

RESOLUTION OENO 5/94

INTERNATIONAL ENOLOGICAL CODEX MONO- AND DIGLYCERIDE ANTIFOAMS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 5, paragraph 4 of the International Convention for Unification of Methods of Analysis and Evaluation of Wines of October 13, 1954,

Based on the proposai of the Conventional Sub-Commission on Unification of the Methods of Analysis and Evaluation of Wines,

DECIDES:

to complete the International Enological Codex with the monograph on “Mono and Diglyceride Antifoams

By mono and diglycerides is meant the mixture of glyceryl mono-and diesters - with a small quantity of triesters - and oil fatty acids and edible fats. The mixture of mono- and diglycerides used as an antifoam essentially consists of oleic acid esters.

The product thus defined may contain small quantities of fatty acids and free glycerol. Its use in technological suitable conditions leaves no measurable traces in wine after filtration.

Characteristics

1. The product exists in the form of an oily, straw-colored liquid, a pasty, ivory-colored product, or a hard, waxy solid of a white to off-white color, with an agreeable odor and taste. The solid form can be présent as flakes, powder, or small grains.

The product used as an antifoam is liquid at ordinary températures but may be viscous at low températures.

2. Solubility: insoluble in water, soluble in ethanol, chloroform and benzene.
3. Identification of fatty acids and glycerol after hydrolysis.

Reflux a lg of sample with a 0.5 M potassium hydroxide solution for 1 hour. Add 15 mL of water, acidify with hydrochloric acid diluted to 30 p. 100 (v/v) (R) (approximately 4 to 5 mL). There is a formation of oily drops or of a white to yellowish-white

precipitate. Extract the fatty acids released with 5mL of hexane, separate the solvent. Repeat the extraction with 5 mL of hexane and combine the two extracts;

Retain the aqueous phase.

3.1. Identify the fatty acids in the hexane extract by gas chromatography. 3.2. Identification of the glycerol.

Introduce 5 mL of the aqueous phase into a test tube. Add an excess of powdered calcium hydroxide and place the test tube in boiling water for 5 min. shaking from time to time. Cool and filter.

Place a drop of filtrate in a test tube and add about 50 mg of potassium hydrogen sulfate. At the mouth of the tube, place a filter paper soaked with a reagent obtained by mixing, immediately before use, a solution consisting of equal volumes of sodium nitrosopentacyanoferrate (R') and piperidine (F). Heat on a small flame. A blue stain on the reagent paper indicates the presence of acrolein.

The stains changes to red after the addition of a 1M Sodium hydroxide solution.

Tests:

Loss on drying at 100 °C

Precisely weigh about 5 g. of the substance to be analyzed in a crystallizing dish 70 mm. in diameter, previously dried in the oven, cooled in a dessicator and tared. Introduce the crystal- lizing dish containing the fat into the oven at 103 °C, and keep it for 30 mn. Remove the crystal- lizing dish, cool in the dessicator and weigh. Put again the sample in the oven for 30 min. and weigh after cooling. The loss in oven is completed when the weight loss does not exceed 0.05% per half-hour of heating.

$$\% \text{ water and volatile matters} = \frac{\text{weight loss} \times 100}{\text{weight of the test sample}}$$

The loss on drying at 100 °C oven must be less than 2%. Sulfate Ash

Determined as indicated on p. 121, on a 5 g sample, the weight of the sulfate ash must be less than 0.5 p. 100 (w/w).

Arsenic

Determined as indicated on p. 126, the arsenic must be less than 3mg/kg based on a 5g sample.

Heavy Metals

Déterminez le contenu en métaux lourds :

- soit après minéralisation à 450 ± 5 °C du résidu laissé après détermination de la perte par séchage. Recueillir la cendre avec 1 mL d'acide hydrochlorique dilué (R) et une goutte d'acide nitrique (R), par chauffage pendant un moment sur un bain-marie afin d'encourager la solution. Décanter dans un flacon à fond rond de 25 mL et rincer le creuset avec de l'eau distillée apportant la solution au trait avec de l'eau distillée.

Prendre un volume v mL de solution correspondant à 2 g de l'échantillon d'essai et déterminer le contenu en métaux lourds comme indiqué sur la p. 130,

- soit après minéralisation d'un échantillon de test précisément pesé d'environ 5 g sous forme liquide avec de l'acide nitrique (R) et du peroxyde d'hydrogène avec l'utilisation possible d'un digérateur à micro-ondes pour accélérer l'opération.

Décanter le liquide obtenu dans un flacon volumétrique de 25 mL et porter au trait avec de l'eau de rinçage. Continuer comme spécifié ci-dessus pour tester les métaux lourds.

Le contenu en métaux lourds, exprimé en plomb, doit être inférieur à 10 mg/kg.

Free fatty acids.

Préparer 125 mL d'un mélange de volumes égaux d'alcool isopropylique et de toluène ; ajouter 2 mL d'une solution à 1 % de phénolphthaleïne dans l'alcool isopropylique et neutraliser avec une solution alcaline jusqu'à ce qu'une faible couleur rose persiste.

Dans un flacon Erlenmeyer de 500 mL, peser précisément environ 5 g de l'échantillon à analyser ; ajouter le mélange solvant neutralisé et dissoudre l'échantillon de test, chauffer si nécessaire. Agiter vigoureusement et ajouter la solution de 0,1 M d'hydroxyde de potassium jusqu'à ce qu'il acquière une couleur rose identique à celle obtenue au moment de la neutralisation du solvant. Le volume utilisé est n mL.

Concentration des acides gras libres exprimée en g d'acide oléique p. 100 (w/w)

$$\frac{n \times 2.8}{g \text{ of assay sample}}$$

La concentration des acides gras libres, exprimée en acide oléique, doit être inférieure à 3 p. 100 (w/w)

Soap

In a 250 mL Erlenmeyer flask, precisely weigh approximately 10 g of the product to be analyzed. Add a mixture of 60 mL of acetone and 0.15 mL of a solution of 0.5 % bromophenol blue in 95% vol. alcohol that was previously neutralized with a solution of 0.1 M hydrochloric acid or a solution of 0.1 M sodium hydroxide. Warm gently in a water bath and titrate with 0.1 M hydrochloric acid solution until the blue colour disappears. Let stand 20 minutes, then warm until any solidified matter is redissolved. If the blue colour reappears, continue the titration.

1 mL of 0.1 M hydrochloric acid in solution corresponds to 0.0304 g of sodium oleate ($\text{NaC}_{18}\text{H}_{33}\text{O}_2$ /

Concentration of soap expressed as grams of sodium oleate per 100 (w/w):

$$\frac{n \times 3.04}{\text{test sample in g}}$$

The concentration of soap, expressed in grams of sodium oleate, must be less 6 p. 100 (w/w).

a-Monoglycerides

Préparation of the sample.

Melt the sample, if it is solid, by warming to a temperature less than 10 from its melting point. Also warm a liquid sample if it is cloudy or turbid in appearance. Mix vigorously.

Procedure

In a 100 mL cylindrical vessel, precisely weigh approximately 1 g of an assay sample, Q. Dissolve it using 25 mL of chloroform. Transfer this solution to a separatory funnel; rinse the cylindrical vessel with 25 mL of chloroform then with 25 mL of water and combine these liquids with those contained in the separatory funnel.

Tightly stopper the funnel; stir for 30 to 60 s; allow the layers to separate (add 1 to 2 mL of glacial acetic acid to break the emulsion). Collect the aqueous phase in a 500 mL glass-stoppered Erlenmeyer flask. Extract the chloroform layer remaining in the separatory funnel with two 25 mL portions of water. Separate the aqueous phase and place in the 500 mL Erlenmeyer flask. These aqueous extracts will be used for the

détermination of free glycerol.

The chloroform is transferred from the separatory funnel to a 500 mL glass stoppered Erlenmeyer flask. Add 50 mL of periodic acetic acid solution (R') and shake.

In two other 500 mL glass stoppered Erlenmeyer flasks intended for used as "blanks", place 50 mL of chloroform and 10 mL of water. Add 50 mL of periodic acetic acid solution (R') and shake both flasks. Allow the three flasks to stand at least 30 min but no longer than 90 min.

To each flask, add 20 mL of potassium iodide solution (R') while swirling. Allow to stand for at least 1 min but no longer than 5 min before titrating. Then add 100 mL of water and titrate with a 0.05 M solution of sodium thiosulfate using of a magnetic stirrer, until the brown color from the aqueous phase disappears; then add 2 mL of starch solution (R) and continue the titration until the disappearance of the iodine in the chloroform layer and the disappearance of the blue color in the aqueous phase.

Calculate the percentage of α -monoglycerides with the formula:

$$\frac{(B - S) \times M \times 17.927}{W}$$

B is the average of the number of mL of sodium thiosulfate solution used to determine blanks containing chloroform.

S is the number of mL of sodium thiosulfate solution used to titrate the sample.

M is the exact molarity of the sodium thiosulfate solution.

W is the weight of the sample to be analyzed contained in the volume of chloroform used for the détermination.

