ACETALDEHYDE

PRINCIPLE

Acetaldehyde is released from the syrup by treatment with dilute phosphoric acid and heat. The liberated acetaldehyde is then measured using a head space gas chromatographic system equipped with a flame ionization detector. Results are reported as 11 dry substance.

SCOPE

The procedure is applicable to corn syrups including those containing fructose, and to most other starch hydrolyzates (Note 1).

SPECIAL APPARATUS

1. Gas Chromatograph: A single column gas chromatograph equipped with a head space sampling assembly and a flame ionization detector is required. An integrator or computer should be part of the system (Note 2).

2. Gas Chromatographic Column-Packed: A 6 ft × ⅛ inch stainless steel column packed with Porapak S, 80-100 mesh.

3. Gas Chromatograph Column-Capillary Column: 30m x 0.53mm Supel-Q PLOT from Supelco, part # 25462 or equivalent.

4. Sample vials, caps, seals and crimping and decapping tools (Note 3).

5. Autopipetor: Units capable of reproducibly delivering aqueous solutions to within ± 0.1% (Note 4).

REAGENTS

1. Purified Water: Chromatographic grade water (distilled or deionized), free of acetaldehyde or other interferences, is necessary.

2. Phosphoric Acid Solution: Add 12.171 g of concentrated phosphoric acid (85% H_3PO_4, sp g 1.68) and 500 mL of purified water to a 1 L volumetric

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flask. Swirl to dissolve, dilute to volume with purified water, and mix (Note 6).

3. Ethylidene Glucose Stock Solution (5.62 ppm) for Calibration: Accurately weigh 56.2 mg of 4,6-0-ethylidene-D-glucose (Aldrich catalogue #E3275-4 or Pfaltz and Bauer catalogue #E11450) into a beaker, and transfer to a one liter volumetric flask using purified water, dilute to volume. Then prepare a 5.62 ppm solution by diluting 100 ml of this solution to one liter with distilled water. Store capped in the refrigerator.

INSTRUMENT PARAMETERS

GC parameters for packed column

Note: As these parameters are instrument dependent, the values below are indicative.

1. Column Oven temperature: 130°C

2. Injector Port Temperature: 200°C

3. Detector Temperature: 200°C

4. Gas Pressure (column dependent): helium 25-35 psi; hydrogen and air, for maximum response (H_2 = ~ 40 ml/min, air = ~ 400 l/min)

5. Headspace Auto Sampler incubation temperature: 60°C

GC parameters for capillary column

Note: As these parameters are instrument dependent, the values below are indicative.

1. Column Oven temperature: 100-130°C

2. Injector Port Temperature: 150-200°C

3. Detector Temperature: 200-275°C

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4. Gas Pressure: He 10-30 psi; optimize hydrogen and air or maximum response

5. Headspace Auto Sampler incubation temperature: 60°C

PROCEDURE

Standardization:

The following instructions are for 20ml vials (See Note 7 for 9ml vials).

Standards (Note 8):

1. Standard 180µg/l acetaldehyde: Pipette 1.5ml std ethylidene glucose (5.62mg/l) + 1.5ml of 1.0% H3PO4 + 7.0 ml H2O into a vial. Cap immediately.

2. Standard 120µg/l acetaldehyde: Pipette 1.0ml std ethylidene glucose (5.62mg/l) + 1.0ml of 1.0% H3PO4 + 8.0 m H2O into a vial. Cap immediately.

3. Standard 90µg/l acetaldehyde: Pipette 0.75ml std ethylidene glucose (5.62m/l) + 0.75ml of 1.0% H3PO4 + 8.5 l H2O into a vial. Cap immediately.

4. Standard 60µg/l acetaldehyde: Pipette 0.5ml std ethylidene glucose (5.62g/l) + 0.5ml of 1.0% H3PO4 + 9.0ml H2O into a vial. Cap immediately.

5. Standard 30µg/l acetaldehyde: Pipette 0.25ml std ethylidene glucose (5.62mg/l) + 0.25ml of 1.0% of H3PO4 + 9.5ml H2O into a vial. Cap immediately.

6. Water/Acid blank: Pipette 10.0ml purified water + 0.5ml of 1.0 of H3PO4 in a vial and cap immediately.

Daily Calibration Check:

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Once a calibration curve has been established (see calculations below), a 60µg/l acetaldehyde standard prepared in the same manner as standard 4 above will be analyzed daily to check the calibration. Expected results for this standard should be 60ppb ± 5ppb.

