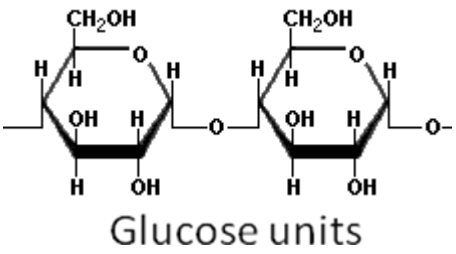
A Simplified Chemical Method for Determining Classes of Rice Amylose Content which Control Cooked Rice Texture

I. Introduction and Theory

Rice produced in the US for either domestic or export market is typically classified into four classes: high-amylose, intermediate-amylose, low-amylose, and waxy (no amylose or glutinous) rice. Amylose is a component of rice starch. The high-amylose rice contains approximately >24 % amylose content of the milled rice, the intermediate-amylose rice contains between 20 – 24 %, the low-amylose rice contains 10 – 19%, and the waxy rice contains < 5%. The amylose content of the milled rice is the major determinant of rice texture – how soft or firm the cooked rice will be. After cooking, the high-amylose and intermediate-amylose rices are firm and fluffy; while the low-amylose and waxy rices are soft, moist, and sticky in texture. During development of new cultivars, rice breeders incorporate various important characteristics, such as high yield, resistance against major fungal diseases, good milling quality and the rice amylose content has to be selected to fit within the long, medium, and short grain market class. The typical U.S. long grain market class has intermediate amylose content, and the medium and short grain market classes have low amylose content. The specialty long grain rice used for par-boiling and canning typically has high amylose content.

Various methods have been developed for determining the amylose content of cereal grains. The routine method used by the USDA-ARS Dale Bumpers National Rice Research Center at Stuttgart, AR is an automated iodine-colorimetric method. This method has been used to screen thousands of rice breeding samples submitted by US breeding programs for quality evaluation. The basis for the method is the formation of a chemical complex between the iodine molecules and the long carbohydrate chains that make up the structure of the amylose molecules. The amylose-iodine complex has the maximum absorbance at the wavelength of 620 nm. The absorbance value of the amylose-iodine complex is proportional to the amylose content of the ground rice. Therefore, a calibration curve can be plotted based on the various rice flour standards with known amylose contents to cognate absorbance values. This calibration curve can be used to determine the amylose content of a rice sample with unknown amylose content. This method requires an instrument that has a visible light source and can read the absorbance value of the amylose-iodine complex of rice sample.



We have developed a simplified method which can roughly predict the amylose classes of the milled rice, i.e. as high-amylose, low-amylose or waxy rice based on the theory stated above. This method uses common inexpensive chemicals and does not require any instrumentation. This simplified method is different from the method stated above in that it is based on the visual observation of the portion of the visible light that is not being absorbed, but reflected by the amylose-iodine complex. The details of this simplified method are presented below.

II. Materials and Solution Preparations

Materials:

1) One 100-mL volumetric flask with snap cap (Cole-Parmer, Pyrex Brand 5580 Volumetric Flask,catalog number: EW-34560-07.)
2) A scale capable of measuring 0.1 – 4.0 gram range
3) Safety goggle and gloves
4) Four weighing papers (Cole-Parmer, Glassine Weighing Paper, Medium, 4 x 4", 500/pk. Catalog number: EW-01338-02.)
5) Six 1-mL disposable syringes (no needle) (Cole-Parmer, B-D ™ Syringes, non-sterile clean, 1 mL Slip-Tip. Catalog number: EW-07944-00.)
6) Three 10-mL disposable syringes (no needle) (Cole-Parmer, B-D™ Syringes, non-sterile clean, 10 mLSlip-Tip, 10 mL. Catalog No.: EW-0794-14)
7) Five 50-mL polypropylene screw-cap tubes (Fisher brand 50 mL Graduated Polypropylene Centrifuge Tube, O.D. x L: 29.5 x 113.8mm; Catalog No.: 14-375-150).
8) Three 25-mLglass tubes (Fisher Scientific, Pyrex Screw Cap Culture Tubes with PTFE lined phenolic cap, 25-mL; O.D. x L: 20 x 150mm; Catalog No.:14-933-1B)

