International Standard 6632

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Fruits, vegetables and derived products — Determination of volatile acidity

Fruits, légumes et produits dérivés — Détermination de l'acidité volatile

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Foreword

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It has been approved by the member bodies of the following countries :

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The member body of the following country expressed disapproval of the document on technical grounds :

USA

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Fruits, vegetables and derived products — Determination of volatile acidity

1 Scope and field of application

This International Standard specifies a method for the determination of volatile acidity in fruits, vegetables and derived products.

The method is applicable to all fresh products and to products preserved without chemical preservatives, as well as to products to which sulphur dioxide has been added with or without one of the following preservatives : sorbic acid, benzoic acid, formic acid.

2 Definition

2.1 volatile acidity : All lower molecular weight fatty acids, such as acetic acid and propionic acid, in free or combined form, with the exception of formic acid.

The volatile acidity, determined by the method specified in this International Standard, is expressed either in milliequivalents per 100 ml or per 100 g of product, or in grams of acetic acid per 100 ml or per 100 g of product.

3 Principle

Acidification of a test portion with tartaric acid, entrainment of the volatile acids by steam distillation, and titration of the distillate with standard volumetric sodium hydroxide solution in the presence of phenolphthalein as indicator. If appropriate, subtraction of the volatile acidity due to added volatile acid antiseptic (preservative) compounds from the volatile acidity thus determined.

4 Reagents

All the reagents shall be of recognized analytical purity. The water used shall be distilled water or water of equivalent purity, free from carbon dioxide.

4.1 Sodium hydroxide, standard volumetric solution, $c(NaOH) = 0{,}1$ mol/l[1].

Prepare just before use and check the concentration by titration immediately before use.

4.2 Phenolphthalein, 10 g/l solution in 95 % (V/V) ethanol.

4.3 Tartaric acid, crystallized.

4.4 Lime water, diluted 1 + 4.

Dilute one volume of the saturated calcium hydroxide solution (4.5) with four volumes of water. Allow the mixture to stand until calcium carbonate is precipitated and decant the limpid solution, which shall be alkaline to the phenolphthalein solution (4.2).

This solution is intended for use in the steam generator (5.2.1).

4.5 Calcium hydroxide, limpid saturated solution.

5 Apparatus

Usual laboratory apparatus, and in particular :

5.1 Mechanical mill.

5.2 Apparatus for extrainment by steam distillation (see the figure), comprising the following elements :

5.2.1 Steam generator, suitable for the production of steam free from carbon dioxide, made of heat-resistant glass or metal, and of capacity about 1 500 ml.

5.2.2 Bubbler, comprising a glass tube, of diameter 30 mm and 270 mm long, the lower part of which is sealed and enlarged to form a sphere of diameter 60 mm in which the test portion is placed. The bubbler shall be placed on a metal disc having an orifice of diameter 40 mm, into which the bottom of the bubbler is fitted.

5.2.3 Fractionating column, comprising a glass tube, of diameter 20 mm and 500 mm long, inside which is a spiral-shaped stainless steel net, No. 100, with a lead of 15 mm.

Any other device having the same fractionating efficiency may be used (see the note to 5.2.4).

1) Hitherto expressed as "0,1 N standard volumetric solution".

5.2.4 Condenser (West type), diameter 16 mm and nominally 400 mm long, placed vertically to ensure condensation of the vapour and complete cooling of the distillate.

NOTE — Apparatus other than that described above may be used, provided it complies with the following requirements :

a) under normal distillation conditions, at least 99,5 % of a known amount of acetic acid added to the sample shall be found in 250 ml of distillate. To check this, use 20 ml of acetic acid solution of concentration, $c(CH_3COOH) = 0{,}1$ mol/l[1];

b) under the same conditions of distillation, no more than 5 parts per thousand of a known amount of lactic acid added to the sample shall be found in the distillate. To check this, use 20 ml of lactic acid solution of concentration, $c(C_2H_5OCOOH) = 1$ mol/l[2];

c) the steam produced by the steam generator shall be free from carbon dioxide. Check this by adding two drops of the phenolphthalein solution (4.2) and 0,1 ml of the sodium hydroxide solution (4.1) to 250 ml of distillate, and verifying that a pink colour (stable for at least 10 s) appears.

5.3 Conical flask, of capacity 500 ml.

5.4 Graduated pipettes, of capacity 20 ml, complying with the requirements of ISO/R 835.

5.5 Burette, of capacity 25 ml, graduated at 0,1 ml intervals, complying with the requirements of ISO/R 385, class B.

5.6 Analytical balance.

6 Procedure (see clause 8 for the particular case of products containing volatile acid preservatives)

6.1 Preparation of test sample

6.1.1 Liquid products and those in which the liquid can be easily separated (juices, syrups, liquid from stewed fruit compot, brines, etc.)

Thoroughly mix the sample. If the sample contains solid particles in suspension, separate the liquid by filtration through a fluted filter paper. If the sample is in the course of fermentation, or if it contains carbon dioxide, transfer, by means of a pipette, 50 to 60 ml of the product into a 500 ml flask. Remove the carbon dioxide by shaking under reduced pressure for 2 to 3 min. To avoid formation of foam, a small amount of antifoaming agent may be added, for example about 0,2 g of tannic acid, directly to the weighed sample.

6.1.2 Viscous or solid products (marmalade, jams, jellies, concentrated juices, dried fruit and vegetables, etc.)

Remove the seeds and hard seed-cavity walls if necessary, and homogenize the sample in the mechanical mill (5.1).

6.1.3 Frozen or deep-frozen products

Allow frozen or deep-frozen products to thaw in a closed vessel and add the liquid formed during this process to the product before mixing.

6.2 Test portion

6.2.1 Liquid products

Transfer, by means of a pipette (5.4), 20 ml of the test sample (6.1.1) to the bubbler (5.2.2). In the case of samples which have strong volatile acidity, a smaller amount may be used and the total volume can be made up to 20 ml by addition of the required amount of water.

6.2.2 Viscous or solid or frozen products

In a beaker, weigh, to the nearest 0,01 g, about 10 g of the test sample (6.1.2 or 6.1.3). Transfer the test portion to the bubbler (5.2.2) with the minimum quantity of water necessary for entrainment of the whole test portion and to render the mixture sufficiently fluid.

6.3 Entrainment of volatile acids

Fill the steam generator (5.2.1) to two-thirds of its volume with the lime water (4.4). Add 0,5 g of the tartaric acid (4.3) to the test portion (6.2) contained in the bubbler (5.2.2). Connect the latter to the steam generator (5.2.1) and to the fractionating column (5.2.3) and condenser (5.2.4). Heat the steam generator and the bubbler simultaneously on burners. If the initial volume of the contents of the bubbler is more than 20 ml, regulate the heating so as to reduce the volume to about 20 ml while the steam is slowly introduced.[3] Reduce the rate of heating of the bubbler to maintain the volume of the contents constant (about 20 ml) for the duration of the distillation during which the output of steam from the generator (5.2.1) is at its maximum. The distillation shall last for 10 to 15 min.

Collect the distillate in the conical flask (5.3), continuing the distillation until about 250 ml of distillate has been collected. The volume of distillate shall be at least 12 times that of the test portion (6.2).

6.4 Titration of volatile acids

Add two drops of the phenolphthalein solution (4.2) to the distillate and titrate with the sodium hydroxide solution (4.1) until a light pink colour, persisting for at least 15 s, appears.

6.5 Number of determinations

Carry out two determinations on the same test sample (6.2).

1) Hitherto expressed as "0,1 N solution".

2) Hitherto expressed as "1 N solution".

3) Using 20 ml of liquid test portion, the volume is not reduced, as the steam is introduced into the flask at the maximum rate at the start of the distillation.

7 Expression of results (see clause 8 for the particular case of products containing volatile acid preservatives)

7.1 Method of calculation and formula

7.1.1 Liquid products

The volatile acidity, expressed in milliequivalents per 100 ml of product, or in grams of acetic acid per 100 ml of product, is given respectively by formulae (1) and (2) :

$$\frac{10 \times V_1}{V_0} \quad \ldots (1)$$

$$\frac{0{,}6 \times V_1}{V_0} \quad \ldots (2)$$

where

V_0 is the volume, in millilitres, of the test portion (6.2.1);

V_1 is the volume, in millilitres, of sodium hydroxide solution (4.1) required for the titration.

