Submission ID #: 61586
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18783778

Title: Analysis and Specification of Starch Granule Size Distributions

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: All screen capture files provided, do not film

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **44**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Ming Gao**: This technique enables a reproducible and statistically valid determination of starch granule sizes. It also provides a proper description of statistically distributed granule sizes **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Ming Gao**: This technique determines starch granule volumes independent of their shapes, uses statistically valid granule-sample sizes for a size distribution, and provides an improved specification of granule size distributions **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Mahta Moussavi**: This method is applicable to plant and food science research studies that involve starch granule sizes in addition to any other starch applications that require granule size information **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Electrolyte Preparation and Standard Operating Method (SOM) and Standard Operating Procedure (SOP) Setup

2.1. Before beginning an analysis, dissolve 25 grams of lithium chloride in 500 milliliters of methanol **[1]** and select an aperture tube with a particle diameter range covering the known granule size range of the starch samples to be analyzed **[2]**.

2.1.1. WIDE: Talent adding lithium chloride to methanol, with lithium chloride and methanol containers visible in frame

2.1.2. Talent selecting tube

2.2. To set up a Standard Operating Method, start the analyzer software **[1]** and, in the Main Manu, click **SOP**(S-O-P) and **Create SOM**(S-O-M) **Wizard** and select the appropriate settings **[1]**.

2.2.1. Talent starting software, with monitor visible in frame
2.2.2. SCREEN: screenshot_1: 00:00-00:35 *Video Editor: please speed up*

2.3. Then click **Edit the SOM** and select the appropriate settings **[1]**.

2.3.1. SCREEN: screenshot_2: 00:00-00:26 *Video Editor: please speed up*

2.4. To select a Preferences file, under **SOP**, click **Create Preferences Wizard** and select the appropriate preference settings **[1]**.

2.4.1. SCREEN: screenshot_3: 00:00-00:18 *Video Editor: please speed up*

2.5. Then click **Create SOP Wizard**, enter a description and author, and select the **SOM** and **Preferences** files to create and save the **SOP [1]**.

2.5.1. SCREEN: screenshot_4: 00:00-00:38 *Video Editor: please speed up*

3. Analyzer Setup

3.1. To set up the analyzer for an analysis, turn on the analyzer **[1]** and verify the **Ready** status in the analyzer software **[2]**.

3.1.1. WIDE: Talent turning on analyzer
3.1.2. SCREEN: screenshot_5

3.2. Fill the electrolyte jar with the previously prepared electrolyte **[1]** and empty the waste jar as necessary **[2]**.

3.2.1. Talent filling jar with electrolyte NOTE: This and next shot in one
3.2.2. Talent emptying waster jar

3.3. Install and secure the selected aperture tube according to the guide in the user's manual **[1]** and push the lock-release clip to unlock the assay platform **[2]**.

3.3.1. Talent installing/securing tube NOTE: This and next shot in one
3.3.2. Lock-release clip being pushed

3.4. Manually lower the platform to the bottom **[1]** and place an analytical beaker containing 100 milliliters of electrolyte onto the platform **[2]**.

3.4.1. Platform being lowered NOTE: This and next shot in one
3.4.2. Beaker being placed onto platform

3.5. Move the stirrer to the stirring position **[1]** and manually raise the platform to the self-locking upper position to immerse the aperture tube and stirrer in the electrolyte **[2]**.

3.5.1. Stirrer being moved NOTE: This and next shot in one
3.5.2. Platform being raised

3.6. Click **Fill** on the bottom instrument Toolbar to automatically fill the system with the electrolyte **[1]** and click **Flush** to automatically flush the system **[2]**.

3.6.1. Talent clicking fill/system being filled
3.6.2. Talent clicking flush/system being flushed

3.7. Then click **SOP** and **Load an SOM** to load the Standard Operating Method **[1]** and click **Sample** and **Enter Sample Info** to enter the sample information for the run **[2-TXT]**.

3.7.1. SCREEN: screenshot_6: 00:00-00:05
3.7.2. SCREEN: screenshot_6: 00:16-00:48 *Video Editor: please speed up*

4. Starch Sample Granule Sizing Analyses

4.1. To prepare a starch-methanol sample sizing suspension, weigh one or two 0.5-gram sample from each of the two or three replicate starch extracts **[1]** and add each aliquot to 5 milliliters of methanol in a 50-milliliter conical centrifuge tube **[2]**.

4.1.1. WIDE: Talent adding sample to balance
4.1.2. Talent adding sample to methanol, with methanol container visible in frame

4.2. Use several pulses of low intensity ultrasound from an ultrasonic processor to fully disperse the starch granules **[1]** and use a disposable transfer pipette to apply about 200 microliters of the first starch-methanol suspension to the 100 milliliters of lithium chloride methanol electrolyte under constant stirring in the beaker **[2]**.

4.2.1. Sample(s) being pulsed *Videographer: Important/difficult step*
4.2.2. Talent adding sample to beaker NOTE: This and next shot in one *Videographer: Important/difficult step*

4.3. Then close the sample compartment door **[1]** and click **Preview** to start a preview run **[2]**.

4.3.1. Talent closing door
4.3.2. SCREEN: screenshot_7: 00:00-00:15 *Video Editor: please speed up*

4.4. In the Status Panel, verify that the dynamically displayed concentration bar is green and shows a 5-8% nominal concentration range for the suspension **[1]**.

