility of the determination of CMC in white wines was defined through the analysis of a white wine by CMC, on 12 occasions at different dates. The results are given in table 2.

	calculated values
reproducibility	
standard deviation	0,082
CV in %	9,6 %
R-limit	0,23
R-limit in %	27 %

Table 2: Reproducibility of CMC determination in white wine

7.4. Specificity

The specificity of CMC determination was verified by adding known quantities of CMC into white wines. The recovery rates thus measure are given in table 3.

Sample	Added concentration (mg/l)	Resulting concentration (mg/l)	Recovery rate
Wine 1	50	33	66 %
Wine 1	50	51	102 %
Wine 1	50	24	77 %
Wine 2	75	78	104 %
Wine 2	75	90	121 %
Wine 2	75	69	92 %
Wine 3	100	109	109 %
Wine 3	100	97	97 %
Wine 3	100	103	103 %
Wine 4	150	163	109 %
Wine 4	150	149	100 %

Wine 4	150	159	106 %

Table 3: Specificity of CMC determination in white wine

7.5. Detection and quantification limits

The detection limits (LD) and quantification limits (LQ) were calculated for an untreated wine that underwent 10 analyses. The detection limit thus determined is of 14 mg/l and the quantification limit is of 61 mg/l.

The method therefore enables to detect the adding of CMC into white wine in quantities exceeding 20 mg/l and to quantify the addition when it exceeds 60 mg/l; this is not highly satisfactory but remains compatible with the maximum authorised dose of 100 mg/l.

7.6. Uncertainty

The uncertainty was calculated at 3 different concentration levels (25, 75 and 150 mg/l) based on the analysis results for wines that have undergone CMC treatment,

using the standard deviation reproducibility. The uncertainty thus obtained is of 40 mg/l, regardless of the CMC determination.

Bibliography

- [1] Regulation (CE) N° 1333/2008 of the 16th of December, 2008 concerning food additives
- [2] Stabilisation tartrique des vins par la carboxyméthylcellulose - Bulletin de l'OIV 2001, vol 74, n°841-842, p151-159.
- [3] Determination of carboxymethycellulose in food products - H.D Graham, Journal of food science 1971, p 1052-1055