INTERNATIONAL STANDARD

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Modified starch — Determination of adipic acid content of acetylated di-starch adipates — Gas chromatographic method

Amidons et fécules modifiés — Détermination de la teneur en acide adipique dans les adipates de diamidon acétylés — Méthode par chromatographie en phase gazeuse

Reference number
ISO 11215:1998(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 11215 was prepared by Technical Committee ISO/TC 93, *Starch (including derivatives and by-products).*

Annexes A to C of this International Standard are for information only.

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Modified starch — Determination of adipic acid content of acetylated di-starch adipates — Gas chromatographic method

1 Scope

This International Standard specifies a method for the gas chromatographic determination of total adipic content and free adipic acid content of acetylated di-starch adipates.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of the publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on the International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 1666:1996, *Starch — Determination of moisture content — Oven-drying method*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

3 Principle

The test portion is dispersed in moderately concentrated sodium hydroxide solution, to hydrolyse fully the adipate from the starch. After acidification, the free adipic acid is extracted with ethyl acetate. The ethyl acetate is removed, and the dry residue is silylated. An aliquot portion of this solution is injected into a gas chromatograph equipped with a capillary column. Pimelic acid is used as internal standard.

Free adipic acid is extracted by washing out the starch with water, acidifying the extract, and extracting the free acid with ethyl acetate.

The determination is performed by silylation and gas chromatography as described above.

4 Reagents and materials

Use only reagents of recognized analytical grade.

4.1 **Water**, complying with at least grade 3 in accordance with ISO 3696.

4.2 **Waxy maize starch**, commercial grade.

NOTE Waxy maize starch is chosen as the base material, as it represents the bulk of starch adipate on the market. This may be substituted with another native starch, if appropriate.

4.3 Adipic acid solution, $\rho(C_6H_{10}O_4)$ = 50,0 mg/l.

4.4 Pimelic acid solution, $\rho(C_7H_{12}O_4)$ = 50,0 mg/l.

4.5 Sodium hydroxide solution, c(NaOH) = 4 mol/l.

4.6 Hydrochloric acid, concentrated, c(HCl) = 12 mol/l.

4.7 Ethyl acetate ($C_4H_8O_2$).

4.8 Nitrogen gas, purity 99 %.

4.9 Acetonitrile

4.10 Silylation reagent: bis(trimethylsilyl)trifluoroacetamide (BSTFA) which includes 1 % trimethylchlorosilane (TMCS).

4.11 Helium gas, purity 99,9999 % (e.g. grade N60).

4.12 Hydrogen gas, purity 99,99 % (e.g. grade N400 or better).

4.13 Air, purity 99,999 % (e.g. grade S).

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Glass reaction tubes, 100 mm x 16 mm, with screw caps fitted with polytetrafluoroethylene (PTFE) covered rubber seals resistant to concentrated hydrochloric acid (4.6).

5.2 Pipettes, adjustable, of 1,00 ml and 5,00 ml capacity, accurate to 0,01 ml.

NOTE The pipettes should be tested to see if they comply to manufacturer's tolerance. Calibration may be required.

5.3 Rotary shaker.

5.4 Pasteur pipettes.

5.5 Heating device, capable of being maintained at (30 ± 2) °C.

5.6 Evaporation device, based on solvent removal with a stream of nitrogen (e.g. Pierce Reacti-Vap III)[1)].

5.7 Ultrasonic bath, power 120 W.

5.8 Gas chromatograph, accommodating capillary columns, fitted with a flame ionization detector, on-column injector, and a computer integrator. See annex A for typical conditions for chromatography.

5.9 Sieve, 800 µm.

5.10 Blade mill.

5.11 Laboratory centrifuge, capable of operating at a radial acceleration of 1 100 g_n.

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1) Pierce Reacti-Vap III is an example of suitable apparatus available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this apparatus.

6 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard.

7 Preparation of test sample

Sieve the laboratory sample through the 800 µm sieve (5.9). If the material does not pass through the sieve, then grind the sample with a blade mill (5.10) until it passes completely through the 800 µm sieve. Homogenize the sample.

8 Procedure

8.1 Calibration for total adipic acid content

8.1.1 Weigh, to the nearest 0,1 mg, approximately 50 mg of waxy maize starch (4.2) into each of four glass reaction tubes (5.1).

8.1.2 Into one tube, pipette (5.2) 1,00 ml of adipic acid solution (4.3) and into the others 0,75 ml, 0,50 ml and 0,25 ml, respectively, of adipic acid solution (4.3).