7.1.2 Viscous or solid or frozen products

The volatile acidity, expressed in milliequivalents per 100 g of product, or in grams of acetic acid per 100 g of product, is given respectively by formulae (3) and (4) :

$$\frac{10 \times V_1}{m_0} \quad \ldots (3)$$

$$\frac{0{,}6 \times V_1}{m_0} \quad \ldots (4)$$

where

m_0 is the mass, in grams, of the test portion (6.2.2);

V_1 has the same meaning as in 7.1.1.

7.1.3 Result

Take as the result the arithmetic mean of the values obtained in the two determinations (6.5), provided that the requirements for precision (see 7.2) are satisfied.

7.2 Precision

7.2.1 Repeatability

The difference between the values obtained in the two determinations (6.5), carried out simultaneously or in rapid succession by the same analyst, shall not exceed 0,2 milliequivalents per 100 ml or per 100 g, or 12 mg of acetic acid per 100 ml or per 100 g.

7.2.2 Reproducibility

The difference between the results obtained in two different laboratories, on the same sample shall not exceed 0,5 milliequivalents per 100 ml or per 100 g, or 30 mg of acetic acid per 100 ml or per 100 g.

8 Particular case of products containing volatile acid preservatives

In the case of products containing sulphur dioxide, with or without sorbic acid, benzoic acid or formic acid, determine the amount of preservative present in the distillate and correct the result of the titration accordingly.

8.1 Determination of sulphur dioxide in the distillate (in the absence of other preservatives)

8.1.1 Reagents

8.1.1.1 Iodine, standard volumetric solution, $c(1/2\ I_2) = 0{,}01$ mol/l[1].

8.1.1.2 Potassium iodide, crystallized.

8.1.1.3 Hydrochloric acid, concentrated.

8.1.1.4 Sodium borate, saturated solution (about 60 g of sodium borate decahydrate per litre).

8.1.1.5 Starch indicator : suspension of 5 g of soluble starch in 1 litre of water containing 200 g of sodium chloride as preservative. Boil the solution for 10 min during preparation.

8.1.2 Titration

Add 1 drop of the hydrochloric acid (8.1.1.3), 5 ml of the starch indicator (8.1.1.5) and about 0,1 g of the potassium iodide (8.1.1.2) to the distillate after titration of the volatile acids (6.4), and titrate the free sulphur dioxide with the iodine solution (8.1.1.1). Record the volume of iodine solution required for this first titration.

Add 20 ml of the sodium borate solution (8.1.1.4) to bring the pH to between 9 and 9,5 and titrate the combined sulphur dioxide with the iodine solution (8.1.1.1) until a violet-blue colour (stable for at least 30 s) is formed. Record the volume of iodine solution required for this second titration.

8.1.3 Expression of results

Correct the volume V_1 of sodium hydroxide solution (4.1) used for the titration, for the acidity of the free and combined

1) Hitherto expressed as "0,01 N standard volumetric solution".

sulphur dioxide, expressed in millilitres of 0,1 mol/l alkaline solution, by means of formula (5) :

$$V_1' = V_1 - \frac{V_2}{10} - \frac{V_3}{20} \quad \ldots (5)$$

where

V_1' is the volume, in millilitres, of sodium hydroxide solution (4.1) corresponding to the corrected volatile acidity;

V_2 is the volume, in millilitres, of iodine solution (8.1.1.1) used for the titration of free sulphur dioxide;

V_3 is the volume, in millilitres, of iodine solution (8.1.1.1) used for the titration of combined sulphur dioxide.

The formulae given in 7.1 may be used directly by substituting V_1' for V_1.

8.2 Determination of sorbic acid in the distillate (with or without the presence of sulphur dioxide)

8.2.1 Reagents

8.2.1.1 Sorbic acid, standard solution corresponding to 20 mg of sorbic acid per litre, or 26,8 mg of potassium sorbate per litre.

8.2.1.2 Oxidizing agent.

Dissolve 0,5 g of sodium hydrogen carbonate and 0,001 g of copper(II) sulphate pentahydrate in 1 000 ml of water.

