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Fruit and vegetable products — Determination of ethanol

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FOREWORD

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Draft International Standards adopted by the Technical Committees are circulated to the Member Bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 2448 was drawn up by Technical Committee ISO/TC 34, *Agricultural food products*, and circulated to the Member Bodies in July 1971.

It has been approved by the Member Bodies of the following countries :

Australia	France	Portugal
Brazil	Hungary	South Africa, Rep. of
Bulgaria	India	Spain
Chile	Iran	Thailand
Czechoslovakia	Korea, Dem.P.Rep. of	Turkey
Egypt, Arab Rep. of	Netherlands	United Kingdom
Finland	Poland	

The Member Body of the following country expressed disapproval of the document on technical grounds :

Germany

Printed in Switzerland

Fruit and vegetable products — Determination of ethanol

1 SCOPE AND FIELD OF APPLICATION

1.1 Scope

This International Standard specifies a method for the chemical determination of ethanol in fruit and vegetable products.

1.2 Field of application

The method is not applicable to products containing more than 5 % (*m*/*m*) of ethanol.

In the case of products containing essential oils, it is necessary to remove the latter (see section 8).

2 DEFINITION

For the purpose of this International Standard, the following definition applies :

ethanol : The whole of the products which are oxidizable under the conditions of the method described.

The ethanol content is expressed as a percentage by mass in the case of solid products and as grams per 100 ml in the case of liquid products.

3 PRINCIPLE

Separation of the ethanol by distillation, followed by oxidation by potassium dichromate in a sulphuric acid medium. Determination of the excess dichromate by ammonium iron(II) sulphate in the presence of ferrous *o*-phenanthroline as indicator.

4 REAGENTS

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equal purity.

4.1 Sulphuric acid, ρ_{20} = 1,836 g/ml.

4.2 Sulphuric acid, ρ_{20} = 1,488 g/ml, solution containing 500 ml of sulphuric acid (4.1) per litre.

4.3 Calcium hydroxide, [$Ca(OH)_2$], suspension obtained by slaking 110 to 112 g of calcium oxide in 1 litre of water.

4.4 Potassium dichromate solution, 42,672 g of $K_2Cr_2O_7$ per litre.

1 ml of this solution is equivalent to 0,01 g of ethanol.

4.5 Potassium permanganate solution, 1,372 g of $KMnO_4$ per litre.

10 ml of this solution is equivalent to 1 ml of ammonium iron(II) sulphate solution (4.6).

4.6 Ammonium iron(II) sulphate hexahydrate [$(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$], 170,2 g/l solution containing 20 ml of sulphuric acid (4.1) per litre, obtained by dissolving the salt in water, adding the sulphuric acid (4.1) and diluting to the mark with water.

Stabilize by the addition of aluminium chips.

2 ml of this solution is equivalent to 1 ml of potassium dichromate solution (4.4).

4.7 Ferrous *o*-phenanthroline solution

Dissolve 0,695 g of iron(II) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) in 100 ml of water, add 1,485 g of *o*-phenanthroline monohydrate and heat to aid solution.

The solution keeps well.

5 APPARATUS

5.1 Distillation apparatus, comprising a 500 ml flask surmounted by a fractionation column and a condenser ending in a slightly tapered extension piece long enough to reach to the bottom of a 100 ml volumetric flask.

Any other steam distillation apparatus may be used, provided that it satisfies the following test :

200 ml of a 10 % ethanol/water mixture distilled five times in succession shall contain at least 9,9 % of ethanol after the last distillation, i.e. no loss of ethanol greater than 0,02 % shall occur in the course of a distillation.

5.2 Heating apparatus which does not cause even slight decomposition of the extractable materials contained in the flask.

5.3 One-mark volumetric flasks, capacity 100 ml, complying with Class A of ISO/R 1042.

5.4 One-mark pipettes, capacities 5, 10 and 20 ml, complying with Class A of ISO/R 648.

5.5 Burettes, fitted with stopcock, capacity 50 ml, complying with Class A of ISO/R 385.

5.6 Wide-necked flasks, capacity 250 ml, fitted with ground glass stoppers, clean, dry, free from grease and airtight (absolute tightness can be ensured with the aid of a polytetrafluorethylene sleeve).

5.7 Blender.

5.8 Analytical balance.

6 PROCEDURE

6.1 Preparation of the test sample.

6.1.1 *Solid or thick products* (purée, marmalade or jam, fruits, vegetables)

Blend mechanically the whole of the sample supplied, taking care not to raise the temperature of the product, and take a sufficient quantity of the product to enable two parallel determinations to be carried out.

6.1.2 *Liquid products* (juices, pulps and syrups)

Thoroughly mix the sample and take a sufficient quantity of the product to enable two parallel determinations to be carried out.

6.2 Test portion

Weigh, to the nearest 0,01 g, a mass of the prepared sample, or take a volume of it, such that the quantity of ethanol collected in 100 ml of distillate is less than 1 g.

6.3 Determination

6.3.1 *Distillation*

Dilute the test portion with about 50 ml of water and transfer it quantitatively to the flask of the distillation apparatus (5.1). Rinse the vessel used to take the test portion with not more than 120 ml of water, collecting the rinsing water in the flask.

Make the product slightly alkaline (pH close to 8) with the calcium hydroxide suspension (4.3), shaken before use.

Add glass beads or pieces of porcelain to control the rate of boiling. Pour 10 ml of water into a 100 ml volumetric flask (5.3) and insert the end of the tapered extension of the distillation apparatus so that it is immersed in the liquid.