17.927 is the molecular weight of the glycerol monostearate divided by 20.

The concentration of α -monoglycerides, expressed as glycerol monostearate, must be greater than 30 p.100 (w/w)

Free glycerol

To each of the aqueous extracts obtained in the détermination of α -monoglycerides, add 50 mL of periodic acetic acid solution (R'). Simultaneously, préparé a blank by adding 50 mL of periodic acetic acid solution (R') to a 500 mL Erlenmeyer flask containing 75 ml of water. Continue the détermination as indicated in the method described for the détermination of α -monoglycerides.

Calculate the percentage of free glycerol by the formula:

$$\frac{(b - S) \times M \times 2.30}{Q}$$

b is the number of mL of sodium thiosulfate used for the détermination of the blank containing 75 ml of water.

S is the number of mL of sodium thiosulfate used in the détermination of aqueous extract.

M is the molarity of the sodium thiosulfate solution.

Q is the weight of the original sample taken to be determined (see détermination of α -monoglycerides).

The free glycerol must be less than 7% (w/w). Reagents (R')

- Sodium (nitrosopentacyanoferrate) in a 5 p. 100 solution (w/v). Aqueous solution containing 5 g of sodium nitrosopentacyanoferrate ($\text{Na}^+\text{O Fe}(\text{CN})_5 2\text{H}_2\text{O}$) per 100 mL, to be prepared immediately before use.

Piperidine in a 20 p. 100 (v/v) solution.

Solution containing 20 mL of piperidine ($\text{C}_5\text{H}_{11}\text{N}$) per 100 mL

- Periodic acetic acid solution

Dissolve 5.4 g of periodic acid (H_5O_6) in 100 ml of water; add 1900 mL of glacial acetic acid. Mix. Store in a brown flask fitted with a ground-glass stopper.

- Chloroform

It must satisfy the following test: in two 500 mL Erlenmeyer flasks, place 50 mL of periodic acetic acid solution. Add 50 mL of chloroform and 10 mL of water to one of the flasks and 50 mL of water to the other. To each flask, add 20 mL of potassium iodide solution (R). Mix gently. Let stand at least 1 min., but less than 5 min. before titrating. Then add then 100 mL of water and titrate with a 0.05 M sodium thiosulfate solution using a magnetic stirrer until the brown color disappears from the aqueous phase; then add mL of starch solution (R) and continue to add the reagent until the disappearance of the iodine in the chloroform layer and the disappearance of the blue colour in the aqueous phase.

The différence between the volumes of thiosulfate used in the two titrations should

not exceed 0.5 mL.

COMMENTS

1. We have compared the spécifications given by JECFA (1978), by the USA (21CFR, para- graphs 184-1505, by the Food Chemical Codex) and by the EEC. See Table 1.

The JECFA spécifications have been retained with the élimination of the IR spectrum and the addition of sulfate ash.

Table I COMPARISON OF MONOGRAPHS ON MONO- AND DIGLYCERIDES

Definitions	1 JECFA-1978	2 21 CFR IUSA	3 EEC
	a-monoglycerides <30%	Identical 90% w/w of glycerides	
Characteristics Appearance Solubility IR spectrum Characterization: • Fatty acids • Glycerol	Liquid to solid + + + +	Liquid to solid + - - -	Liquid to solid - - - -
Assay Water content Sulfate ash Arsenic mg/kg Heavy metal mg/kg Lead mg/kg Acid index	<2% (K. Fischer) - 3 10 - 6	- <0.5% 3 10 - 6	<2% (K. Fischer) <0.5% 3 No hazardous level 10 3% oleic acid (equivalent to acide index of 6)

Soap	<6% sodium oleate	-	-
Free glycerol	<7%	<7%	<7%
Mono and diesters	-	-	>70%
Polyglycerols	<30%	These specifications must conform to the characteristics furnished by the manufacturer	<4% of total glycerol
α -Monoglycerides			-
Total			-
Monoglycerides			-
Iodine Index			-
Hydroxide Index			-
Saponification Index			-

2. Depending on their unsaturated fatty acid concentration, the mono- and diglycerides may occur in either a liquid or solid form.

The mono- and diglycerides used as antifoams are derived from oleic acid and, therefore, are essentially liquids. They are surfactants.

3. The procedure for the détermination of parameters is that mentioned by JECFA taking into account, when it exists, the description mentioned in the Food Chemical Codex or the Official Methods of Analysis of the AOAC.
4. The reagents marked (R) are included amongst the reagents described in the 1978 édition of the International Fnoological Codex.

The reagents marked (R) are new, and described in the paper appended to the mono-graph.