8.2.1.3 Copper(II) sulphate, 0,1 g/l solution.

8.2.1.4 Chromic-sulphuric acid solution.

Dissolve 50 mg of potassium dichromate in about 90 ml of water in a 200 ml one-mark volumetric flask. Add 10 ml of sulphuric acid solution, $c(1/2\ H_2SO_4) = 0{,}3$ mol/l [1], and dilute to the mark with water.

NOTE — 1 litre of sulphuric acid solution, $c(1/2\ H_2SO_4) = 0{,}3$ mol/l [1], contains 14,7 g of sulphuric acid, or 8 ml of sulphuric acid, $\varrho_{20} = 1{,}84$ g/ml.

8.2.1.5 Thiobarbituric acid solution.

Dissolve 0,5 g of thiobarbituric acid in 50 ml of water and add 10 ml of sodium hydroxide solution, $c(NaOH) = 1$ mol/l [2]. Transfer to a 100 ml one-mark volumetric flask, add 11 ml of hydrochloric acid solution, $c(HCl) = 1$ mol/l [2], and dilute to the mark with water.

This solution is unstable and shall be used within 5 h of preparation.

8.2.2 Apparatus

8.2.2.1 Dish, of porcelain or heat-resistant glass, and of diameter 55 mm.

8.2.2.2 One-mark volumetric flasks, of capacities 10 — 20 and 250 ml.

8.2.2.3 Boiling water bath.

8.2.2.4 Spectrophotometer, suitable for use in the visible and ultraviolet regions of the spectrum, fitted with silica cells of optical path length 1 cm.

8.2.3 Identification and determination

Collect a known volume V (about 250 ml) of distillate as described in 6.3. Confirm the presence of sorbic acid and determine the sorbic acid content before determination of the volatile acidity.

8.2.3.1 Identification

8.2.3.1.1 Place 10 ml of the distillate in the dish (8.2.2.1). Make alkaline by adding 1 ml of the lime water (4.4) and add 1 drop of the copper(II) sulphate solution (8.2.1.3). (This solution catalyses the oxidation of sulphur dioxide by atmospheric oxygen.) Evaporate to dryness on the boiling water bath (8.2.2.3), thus oxidizing the sulphur dioxide, volatilizing the ethanol and other alcohols, aldehydes, ketones and aromatic esters and fixing the acids as calcium salts. Dissolve the residue in 10 ml of water.

8.2.3.1.2 Transfer 5 ml of this solution to a 10 ml volumetric flask, and add 2 ml of the chromic-sulphuric acid solution (8.2.1.4). Keep the flask in the boiling water bath for 10 min. Add 3 ml of the thiobarbituric acid solution (8.2.1.5) and keep in the boiling water bath for a further 20 min.

The appearence of a pink colour confirms the presence of sorbic acid.

8.2.3.2 Determination

Carry out the determination of sorbic acid in the distillate by one of the following methods.

8.2.3.2.1 Colorimetric method

After 20 min in the boiling water bath, cool the pink-coloured solution obtained in 8.2.3.1.2 in an ice-water bath. Dilute to

1) Hitherto expressed as "0,3 N solution".

2) Hitherto expressed as "1 N solution".

10 ml with water and measure the absorbance of the solution by means of the spectrophotometer (8.2.2.4) at a wavelength of 532 nm, using, as the reference liquid, a blank solution prepared by the same procedure (8.2.3.1.2) but using 5 ml of distilled water in place of the distillate.

Compare the absorbance with that of suitably diluted standard sorbic acid solution (8.2.1.1), treated as described in 8.2.3.1.2.

8.2.3.2.2 Ultraviolet absorption method[1)]

Dilute an aliquot portion of the distillate 1 + 3 or 1 + 9 or 1 + 19 with the oxidizing agent (8.2.1.2) in a 10 ml or 20 ml volumetric flask (8.2.2.2). (This solution catalyses the oxidation of sulphur dioxide by atmospheric oxygen.) Mix and leave exposed to the air for a few minutes. Fill one of the silica cells (see 8.2.2.4) with this solution.