4.4.1. SCREEN: screenshot_8: 00:17-00:27 *Video Editor: please emphasize concentration bar and concentration range when mentioned*

4.5. Click **Stop** to stop the Preview **[1-TXT]** and click **Start** to start the analysis. The analyzer will automatically complete the run once the total count of sized granules, which is displayed along with the run time on the **Status Panel** in a run **[2]**, reaches the Total Count as set by the **Control Mode** of the SOM **[3]**.

4.5.1. SCREEN: screenshot_7: 00:15-00:20 **TEXT: Dilute starch-electrolyte as necessary**
4.5.2. SCREEN: screenshot_8: 00:01-00:40 NOTE: Looks like videographer shot this as well, 4.5.2 and 4.5.3 in one shot *Video Editor: please speed up and emphasizes Run time and Total count when mentioned*
4.5.3. SCREEN: screenshot_9: 00:41-00:48

4.6. To perform a technical repeat run using the same starch-electrolyte suspension, click **Start** or **Repeat** on the bottom Toolbar **[1]**.

4.6.1. SCREEN: screenshot_9: 00:54-01:37 *Video Editor: please speed up*

4.7. At the end of the analysis, empty the beaker **[1]**, rinse it with methanol **[2]**, and refill it with 100 milliliters fresh electrolyte solution for analysis of the next sample **[3]**.

4.7.1. Talent emptying beaker
4.7.2. Talent rinsing beaker, with methanol container visible in frame
4.7.3. Talent adding solution to beaker, with solution container visible in frame

5. Data Analysis

5.1. If an SOM was used to control the runs, use the **Create Preferences Wizard** to select the **Preferences** settings as desired for viewing, printing, and statistical analyses of the results **[1]**.

5.1.1. WIDE: Talent selecting Create Preferences Wizard, with monitor visible in frame
5.1.2. SCREEN:

5.2. To overlay the results from multiple runs on a single graph, in the **Overlay** window, select the results to be overlaid **[1]**.

5.2.1. SCREEN: screenshot_10: 00:10-00:19 *Video Editor: please speed up*

5.3. Click **Add** to move the files to the Selected Files box and click **OK** to overlay the selected results on a single graph **[1]**.

5.3.1. SCREEN: screenshot_10: 00:20-00:52 NOTE: This and next shot in one

5.4. To add a file to an open overlay, click **RunFile** and **Open for Overlay** to access the Overlay window, select the desired file, and click to add **[1]**.

5.4.1. SCREEN: screenshot_10: 01:09-01:21

5.5. Click **File**, **FileTool**, and **Average** to open the Average window and navigate to and select multiple desired result files in the Files box **[1]**.

5.5.1. SCREEN: screenshot_11: 00:00-00:12 NOTE: This and next shot in one

5.6. Click **Add** to move the files to the Selected Files box and click **OK** to average the selected results for display in a single graph **[1]**.

5.6.1. SCREEN: screenshot_11: 00:12-00:55 *Video Editor: please speed up*

5.7. To include an additional result file in an average distribution, click **RunFile** and **Open and Add to Average** to open the Add to Average window and add the file of interest. The new average will appear on the graph in the **Run** window **[1]**.

5.7.1. SCREEN: screenshot_11: 00:56-01:20

5.8. To obtain the graphic geometric mean and standard deviation for specifying the average distribution, click **Calculate** and **Averaged Statistics** to open the statistics summary window to display the means and standard deviations of the data **[2]**.

5.8.1. SCREEN: screenshot_12: 00:06-00:12 *Video Editor: please emphasize Mean and SD column when mentioned*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

4.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?

4.2., 4.3. The single most difficult aspect of the procedure is to achieve an assay suspension of 5-8% nominal concentration with minimal granule aggregates. If frequent aperture blockages lead to abortion of a run, step 4.2.1 may have to be repeated, and the assay suspension re-made.

Results

6. Results: Representative Volume-Percentage Volume-Equivalent-Sphere Size Distribution Analyses

6.1. Here differential volume-percentage volume-equivalent-sphere-diameter distributions **[1]** for the four replicate sizing analyses of representative sweet potato starch samples **[2]** and their average distributions are shown **[3]**.

6.1.1. LAB MEDIA: Figure 1 *Video Editor: please emphasize Figure key*
6.1.2. LAB MEDIA: Figure 1 *Video Editor: please emphasize orange, green, purple, and blue data lines*
6.1.3. LAB MEDIA: Figure 1 *Video Editor: please emphasize black data line*

6.2. In this figure **[1]**, the average cumulative number- **[2]** and volume-percentage size distributions of the four replicate sizing analyses, which were transformation views of the average differential volume-percentage size distribution, can be observed **[3]**.

6.2.1. LAB MEDIA: Figure 2
6.2.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize black data line*
6.2.3. LAB MEDIA: Figure 2 *Video Editor: please emphasize red data line*

6.3. Comparing the cumulative number and volume percentages of these starch granule samples **[1]** revealed that the granules with smaller volume-equivalent-sphere diameters accounted for much larger percentages of the total count than the total volume **[2]**.

6.3.1. LAB MEDIA: Figure 2
6.3.2. LAB MEDIA: Figure 2 *Video Editor: please add/emphasize dashed vertical line*

Conclusion

7. Conclusion Interview Statements

7.1. **Ming Gao**: It is important to minimize the granule aggregates in the starch sample and assay suspensions and to attain a 5-8% nominal concentration range for the starch-electrolyte suspension **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (Steps 4.2., 4.4.)

7.2. **Ming Gao**: Assays for characterizing the physico-chemical and thermal properties of starch samples and for correlating the granule sizes with starch synthesis characteristics in the source plants can also be performed **[1]**.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera