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

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precipitate. Extract the fatty acids released with 5mL of hexane, separate the solvant. 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 o f 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 bp 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.

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

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.

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Heavy Metals

Détermine the heavy metal content:

- either after mineralization at 450 + 5 °C of the residue left after determining the loss by drying. Collect the ash with 1 mL dilute hydrochloric acid (R) and a drop of nitric acid (R), by heating for a while on a water bath in order to encourage solution. Decant into a 25 mL round- bottomed flask and rinse the dish with distilled water bringing the solution to the mark with dis- tilled water.

Take a v mL volume of solution corresponding to 2 g of the assay sample and détermine the heavy métal content as indicated on p. 130,

- either after mineralization of a precisely weighed test sample of about 5 g in the liquid form with nitric acid (R) and hydrogen peroxide with the possible use of a microwave digester to accelerate the opération.

Decant the liquid obtained in a 25 mL volumetric flask and bring to the mark with rinse water. Continue as specified above to test for heavy metals.

The heavy métal content, expressed in lead, must be less 10 mg/kg.

Free fatty acids.

Prepare 125 mL of a mixture of equal volumes of isopropyl alcohol and toluene; add 2mL of a 1% solution of phenolphtalein in isopropylic alcohol and neutralize with an alkaline solution until a weak rose color persists.

In a 500 mL Erlenmeyer flask, precisely weigh approximatively 5 g of the sample to be analyzed; add the neutralized solvent mixture and dissolve the test sample, warming if necessary. Shake vigorously and add the solution of 0.1 M potassium hydroxide until it acquires an identical rose color to that obtained at the time of the neutralization of the solvent. The volume used is n mL.

Concentration of free fatty acids expressed in g of oleic acid p. 100 (w/w)

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

The concentration of free fatty acids, expressed as oleic acid, must be less than 3 p. 100 (w/w)

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Soap

In a 250 mL Erlenmeyer flask, precisely weigh approximatively 10 g of the product to be ana- lyzed. 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 batln and titrate with 0.1 M hydro- chloric 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 (NaC18H330 /

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

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

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 température 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 approximatively 1 g of an assay sample, Q. Dis solve it using 25 mL of chloroform. Transfer this solution to a separatory funnel; rinse the cylin drical 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 émulsion). 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

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détermination of free glycerol.

The chloroform is transferred from the separatory funnel to a 500 mL glass stoppered Erlen meyer flask. Add 50 mL of periodic acetic acid solution (R'f 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 wirling. 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 —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 détermine 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 —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 a-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 a-monoglycerides.

Calculate the percentage of free glycerol by the formula:

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

b is the number of mL of sodium thiosulfate used for the détermination of the blank containing75 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 a-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 (Na^O Fe(CN)5 2H£0) per 100 mL, to be prepared immediately before use.

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

Solution containing 20 mL of piperidine (C5HnN) per 100 mL

- Periodic acetic acid solution

Dissolve 5.4 g of periodic acid (H^O^ 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 disappearence 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

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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)

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

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 procédure for the déterminationof 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 1978édition of the International Fnological Codex.

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

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