Distil in such a way that the distillate, when it reaches the volumetric flask, is at a relatively low temperature (15 to 20 °C).

Collect about 80 to 85 ml of distillate. Stop the distillation and rinse the condenser and extension with a few millilitres of water.

Shake the volumetric flask to mix the contents. If necessary immerse the flask in cold water (15 to 20 °C) for a few minutes.

Dilute the contents of the volumetric flask to the mark with water and shake.

6.3.2 *Oxidation*

Pour 20 ml (volume V_1) of potassium dichromate solution (4.4), accurately measured (see 6.5.1), and 20 ml of sulphuric acid solution (4.2) into a 250 ml flask with ground glass stopper (5.6) and shake.

Add 10 ml (volume V_0) of distillate, accurately measured. Stopper the flask, moistening the stopper with a drop of sulphuric acid (4.1), shake and wait for at least 30 min, shaking the flask from time to time.

The resultant mixture should in no case assume the green coloration of the chromium cation, indicating that the ethanol content of the test portion is too high. If this occurs, recommence the oxidation taking a smaller amount of distillate (for example 5 ml). If necessary, recommence both distillation and oxidation taking a smaller test portion. Take account of any such changes in the calculations.

6.3.3 *Titration*

Titrate the excess of dichromate, using the ammonium iron(II) sulphate solution (4.6). The excess of dichromate should be at least equal to 20 % of the quantity used for the blank test. Shake the flask after each addition.

When the colour changes to greenish blue, add 4 drops of ferrous *o*-phenanthroline solution (4.7). (Another appropriate coloured indicator may be used, at the discretion of the operator, see 6.5.2.) Continue the addition of the ammonium iron(II) sulphate solution (4.6) until the colour of the medium changes from greenish blue to brown. If the end-point is passed, return to it precisely by adding potassium permanganate solution (4.5). Deduct, from the volume of the ammonium iron(II) sulphate solution (4.6) used, one tenth of the volume of potassium permanganate solution (4.5) added. Let V_2 be this difference, which represents the exact volume of ammonium iron(II) sulphate solution (4.6) equivalent to the excess potassium dichromate.

6.3.4 Carry out two determinations on the same prepared sample.

6.4 Blank test

Carry out a blank test in the same conditions as for the titration, replacing the volume, V_0, of distillate by the same volume of distilled water. Let V_3 be the volume of ammonium iron(II) sulphate solution (4.6) used following the procedure of 6.3.3.

6.5 Notes on procedure

6.5.1 If the test portion contains too little ethanol, a smaller amount of potassium dichromate solution (4.4) may be used, i.e. 10 or 5 ml of this solution, diluted with 10 or 15 ml of distilled water, respectively. Take account of any such change in the calculations.

6.5.2 The titration of the excess of dichromate with the solution of ammonium iron(II) sulphate (4.6) can also be carried out in the presence of a mixture of

- 1 ml of orthophosphoric acid (85 %), $\rho_{20} = 1{,}71$ g/ml;

and

- 1 ml of a solution of barium diphenylaminesulphonate containing 0,5 g per 100 ml.

7 EXPRESSION OF RESULTS

7.1 Method of calculation and formula

7.1.1 *Solid products*

The ethanol content, expressed as a percentage by mass, is equal to :

$$0{,}01\ V_1 \times \frac{(V_3 - V_2)}{V_3} \times \frac{100}{V_0} \times \frac{100}{m}$$

where

m is the mass, in grams, of the test portion;

V_0 is the volume, in millilitres, of distillate taken for the titration;

V_1 is the volume, in millilitres, of potassium dichromate solution used for the oxidation;

V_2 is the volume, in millilitres, of ammonium iron(II) sulphate solution used for the back titration of the dichromate;

V_3 is the volume, in millilitres, of ammonium iron(II) sulphate solution used in the blank test.

7.1.2 *Liquid products*

The ethanol content, expressed in grams per 100 ml of product, is equal to :

$$0{,}01\ V_1 \times \frac{(V_3 - V_2)}{V_3} \times \frac{100}{V_0} \times \frac{100}{V_4}$$

where

V_0, V_1, V_2 and V_3 have the same meaning as in 7.1.1;

V_4 is the volume, in millilitres, of the test portion.

Take as the result the arithmetic mean of the two determinations if the requirement concerning repeatability (see 7.2) is satisfied. If this condition is not met, repeat the determinations.

Report the result to two significant figures.

7.2 Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 1 % of the mean value.

8 SPECIAL CASE : PRODUCT CONTAINING ESSENTIAL OILS

In the presence of essential oils, the distillate is turbid, with drops of essential oil floating on the surface. The procedure should be modified as follows :

Collect the distillate in the 100 ml volumetric flask and allow it to stand for 2 h. Dilute to the mark with water, the interface between the two phases, essential oil and water, being at the level of the mark. Allow to stand for a further 1 to 2 h.

Discard the small quantity of essential oil collected on the surface, either by suction with a fine pipette, or by filtration through paper in a covered funnel.

Transfer the still turbid filtrate to a 150 ml flask with 10 g of polystyrene granules (granule size 1 to 2 mm). Shake the stoppered flask for 15 min and then filter the mixture through gauze in a covered funnel; the liquid should then have become clear and have lost its odour almost completely. Proceed with the determination on this liquid.

9 TEST REPORT

The test report shall show the method used and the result obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the result.

The report shall include all details for complete identification of the sample.