8.1.3 Adjust the volume in each tube to 1,5 ml with water (4.1) and add 1,00 ml of pimelic acid solution (4.4) to each tube. Each tube then contains 50 µg of pimelic acid, and 50,0 µg, 37,5 µg, 25,0 µg and 12,5 µg respectively of adipic acid.

NOTE It is possible that pimelic acid contains some adipic acid. If this is proven, a fifth tube should be prepared in a similar fashion but without addition of adipic acid solution (4.3).

8.1.4 Agitate the tubes manually to disperse the starch fully. Add 2,5 ml of sodium hydroxide solution (4.5).

8.1.5 Seal the tubes well and place on the rotary shaker (5.3) for 5 min.

8.1.6 Remove the tubes and, with cooling, add 1,0 ml of hydrochloric acid (4.6). Mix well.

8.1.7 Add 5 ml of ethyl acetate (4.7). Seal the tubes tightly and shake vigorously for 1 min.

8.1.8 Let the tubes stand until good phase separation is achieved. With a Pasteur pipette (5.4), transfer the supernatant layer (ethyl acetate) to a clean screw-top tube.

Make sure that none of the aqueous layer is carried over with the organic layer.

8.1.9 Place the tubes in the heating device (5.5) set at 30 °C, and evaporate the ethyl acetate completely under a stream of nitrogen (4.8) with the evaporation device (5.6).

8.1.10 Repeat steps 8.1.7 to 8.1.9 three times more, accumulating the dried residue in the same tube.

8.1.11 Dissolve the total residue in 0,6 ml of acetonitrile (4.9) and place the closed tubes in the ultrasonic bath (5.7) for 2 min.

8.1.12 Add 0,3 ml of the silylation reagent (4.10). Close the tubes and homogenize in the ultrasonic bath for 2 min.

8.1.13 Place the tubes in the heating device (5.5) set at 30 °C, for 30 min to complete derivatization.

8.1.14 Inject 0,5 µl of the solution into the gas chromatograph. See annex A for typical conditions for chromatography.

8.2 Total adipic acid content

8.2.1 Weigh, to the nearest 0,1 mg, approximately 50 mg of the prepared test sample into a glass reaction tube (5.1).

8.2.2 Add 1,5 ml of water (4.1) and 1,00 ml of pimelic acid solution (4.4) and shake well to disperse fully the test portion.

8.2.3 Proceed in accordance with 8.1.4 up to and including 8.1.14.

8.3 Calibration for free adipic acid content

8.3.1 Weigh, to the nearest 0,1 mg, approximately 500 mg of waxy maize starch (4.2) into each of four glass reaction tubes (5.1).

8.3.2 Into one tube, pipette (5.2) 1,00 ml of adipic acid solution (4.3) and into the others 0,75 ml, 0,50 ml and 0,25 ml, respectively, of adipic acid solution (4.3).

8.3.3 Adjust the volume in each tube to 4,0 ml with water (4.1) and add 1,00 ml of pimelic acid solution (4.4) to each tube. Each tube then contains 50 µg of pimelic acid, and 50,0 µg, 37,5 µg, 25,0 µg and 12,5 µg, respectively, of adipic acid.

NOTE It is possible that pimelic acid contains some adipic acid. If this is proven, a fifth tube should be prepared in a similar fashion but without addition of adipic acid solution (4.3).

8.3.4 Seal the tubes and agitate for 16 h in a shaker.

8.3.5 Remove the tubes from the shaker and centrifuge for 5 min at a radial acceleration of 1 100 g_n in the centrifuge (5.11).

8.3.6 Transfer the clear supernatant liquid into a clean glass reaction tube (5.1). Add 50 µl of hydrochloric acid (4.6) and 5 ml of ethyl acetate (4.7).

8.3.7 Seal the tubes tightly and shake vigorously for 1 min.

8.3.8 Proceed in accordance with 8.1.8 up to and including 8.1.14.

8.4 Free adipic acid content

8.4.1 Weigh, to the nearest 0,1 mg, approximately 500 mg of the prepared test sample into a glass reaction tube (5.1).

8.4.2 Add 4,0 ml of water (4.1) and 1,00 ml of pimelic acid solution (4.4) and shake well to disperse fully the test portion.

8.4.3 Proceed in accordance with 8.3.4 up to and including 8.3.8.

8.5 Moisture content

Determine the moisture content of the test sample in accordance with ISO 1666.