Measure the absorbance of the solution by means of the spectrophotometer (8.2.2.4) at a wavelength of 256 nm, using, as the reference liquid, a blank solution prepared by the same procedure but using water in place of the distillate. The measured absorbance shall be less than 0,7; this determines the order of dilution to adopt.

Compare the absorbance with that of the standard sorbic acid solution (8.2.1.1) diluted 1 + 3 with the oxidizing agent (8.2.1.2).

8.2.4 Expression of results

The sorbic acid content of the distillate, expressed in milligrams per litre, is given by formula (6) :

$$20 \times \frac{A_x}{A_r} \qquad \ldots (6)$$

where

A_x is the absorbance of the diluted distillate, at 532 nm (see 8.2.3.2.1) or at 256 nm (see 8.2.3.2.2);

A_r is the absorbance of the reference solution (8.2.1.1), diluted in the same manner, at 532 nm (see 8.2.3.2.1) or at 256 nm (see 8.2.3.2.2).

8.2.5 Determination of distillate acidity

The acidity of the sorbic acid, expressed in millilitres of 0,1 mol/l alkaline solution, is given by formula (7) :

$$\frac{20}{11{,}2} \times \frac{A_x}{A_r} \times \frac{V'}{1\,000} \qquad \ldots (7)$$

where

V' is the remaining volume, in millilitres, of distillate, slightly less than V (the volume of distillate collected — see 8.2.3);

A_x and A_r have the same meanings as in 8.2.4;

11,2 is the mass, in milligrams, of sorbic acid equivalent to 1 ml of 0,1 mol/l sodium hydroxide solution.

8.2.6 Method of calculation and formulae

Correct the volume V_4 of sodium hydroxide solution (4.1) used for the titration for the acidity of the sorbic acid as follows.

8.2.6.1 Liquid products

The volatile acidity of the sample, expressed in milliequivalents per 100 ml, or in grams of acetic acid per 100 ml, is given respectively by formulae (8) and (9) :

$$\frac{10}{V_0}\left[V_4 - \left(\frac{20}{11{,}2} \times \frac{A_x}{A_r} \times \frac{V'}{1\,000}\right)\right]\frac{V}{V'} \qquad \ldots (8)$$

$$\frac{0{,}6}{V_0}\left[V_4 - \left(\frac{20}{11{,}2} \times \frac{A_x}{A_r} \times \frac{V'}{1\,000}\right)\right]\frac{V}{V'} \qquad \ldots (9)$$

where

V_4 is the corrected volume, in millilitres, of sodium hydroxide solution (4.1) used for the titration;

A_x and A_r have the same meanings as in 8.2.4;

V and V' have the same meanings as in 8.2.5;

V_0 has the same meaning as in 7.1.1.

8.2.6.2 Viscous or solid or frozen products

The volatile acidity of the sample, expressed in milliequivalents per 100 g, or in grams of acetic acid per 100 g, is given respectively by formulae (10) and (11) :

$$\frac{10}{m_0}\left[V_4 - \left(\frac{20}{11{,}2} \times \frac{A_x}{A_r} \times \frac{V'}{1\,000}\right)\right]\frac{V}{V'} \qquad \ldots (10)$$

$$\frac{0{,}6}{m_0}\left[V_4 - \left(\frac{20}{11{,}2} \times \frac{A_x}{A_r} \times \frac{V'}{1\,000}\right)\right]\frac{V}{V'} \qquad \ldots (11)$$

where

V_4 has the same meaning as in 8.2.6.1;

A_x and A_r have the same meanings as in 8.2.4;

V and V' have the same meanings as in 8.2.5;

m_0 has the same meaning as in 7.1.2.

1) For products containing volatile oils and volatile aromatic compounds (such as the aurantiaceae), only the colorimetric method may be used.

8.3 Determination of benzoic acid in the distillate
(with or without the presence of sulphur dioxide)

8.3.1 Reagent

8.3.1.1 Benzoic acid, standard solution containing 20 mg of benzoic acid, or 23,6 mg of sodium benzoate, per litre.

8.3.2 Identification and determination

Collect a known volume V (about 250 ml) of distillate as described in 6.3. Confirm the presence of benzoic acid and determine the benzoic acid content before the determination of volatile acidity.

