FAT, TOTAL (Hydrolysis)

PRINCIPLE

The major portions of the native fats in corn starch are bound in a manner as to render them unextractable by the usual methods of solvent extraction. When the starch is partially hydrolyzed with acid at an elevated temperature then cooled, fatty acids precipitate together with other residual material. These are removed by filtration and dried. The fatty acids are subsequently isolated by solvent extraction.

SCOPE

This method is applicable to starch and its modifications except for 1-octenyl succinic anhydride modified food starch,corn gluten feed, and corn gluten meal and other products derived from corn which contain complexed and/or bound fat.

SAFETY PRECAUTIONS

- N-hexane and petroleum ether are extremely flammable. Always work with these solvents under a fume hood (See Note 1).

- Hydrochloric Acid, 37%**:** This substance is classified as a POISON under the Federal Caustic Poison Act. Corrosive. Liquid and mist cause severe burns to all body tissue. May be fatal if swallowed. Harmful if inhaled.

- For further information, refer to the MSDS for these chemicals.

SPECIAL APPARATUS

1. Heater: An electric hot plate is recommended, having at least 300 watt capacity and continuously-variable regulation. Multi-unit extraction assemblies are available commercially.

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2. Extraction Apparatus: This consists of a Butt extractor (see sketch) with a Ts 34/45 female joint at upper end for attaching a Friedrichs or Hopkins condenser, and a Ts 24/40 male joint at lower end for attaching a 125 mL Erlenmeyer flask. (Available from H. S. Martin & Company, P. O. Box 661, Rhineland, New Jersey 08360).

3. Extraction Apparatus: This consists of a Soxhlet type extractor (see sketch) with a Ts 34/45 female joint at upper end for attaching an Allihn or Friedrichs type condenser, and a Ts 24/40 male joint at the lower end for attaching a 125 mL Erlenmeyer flask.

4. Extraction Shells: Paper, 80 x 22 mm (Note 2)

5. Vacuum Oven: Operate at 75 °C, and at a pressure below 100 torr.

REAGENTS

1. Petroleum Ether: Boiling range, 40 to 60 °C; bromine value, less than 1; residue on evaporation, less than 2 mg/100 mL

2. Hydrochloric Acid, Concentrated: Reagent grade, 37% HCl, sp g 1.19

3. Hydrochloric Acid, 3.0*N*: Dilute 1 part concentrated hydrochloric acid with 3 parts of purified water and mix thoroughly.

4. n-Hexane: ACS Reagent grade (Note 3).

5. Filter Aid: Kieselgur, Hyflo Super-Cel, or equivalent

6. Filter Paper: Whatman Number 1, or equivalent

PROCEDURE

For feedstuffs:
Weigh 2.5 g (Note 4) of sample (Note 5) to the nearest 1 mg, place in a 400 mL beaker, add 100 mL of 3*N* hydrochloric acid and several boiling chips. Cover

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beaker with a watch glass and place on the electric hot plate in a hood. Bring the mixture to a boil and continue boiling gently for 1 hour (Note 6).

Cool and add sufficient filter aid to prevent loss of any fat or oil during filtration. Gravity filter through a moistened double filter paper. Wash the residue with purified water until the filtrate is neutral to litmus paper or methyl orange indicator (Notes 7 and 8).

Place the double filter paper containing the residue on a watch glass and dry 1.5 hours in the vacuum oven at 75 °C. After drying, place the double filter paper containing the dry residue in an extraction shell, plug the shell with fat-free cotton wool, and place in the extractor. Attach a previously-dried and weighed Erlenmeyer flask containing about 50 mL of petroleum ether. Attach water-cooled condenser and place assembly on electric heater (Note 9). Adjust heat to produce 150 to 200 drops of condensed solvent per minute and extract for 6 hours.

Disconnect flask and evaporate all solvent on a steam bath (in hood). Dry residue and flask 1.5 hours in vacuum oven at 75 °C. Cool in desiccator and weigh. Redry 30 minutes in vacuum oven at 75 °C, cool and reweigh to insure that the fat residue weight is constant (Note 10).

For Starch:
Weigh 25 g (± 0.1 g) of sample, transfer to a 600 mL beaker, and disperse sample in 100 mL of purified water. Mix 100 mL of hydrochloric acid with 200 mL of purified water, heat to boiling, and add to the starch suspension. Heat acidified starch sample to boiling and boil for 5 mins. or until a negative starch test is obtained upon addition of a weak iodine solution. Place in a cold-water bath (below 25 °C) for 30 minutes to coagulate fatty acids.

Gravity filter reaction mixture through Whatman No. 1 filter paper and wash residue with purified water at room temperature until the filtrate is neutral to methyl orange indicator. Wipe adhering fat from inside of beaker with a clean filter paper and combine with main residue. Fold filter paper containing the residue, place on a watch glass and dry 3 hrs. in an air oven at 50 °C, or over night in a warm place.

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Place folded filter paper containing dried residue in extraction shell. Plug top of shell with cotton extracted previously with hexane, and place in extractor. Attach a previously-dried and weighed Erlenmeyer flask containing about 50 mL of hexane. Attach water-cooled condenser and place assembly on heater (Note 3). Adjust heat to produce 150 to 200 drops of condensed solvent per min. and extract for 3 hrs.

Disconnect flask and evaporate solvent in steam bath (in hood) until no odor of solvent remains. Place in vacuum oven for 1 hour at 100 °C (Note 11). Cool in desiccator and weigh.

CALCULATION

$$\%\ \text{Total Fat (as is)} = \frac{\text{Dry Residue Wt. (g)} \times 100}{\text{Sample Wt. (g)}}$$

NOTES AND PRECAUTIONS

1. Hexane is volatile and flammable. Cooling water temperature must not exceed 20 °C. The extraction assembly should be located in a hood. Be sure all connections are tight.

2. Extraction shells such as paper cones and alundum thimbles are suitable.

3. Although n-hexane is preferred, the yield of extractables with petroleum ether (B.D. 30 °-60 °C) is substantially the same.

4. Increase sample size to 5 g when the product contains less than 2% total fat.

5. Grind the sample with a suitable mill if the product is in the form of pellets or large particles.

6. Stir the dispersion occasionally to avoid adherence of sample particles to the wall of the beaker.

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7. Check the filtrate to insure that it contains no oil. If the filtrate contains oil, the sample must be extracted prior to hydrolysis.

8. Neutral is defined here as the pH of the purified wash water.

9. Petroleum ether is volatile and flammable. Cooling water temperature must not exceed 20 °C. Perform extraction in a properly ventilated hood.

10. The loss in residue weight between two successive weighings must not exceed 1 mg.

11. Prolonged drying of the extract at elevated temperatures may cause higher results due to fat oxidation.

REFERENCE

"Determination of Crude Oils and Fats," *Official Journal of the European Communities*, No. L 15/29, 18.1.84

METHOD HISTORY

Combined the Total Fat (Hydrolysis) methods for Corn Starch (Unmodified) (B-20) and Feedstuffs (G-9) on 11-09-2010.

Corn Starch (Unmodified), Fat, Total (B-20), Date of Acceptance 11-14-1955, Revised 3-29-2006.

Feedstuffs, Total Fat (G-9), Date of Acceptance 4-25-1990.

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