International Standard 7466

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Fruit and vegetable products — Determination of 5-hydroxymethylfurfural (5-HMF) content

Produits dérivés des fruits et légumes — Détermination de la teneur en hydroxy-5 méthylfurfural (5-HMF)

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Foreword

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Fruit and vegetable products — Determination of 5-hydroxymethylfurfural (5-HMF) content

1 Scope and field of application

This International Standard specifies a method for the determination of the 5-hydroxymethylfurfural (5-HMF) content of products produced by the thermal processing of fruits and vegetables.

2 Principle

Preparation of a test solution by adding water to a test portion.

In the case of products containing more than 10 mg of free sulfur dioxide per litre, addition of acetaldehyde.

Addition of *p*-toluidine and barbituric acid solution to an aliquot portion of the test solution, thus forming a red coloration by reaction with the 5-hydroxymethylfurfural present.

Spectrometric measurement at a wavelength of 550 nm.

3 Reagents

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity, recently boiled.

3.1 Carrez (I) solution.

Dissolve 150 g of potassium hexacyanoferrate(II) in water and dilute to 1 000 ml.

3.2 Carrez (II) solution.

Dissolve 300 g of zinc sulfate heptahydrate in water and dilute to 1 000 ml.

3.3 Barbituric acid solution.

Dissolve 0,500 g of barbituric acid in about 70 ml of water, by heating on a water bath if necessary, transfer quantitatively to a 100 ml one-mark volumetric flask, allow to cool if necessary, and dilute to the mark.

This solution shall be stored in a refrigerator.

3.4 *p*-toluidine reagent.

Dissolve 10,0 g of *p*-toluidine (of melting point 45 °C) in about 50 ml of propan-2-ol, by heating on a water bath if necessary, add 10 ml of glacial acetic acid, transfer quantitatively to a 100 ml one-mark volumetric flask, allow to cool if necessary, and dilute to the mark with propan-2-ol.

This reagent may be stored in a dark brown bottle in a refrigerator for 1 month.

3.5 Acetaldehyde, 1 % (*V*/*V*) solution (in the case of free sulfur dioxide contents exceeding 10 mg/l).

3.6 5-hydroxymethylfurfural (5-HMF), standard solutions.

NOTE — Commercial grades of unknown purity may be purified by vacuum distillation (115 ± 3 °C; 1,333 Pa) and stored under nitrogen.

3.6.1 Stock solution

Dissolve 200 mg of 5-HMF in water, transfer quantitatively to a 200 ml one-mark volumetric flask, dilute to the mark and mix well.

3.6.2 Working solution

Transfer 1 ml of the stock solution (3.6.1) to a 100 ml one-mark volumetric flask, dilute to the mark with water and mix well.

4 Apparatus

Usual laboratory equipment, and in particular :

4.1 Spectrometer, suitable for making measurements at 550 nm, and equipped with cells of optical path length 1 cm.

4.2 One-mark volumetric flasks, of capacities 25, 100 and 200 ml.

4.3 Test tubes, graduated at 10 ml, with ground stoppers.

4.4 Water bath, thermostatically controlled at 20 ± 1 °C.

4.5 Analytical balance.

5 Procedure

5.1 Preparation of the test sample

If necessary remove seeds and hard seed-cavity walls, and then thoroughly mix the sample. Certain products will require no more than filtration.

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.

5.2 Test portion

Take 20 ml or 20 g of the test sample.

NOTE — In the case of products with high 5-HMF contents, smaller test portions, for example of 10 ml or 10 g, or 5 ml or 5 g, may be taken.

5.3 Preparation of the test solution

5.3.1 Colourless or lightly coloured homogeneous products

Transfer the test portion to a 100 ml one-mark volumetric flask (4.2). Place on the water bath (4.4), controlled at 20 ± 1 °C. Dilute to the mark with water.

If necessary, filter the solution, discarding the first 20 ml of filtrate.

5.3.2 Products which have been homogenized, and strongly coloured products

Transfer the test portion to a 100 ml one-mark volumetric flask (4.2) and add 50 ml of water.

By means of pipettes, and operating with care, add 2 ml of the Carrez (I) solution (3.1) and 2 ml of the Carrez (II) solution (3.2). Dilute to the mark with water and mix. Filter, and discard the first 20 ml of filtrate.

5.4 Determination

5.4.1 Products having free sulfur dioxide contents less than 10 mg/l

5.4.1.1 Transfer, by means of a pipette, 2 ml of the test solution (5.3.1 or 5.3.2) to each of two test tubes (4.3) (A and B). Add 5 ml of the *p*-toluidine reagent (3.4), stopper and shake. Leave for 1 to 2 min.

5.4.1.2 Add to the contents of tube A, 1 ml of water and shake. Fill a spectrometric cell with this solution (reference solution).

5.4.1.3 Add to the contents of tube B, 1 ml of the barbituric acid solution (3.3) and shake. Fill a spectrometric cell with this solution and measure its absorbance at 550 nm, within 3 to 4 min of the addition of the barbituric acid solution, against the reference solution (5.4.1.2).

NOTE — The absorbance reaches a maximum 3 to 4 min after the addition of the barbituric acid solution and then falls abruptly. The reading should be taken at maximum absorbance.

5.4.2 Products having free sulfur dioxide contents greater than 10 mg/l

5.4.2.1 Preliminary elimination of sulfur dioxide.

Transfer 15 ml of the test solution (5.3.1 or 5.3.2) to a 25 ml one-mark volumetric flask, add 2 ml of the acetaldehyde solution (3.5), shake, dilute to the mark with water and shake again.

5.4.2.2 Proceed as specified in 5.4.1.

5.5 Preparation of the calibration graph

Transfer to a series of six tubes (4.3) 0,2 — 0,6 — 1,0 — 1,4 — 1,8 and 2,0 ml, respectively, of the standard 5-HMF working solution (3.6.2). Add water to each tube (except the last) to bring the total volume to 2 ml.

These solutions contain, respectively, 2 — 6 — 10 — 14 — 18 and 20 µg of 5-HMF.

Proceed as described in 5.4.1.

Plot a graph of the 5-HMF contents, in micrograms, against the corresponding absorbances.

6 Expression of results

The 5-HMF content, expressed in milligrams per litre or in milligrams per kilogram, is equal to

$$\frac{m_1 \times V_1}{V_2 \times V_0}$$

or

$$\frac{m_1 \times V_1}{V_2 \times m_0}$$

where

m_0 is the mass, in grams, of the test portion (in the case of test portions taken by mass);

m_1 is the mass, in micrograms, of 5-HMF corresponding to the absorbance measured in 5.4.1 or 5.4.2, read from the calibration graph;

V_0 is the volume, in millilitres, of the test portion (in the case of test portions taken by volume);

V_1 is the volume, in millilitres, of the test solution (i.e. 100 ml);

V_2 is the volume, in millilitres, of the aliquot portion taken for the determination (i.e 2 ml).

Express the result to the nearest whole integer, as follows :

a) for fruit juices, in milligrams per litre;

b) for all other products, in milligrams per litre or per kilogram.

7 Test report

The test report shall show the method used and the results obtained. It shall also mention any operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances likely to have affected the results.

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

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