8.3.2.1 Identification[1)]

Place 2 ml of distillate in a silica cell (see 8.2.2.4), and record the absorption spectrum between 220 and 300 nm. If the absorbance exceeds 0,7, carry out the same examination on the distillate diluted 1 + 1, 1 + 3 or 1 + 9, with water.

Benzoic acid is characterized in this solvent by two maximum absorption bands, one between 225 and 230 nm, and the other at 268 nm.

8.3.2.2 Determination

8.3.2.2.1 In the absence of volatile oils, aromatic compounds and sulphur dioxide, measure the maximum absorbance, between 225 and 230 nm, of the distillate, suitably diluted, in a silica cell (see 8.2.2.4), using water as the reference liquid.

Compare the absorbance with that of the standard benzoic acid solution (8.3.1.1) diluted 1 + 1 with water.

8.3.2.2.2 If the product contains sulphur dioxide, dilute the distillate 1 + 1, 1 + 3 or 1 + 9 with the oxidizing agent (8.2.1.2), shake and leave exposed to the air for a few minutes.

Measure the maximum absorbance, between 225 and 230 nm, using, as the reference liquid, a blank solution prepared by diluting the oxidizing agent (8.2.1.2) with water, to replace the distillate.

8.3.2.2.3 If the product contains volatile oils or volatile aromatic compounds (such as the aurantiaceae), with or without sulphur dioxide, place 10 ml of the distillate in the dish (8.2.2.1). Make alkaline by adding 1 ml of the lime water (4.4), add 1 drop of the copper(II) sulphate solution (8.2.1.3) and evaporate to dryness on the boiling water bath (8.2.2.3). Dissolve the residue in 10 ml of water.

Measure the maximum absorbance of the solution at about 230 nm using, as the reference liquid, a blank solution prepared by the same procedure, but using 10 ml of water in place of the distillate.

Compare the absorbance with that of the standard benzoic acid solution (8.3.1.1) diluted 1 + 1 with water.

8.3.3 Expression of results

The benzoic acid content of the distillate, expressed in milligrams per litre, is given by formula (6') :

$$20 \times \frac{A_x}{A_r} \qquad \ldots (6')$$

where

A_x is the absorbance of the diluted distillate (see 8.3.2.2);

A_r is the absorbance of the reference solution (8.3.1.1), diluted in the same manner (see 8.3.2.2).

8.3.4 Determination of distillate acidity

The acidity of benzoic acid, expressed in millilitres of 0,1 mol/l alkaline solution, is given by formula (12) :

$$\frac{20}{12,2} \times \frac{A_x}{A_r} \times \frac{V''}{1\,000} \qquad \ldots (12)$$

where

V'' is the remaining volume, in millilitres, of distillate, slightly less than V (the volume of distillate collected — see 8.3.2);

A_x and A_r have the same meanings as in 8.3.3;

12,2 is the mass, in milligrams, of benzoic acid equivalent to 1 ml of 0,1 mol/l sodium hydroxide solution.

8.3.5 Method of calculation and formulae

Correct the volume V_5 of sodium hydroxide solution (4.1) used for the titration for the acidity of the benzoic acid as follows.

8.3.5.1 Liquid products

The volatile acidity of the sample, expressed in milliequivalents per 100 ml, or in grams of acetic acid per 100 ml, is given respectively by formulae (13) and (14) :

$$\frac{10}{V_0}\left[V_5 - \left(\frac{20}{12,2} \times \frac{A_x}{A_r} \times \frac{V''}{1\,000}\right)\right]\frac{V}{V''} \qquad \ldots (13)$$

$$\frac{0,6}{V_0}\left[V_5 - \left(\frac{20}{12,2} \times \frac{A_x}{A_r} \times \frac{V''}{1\,000}\right)\right]\frac{V}{V''} \qquad \ldots (14)$$

1) For products containing volatile oils and volatile aromatic compounds (such as the aurantiaceae), the distillate should be treated as indicated in 8.3.2.2.3 before examination of the ultraviolet spectrum.

where

V_5 is the corrected volume, in millilitres, of sodium hydroxide solution (4.1) used for the titration;

A_x and A_r have the same meanings as in 8.3.3;

V and V'' have the same meanings as in 8.3.4;

V_0 has the same meaning as in 7.1.1.