Sources of the chemicals:

1) Isopropyl alcohol (99%)
2) Sodium Hydroxide pellets, (Fisher Scientific Cat. # A250-500)
3) Vinegar, 5% acidity (5% acetic acid; Heinz vinegar preferred)
4) Povidone, 1% available iodine ingredient (found in pharmacy stores)
5) Rice: waxy rice(commonly found in Asian imported food stores), low amylose rice (medium grain rice), and high-amylose rice (long grain, high amylose rice)
6) Deionized water

Preparation of the solutions:

- **1 N Sodium Hydroxide (NaOH):** Fill a clean, 100-milliliter flask ¾-full with the deionized water. Weigh accurately and rapidly 4 grams of NaOH, and transfer it to the 100-milliliter flask. Mix the contents to dissolve the NaOH. As this is an exothermic reaction, allow to cool to room temperature. Add deionized water to the 100-milliliter mark of the flask. Mix the content thoroughly, and transfer it to a clean, reagent-storage bottle.

III. Steps to Digest or Solublize Amylose of Rice Sample

- **Day 1:** Weigh 100mg of milled, ground (pulverized to a powder)-rice sample, and transfer it to a 50-ml tube with a screw cap. Then, add 0.5ml isopropyl alcohol down the side of the tube to wash down any sample that has adhered to the side of the tube. Before proceeding to the next step, make sure the sample is completely wetted with the added isopropyl alcohol by swirling the tube gently. Ground rice that is not wet with ethanol before coming in contact with NaOH, will clump. Add 4.5ml NaOH (1 N) down the side of the tube trying to wash as much as possible of the remaining sample down to the bottom of the tube. Once the NaOH has been added to the sample, the tube should be kept stable by placing in a test tube rack This will allow the starch in the rice sample to be digested completely. Put the cap on and then allow the sample to digest overnight at room temperature.

- **DAY 2:** Add deionized water to the tube up to the 50mL mark and invert the tube several times to thoroughly mix the content. It is ready for analysis below.

IV. Steps to Develop Color for Rice Classification:

- **Day 2**:
    a) **Dilute the vinegar 1:60 (v/v) with deionized water:**
        i. Have an -cap-50mL tube with graduated marks ready.
        ii. Add 0.5mL vinegar (using a 1mL syringe) into the tube.
        iii. Add deionized water to the 30mL mark.
        iv. Cap the tube and invert it to mix the content.