Sample Preparation (20ml vials):

For HFS 55 with 77% dry substance:
Using an analytical balance weigh 1.5g of sample into a vial. Add 0.5ml of 1.0% H3PO4 using an automatic pipette. Add 8.5ml of purified water using a dispensette or pipette. Cap immediately. Note: all vials need to be mixed thoroughly. To insure complete mixing, check for striations in sample.

For HFS 42 with 71% dry substance:
Using an analytical balance weigh 1.6g of sample into a vial. Add 0.5ml of 1.0% H3PO4 using an automatic pipette. Add 8.4ml of purified water using a dispensette. Cap immediately.

Note: all vials need to be mixed thoroughly. To insure complete mixing check for striations in sample.

For samples with other % dry substance, the sample and water amount must be recalculated. For a sample with a% dry substance, the amount of syrup to be weighed is:

$$\frac{77 \times 1.5}{a}\ g$$

the amount of water to be added is:

$$10 - \frac{77 \times 1.5}{a}\ ml$$

Incubate all vials, standards and samples, in a boiling water bath for 60 minutes. Place samples and vials into the headspace auto sampler. Analyze samples using conditions as described under "Instrument parameters" (Note 9).

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Inject sample via headspace autosampler and measure peak height or peak area of acetaldehyde.

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CALCULATIONS

The concentration of acetaldehyde (A2) expressed in nanog per gram (ppb) of 11 dry substance sample, detected after heating at 60°C for one hour, is obtained by the following:

Acetaldehyde, ppb (11dry substance basis): Make a calibration curve (linear regression) using the peak height/areas of the acetaldehyde height/peak in the standards and the concentration of acetaldehyde in the standards. Calculate the level of acetaldehyde in the unknown samples with this calibration curve.

The calibration curve uses the standard formula of Y= mx+b generated by the instrument software where:

Y= peak height/area
x= nanog per gram acetaldehyde, 11 dry substance basis
m= slope of line
b= the y-intercept

NOTES AND PRECAUTIONS

1. Sulfur dioxide levels in excess of 10 ppm may interfere.

2. Automated head space gas chromatographic systems are recommended. PE AutoSystem XL GC with an HS40 XL headspace sampler or equivalent.

3. Seals give off various volatile impurities, and should be selected to minimize interferences during acetaldehyde measurements. The vials and seals used in this method are rated significantly higher than the 30 psig pressurization during analysis and the pressure generated during heating at 100ºC during hydrolysis of acetaldehyde adducts. However, care should be taken during heating to avoid superheating and violent bumping between vials or the sides of the heating vessel. A wire holder for the vials and a shatterproof screen for the heating bath are advised.

4. Autopipetors such as the BrandTech “Dispensette III,” available through Fisher Scientific are recommended.

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5. Purified water must be used to prepare all reagents and blanks to avoid contamination.

6. 85% phosphoric acid: Wash thoroughly after handling. Remove contaminated clothing and wash before re-use. Use only in a well ventilated area. Do not get into eyes, on skin, or on clothing. Do not ingest or inhale. Use only in a chemical fume hood. For more information, consult the Material Safety Data Sheet.

7. With 9.0ml vials, water blanks and acetaldehyde standards are prepared using 5.00ml total. Samples are prepared using one-half the amounts stated in the test for 20ml vials.

8. Dilute solutions of ethylidene glucose (molecular weight = 206) hydrolyze under the influence of acid and heat to generate an equimolar amount of acetaldehyde (molecular weight =44). Thus, a range of standards are prepared using equal amounts of 5.62ppm ethylidene glucose and 1% H3PO4 diluted to 10ml, sealed in head space vials and boiled for one hour to produce the desired µg/l acetaldehyde.

9. If the number of vials used significantly drops the temperature of the boiling water bath, the timer for the one hour equilibrium time should be started only after boiling is re-attained. If the equilibrated syrup samples are not to be run within three hours after boiling, vials should be refrigerated.

10. Attenuation of chromatograph may be necessary to keep the A2 peak on scale (retention time should be approximately 1.9 to 3.0 min, depending on column and flow rate.

METHOD HISTORY

Corn Syrup, Acetaldehyde (E-1), Date of Acceptance 4-15-1986, Revised 12-19-2006.