8.3.5.2 Viscous or solid or frozen products

The volatile acidity of the sample, expressed in milliequivalents per 100 g, or in grams of acetic acid per 100 g, is given respectively by formulae (15) and (16) :

$$\frac{10}{m_0}\left[V_5 - \left(\frac{20}{12{,}2} \times \frac{A_x}{A_r} \times \frac{V''}{1\,000}\right)\right]\frac{V}{V''} \qquad \ldots (15)$$

$$\frac{0{,}6}{m_0}\left[V_5 - \left(\frac{20}{12{,}2} \times \frac{A_x}{A_r} \times \frac{V''}{1\,000}\right)\right]\frac{V}{V''} \qquad \ldots (16)$$

where

V_5 has the same meaning as in 8.3.5;

A_x and A_r have the same meanings as in 8.3.3;

V and V'' have the same meanings as in 8.3.4;

m_0 has the same meaning as in 7.1.2.

8.4 Determination of volatile acidity in the presence of formic acid (with or without the presence of sulphur dioxide)

8.4.1 Reagents

8.4.1.1 Sodium acetate, crystallized.

8.4.1.2 Mercury(II) chloride solution.

Dissolve 1,5 g of mercury(II) chloride in 100 ml of water.

8.4.1.3 Potassium dichromate, 33,79 g/l solution.

8.4.1.4 Sulphuric acid, diluted 2 + 1.

8.4.1.5 Iron(II) sulphate.

8.4.2 Apparatus

8.4.2.1 Microscope.

8.4.2.2 Boiling water bath.

8.4.2.3 Heat-resistant dish, of capacity 500 ml.

8.4.3 Identification of formic acid and determination of volatile acidity

8.4.3.1 Identification of formic acid

Collect a known volume V (about 250 ml) of distillate as described in 6.3. Confirm the presence of formic acid before the determination of volatile acidity.

Transfer 2 ml of distillate[1] to a test tube. Add 0,25 g of the sodium acetate (8.4.1.1) and 1 ml of the mercury(II) chloride solution (8.4.1.2). Heat on the boiling water bath (8.4.2.2) for 10 min. After cooling, examine the crystalline precipitate of mercury(II) chloride under the microscope for cross- or X-shaped crystals which characterize the presence of formic acid.

Under the conditions indicated, the presence of formic acid is detected in the initial product (6.2) at a minimum level of detection of 0,5 g/l, which is much less than the effective antiseptic dose. However, smaller traces of formic acid which may occur naturally in vegetable products cannot be detected in this way.

8.4.3.2 Oxidation of formic acid and of any sulphur dioxide

Titrate the volatile acidity of the distillate with the sodium hydroxide solution (4.1) using the phenolphthalein solution (4.2) as indicator. Add 10 ml of the sodium hydroxide solution to make the distillate strongly alkaline, and concentrate the volume in the dish (8.4.2.3) on the boiling water bath (8.4.2.2) to obtain 2 to 5 ml of liquid residue. Alcohols, aldehydes, ketones, esters, volatile oils and aromatic compounds are thus removed. Put the residue in the bubbler (5.2.2) of the steam distillation apparatus (5.2), rinse the dish with 10 ml of the potassium dichromate solution (8.4.1.3), then with 10 ml of the sulphuric acid solution (8.4.1.4), collecting these liquids successively in the bubbler. Shake and leave for at least 15 h.

Under these conditions, the formic acid and sulphur dioxide are totally oxidized while the acetic acid and other higher homologous compounds which constitute the volatile acidity remain unaffected. (This procedure does not allow specific determination of the formic acid contained in the distillate.)

Reduce the pressure in the bubbler, and shake to remove the carbon dioxide formed. Reduce the excess of potassium dichromate by adding 2 to 3 g of the iron(II) sulphate (8.4.1.5), collect the distillate and titrate the volatile acids by the procedure described in 6.3 and 6.4.

8.4.4 Expression of results

See 7.1.

1) Taking 2 ml of distillate for the identification of the formic acid (8.4.3.1) introduces a negligible error in the result of the determination (8.4.3.2).