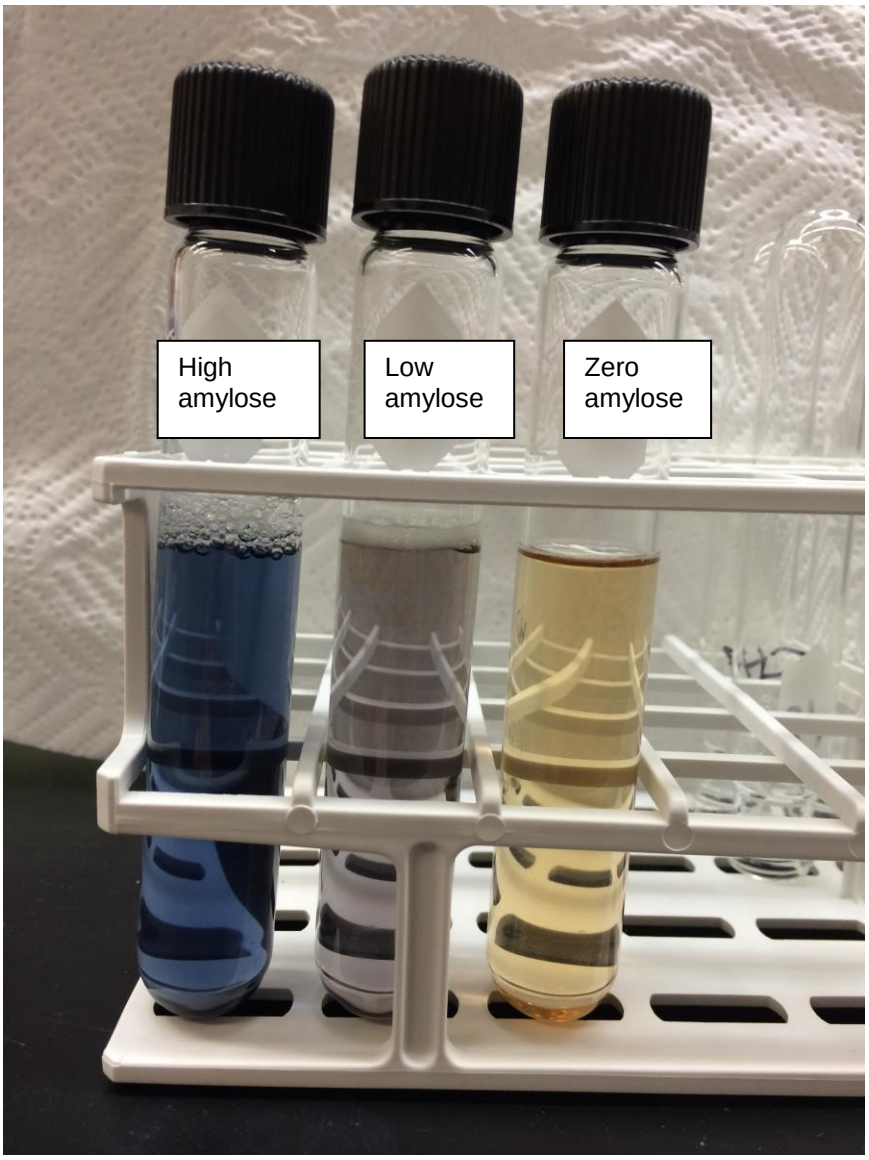
    b) **Dilute the Povidone 1:100 (v/v) with deionized water:**
        I. Have an -cap-50mL tube with graduated marks ready.
        II. Add 0.5mL Povidone (using a 1mL syringe) into the tube.
        III. Add deionized water to the 50mL mark.
        IV. Cap the tube and invert it to mix the content.

    c) Color reaction:
        I. Pipet 1mL of digested starch solutions, one waxy rice, one low-amylose rice and one high amylose rice, into three separate tubes (a 50mL test tube with a cap).Invert to mix.
        II. Into each tube, add 10mL of the diluted vinegar (from Day 2, step (a) above). Invert to mix.
        III. Then into each tube, add 4.5ml mL of the diluted Povidone (from Day 2, step (b) above). Invert to mix.
        IV. Color will be developed within seconds.

    Note differences in color in tubes. Blue: high-amylose rice, which cooks up firm and fluffy. Grayish blue: low-amylose rice, which is soft and moist after cooking. Brownish yellow: glutinous rice containing no amylose, which is sticky in texture.

    The chemical analysis can be complemented by cooking samples of long grain, medium grain, and waxy rice and having students taste the cooked rice for differences in texture. Each rice sample should be cooked in the same way (1 rice:2 water) for the same amount of time (about 20 minutes) and served at the same time. When tasted, the long grain rice should be

relatively firm, the medium grain relatively soft, and the waxy rice very soft and sticky. They will also differ in appearance. One can describe differences in texture and appearance using these cooked rice samples (descriptor analysis) and ask students which one they liked best (preference test). The cooked samples can be cooled and tasted again. The texture differences will be greatly magnified.



Should you have questions or would like small quantities of rice flour having different amylose contents, please contact:

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