# STARCH IDENTIFICATION (Microscopy)

## PRINCIPLE

Starch granules are suspended in aqueous or nonaqueous media for polarized light microscopy, or are mounted dry on a stub for scanning electron microscopy. The botanical source of the starch is identified according to size and shape of granules, granule surface markings, position of hilum iodine stain color and the presence of maltese crosses under polarized light (Note 1).

## SCOPE

The method is applicable to all starches, but is intended especially for the identification of separated, unmodified, native or commercial starches.

## SPECIAL APPARATUS

1. Light microscope with bright and polarized light capabilities, equipped with 4X, 10X, 20X, 40X objectives and 8 or 10X wide field eyepieces (Note 2)

2. Precision grade stage and eyepiece micrometers to calibrate microscope magnification (Note 3)

3. Scanning electron microscope with variable KV setting and associated accessories (Note 2)

4. Triode-type sputter coater equipped with gold-palladium target and carbon thread attachment

## REAGENTS

1. Iodine solution (0.5*N*): Dissolve 95 g of potassium iodide in 60 mL of purified water, add 32 g iodine crystals, stir until completely in solution, transfer to 500 mL volumetric flask with the aid of purified water, dilute to volume and mix.

2. Iodine solution (0.1 *N*) : Transfer 100 mL of 0.5 N iodine solution into a 500 mL volumetric flask, dilute to mark with purified water and mix.

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3. Buffer solution, pH 5.0

4. Glycerine solution, 50% in water (v/v)

### PROCEDURE

**Polarized Light Microscopy:** Place a small drop of water on one side of a standard microscope slide. Use a narrow pointed spatula or dissecting needle to transfer about 5 mg of sample onto the water. Mix thoroughly to disperse the starch (Note 4). Place a cover slip over the suspension taking care to avoid entrapment of air bubbles; bleed or wick off excess water with a small piece of tissue paper held at the edge of the cover slip to obtain a thin film (Note 5). Prepare a second mount on the other half of the same slide using glycerol or mineral oil. Compare appearances of the specimen under bright and polarized light in both media (Note 6).

Examine by looking at several fields at 150-300X magnification and select a field that is representative of the sample. Note and record (by means of photomicrographs if possible):

1) Granule size range - 5 to 10-fold spread, or bimodal

2) Granule shape - polygonal, oval, truncated or flat

3) Appearance under polarized light

4) Visibility and position of hilum - centered or off-center (Note 7).

**Scanning Electron Microscopy:** To prepare a specimen for viewing, attach a piece of double-faced adhesive tape large enough to cover a stub. Electrically ground the tape to the stub at one edge with carbon or silver paint. Dust a small quantity of starch lightly on the tape and rap the stub sharply on a hard surface to "seat" the sample securely. Turn the stub sideways and rap again to remove loose particles. A compressed air gun is helpful in embedding the specimen particles into the adhesive tape and in removing the excess (Note 8). Using sputter coater,

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coat the sample with a thin layer of gold-palladium in an argon atmosphere as in preparing any nonconducting material (Note 9). Avoid damage to test sample by viewing at energy levels no higher than 10-15 KV. Even at low voltages, special preparation may be required to optimize image quality and to reduce static charging of individual particles in the beam. Three methods can be tried to overcome excessive static charging: 1) Mount a second sample of the material on copper tape rather than the normal adhesive tape in order to ground charged particles; 2) Coat the sample with carbon first, followed by gold-palladium (Note 10); or 3) Lower the KV setting. The latest scanning electron microscopes can perform at much lower settings and still give satisfactory images.

Look at several fields under 150-300X magnification and select a field that is representative of the sample. Once a field is selected, increase magnification to the level necessary to view surface properties, usually 750-1500X. A scanning electron microscope provides for depth of focus, showing the 3-dimensional structure of starch granules with surface irregularities standing out. Indentations left by zein distinguish maize from pock-marked sorghum (Note 11). An equatorial groove can be seen around the perimeter of wheat starch granules. Tiny holes may indicate initial enzyme attack.

### RESULTS

The following is a guide to identify botanical origin of starch:

**Polygonal Shape:** Oat, rice, all varieties of corn (maize) and sorghum starches are polygonal in shape, but oat and rice are about one half the size of the others. The hilum of all polygonal granules is centric. Waxy varieties and high amylose starches may be identified by iodine staining, described below. High amylose starches are differentiated from regular corn starch by small amounts of abnormal-looking granules long, irregular shaped tubes that show little birefringence under polarized light. Their population tends to increase with the level of amylose. Hence, they are readily seen in 50% amylose starch, but a careful inspection of several fields may be required before they can be seen in 30% amylose starch.

**Elliptical, Oval or Ovoid:** Arrowroot, potato, sago and canna starches are oval in shape and show an off-center hilum. Concentric rings or striations are evident on most of the granules in these species under bright and, particularly, under polarized

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light. Arrowroot starch is the smallest in size, and canna starch is the largest. Most legume starches are also oval in shape, but tend to have centric hilums. Chick pea (garbanzo) starch is difficult to distinguish from arrowroot starch.

**Dome, Cap-Shaped or Truncated:** Tapioca (manioc, cassava) starch contains characteristic cap-shaped, truncated granules, a centric hilum and a granule size distribution similar to that of corn starch. Both tapioca and corn starch contain round granules; therefore, mixtures of tapioca and corn starch can be difficult to characterize except for the truncated granules. Arrowroot starch also contains many truncated granules, but the average diameter is almost double that of tapioca starch. Truncated and dome-shaped granules are seen in other less common starches (from babassu palm, white sweet potato, maranta), but most are larger than the granules of tapioca starch.

**Granule Size (Table 1):** Most starches show a 5 to 10-fold granule size range, but wheat, barley and rye starch granules tend to have a bimodal distribution. Barley starch is typical, with one fraction containing granules about 1-3 µm and the other 6-35 µm. Wheat is also referred to as 2-sized (A and B granules) and is the most heterogeneous of the three. Wheat, barley and rye granules actually have a lenticular shape, and are thin and very bright. They polarize light when standing on edge and are round and dim when laying flat.

Additional information about starch quality and processing can be gleaned by observing starch granule damage, gelatinization properties and the presence of adjuncts. For instance, flours contain endosperm, protein and fiber along with starch (Note 12).

**Iodine Staining (Waxy/Non-Waxy):** The dilute iodine solution (0.1 N) is used to distinguish red-staining waxy starch granules from blue-staining regular or high amylose starch granules under bright light. The starch (0.3 g) is suspended in 15 mL of water, stirred magnetically and about 3 mL of iodine solution in 85 mL of water is added rapidly. A drop of the suspension is transferred to a microscope slide, which is covered and brought into focus at about 215X for granule counting (Note 13). Count red-stained and blue-stained granules separately until 300-500 granules have been counted. Express as percent waxy and percent non-waxy. A non-waxy starch content of 10% or less is considered normal contamination.

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**Iodine Staining (High Amylose):** For high amylose starch characterization, use 0.5 N iodine solution and view under polarized light. Mix 1 gram starch, 14.0 mL of purified water and 1 mL of buffer (pH 5.0) in a 250 mL beaker. In a separate beaker combine 84 mL purified water and 1.2 mL 0.5 *N* iodine (Note 14). Place the 250 mL beaker on a magnetic stirrer and, while stirring, quickly add the iodine mixture. Mix well. Place one drop of stained slurry and one drop of glycerine solution on a slide (Note 15). Blend and place cover slip on slide. Blot excess liquid to reduce thickness. At 400-500X optimize illumination and polarized light. Adjust condenser aperture for contrast to give a lightly hued background.

Common starch granules will be blue, while the high amylose granules will appear reddish or red variegated with blue. Count 500-600 granules and calculate composition as percent high amylose.

### NOTES AND PRECAUTIONS

1. Some knowledge of starch is helpful; experience of light and electron microscopy is presumed and necessary.

2. Photomicrography accessory and attachments are recommended for aid in communication and documentation of results.

3. Objective and eyepiece combinations must be calibrated before use to obtain accurate particle size estimates. The calibration is valid on any microscope and remains so until optical components are changed or replaced.

4. A 0.2-0.3% suspension of starch in water gives a field suitable for viewing. Hundreds of well dispersed granules (without overlap or crowding) should be observable.

5. Do not place too much pressure on the cover slip which may result in crushed granules. Encircling the cover slip with a thin ring of oil or glycerol will stabilize the field against evaporation of water during examination.

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6. When looking at a series of samples, make up slides one at a time and look at each immediately before field appearance changes.

7. The hilum may not be obvious under bright light for some starches. The center of the maltese cross under polarized light marks its location.

8. Compressed air guns are available from most microscopy supply houses and photographic equipment suppliers.

9. Gold-palladium in argon produces the most finely divided film. The aim is to make the specimen conductive, but not so heavily coated as to obscure surface detail. Starches are generally coated at about 15 mA in 4 minutes.

10. This approach is particularly helpful with static charging of large starch granules as in potato or canna.

11. Measuring gelatinization temperature range may be the only way to distinguish maize and sorghum starch, as some sorghum starches are not pock-marked.

12. Refer to Reference 3 for additional information.

13. A hematocytometer slide may be used to facilitate counting over the ruled grid.

14. Iodine concentration is critical for this procedure. Adjustment of the iodine concentration may be necessary to give the desired colors.

15. Glycerine tends to enhance the red color under polarized light.

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**TABLE I**
**GRANULE SIZE ANALYSIS OF COMMERCIAL UNMODIFIED STARCHES**
**BY LIGHT MICROSCOPE AND COULTER COUNTER**

| Starch Species | Granule Size Range, µm: Light Microscope | Granule Size Range, µm: Coulter Counter | Diameter of gran. of avg. wt., µm (Coulter Data) |
|---|---|---|---|
| Waxy rice | 2 - 10 | 2 – 13 | 5.4, 5.6 |
| High amylose corn 70% amylose | -- | | |
| 55% amylose | 5 - 35 | 4 – 22 | 9.6, 10.0 |
| | | 3 – 22 | 10.3, 10.7 |
| Corn | 5 - 30 | 5 – 25 | 13.8, 14.3, 14.5 |
| Waxy corn | 5 - 25 | 4 – 28 | 13.9, 14.2 |
| Tapioca | -- | 3 – 28 | 13.8, 14.2 |
| Grain sorghum | -- | 3 – 27 | 16.0, 16.2 |
| Waxy sorghum | -- | 4 – 27 | 15.0, 16.5, 16.9 |
| Wheat | 5 - 35 | 3 – 34 | 16.4, 16.6 |
| Barley | 2 - 3<br>35 - 40[a] | 6 – 35 | 16.5, 16.7 |
| Sweet potato | -- | 4 – 40 | 18.0, 18.6 |
| Garbanzo or chick pea | -- | 7 – 54 | 20.3, 21.1 |
| Shoti | 10 - 60[b] | 9 – 34 | 21.0, 22.3 |
| Arrowroot | 10 - 50 | 9 – 40 | 22.9, 23.9 |
| Sago flour | 20 - 65 | 15 – 50 | 33.1 |
| Potato | 10 - 100 | 10 – 70 | 34.0, 36.0 |
| Canna or Australian Arrowroot | 30 - 130 | 22 – 85 | 53.0, 53.0 |

[a] Sharply bimodal

[b] Non-spherical, flat granules

### REFERENCES

1. McCrone, W. C. and J. G. Delly, The Particle Atlas, 2nd ed., Vol. 2, pp. 457-462, Ann Arbor Science Publishers, Ann Arbor, Michigan, 1973.

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2. "Polarization Color of Maize Starches Varying in Amylose Content and Genetic Background," H. L. Seckinger and M. I. Wolf, Die Starke, No. 1, Jahrgang 18, pp. 2-5, 1966.

3. Snyder, E. M., Industrial Microscopy of Starches, p. 661 in Starch: Chemistry and Technology, 2nd ed., R. L. Whistler, J. N. BeMiller, and E. F. Paschall, editors, Academic Press, New York, N.Y., 1984.

4. Fitt, L. E. and E. M. Snyder, Photomicrographs of Starches, p. 675, ibid.

### METHOD HISTORY

Starch (Unmodified), Starch Identification (Microscopy) (B-25), Date of Acceptance 6-20-1991.