QUANTIFICATION OF SOLUBLE STARCH FROM FRESH POTATOES USING PHOTOPETTE

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- *Educational experiment using Photopette® to measure soluble starch in potatoes and other plants.*
- *Simple extraction method combined with iodine reagent allows easy starch quantification.*

OBJECTIVE

This application note provides an educational experiment to quantify soluble starch in fresh potatoes using the Photopette® hand held photometer at 600 nm wavelength.

INTRODUCTION

Starch is a carbohydrate based energy storage molecule found in plants. There are two types of starch - amylose (highly soluble in water) and amylopectin (slightly soluble in water) [1]. Both types of starch are made from glucose monomers but with a different linkage.

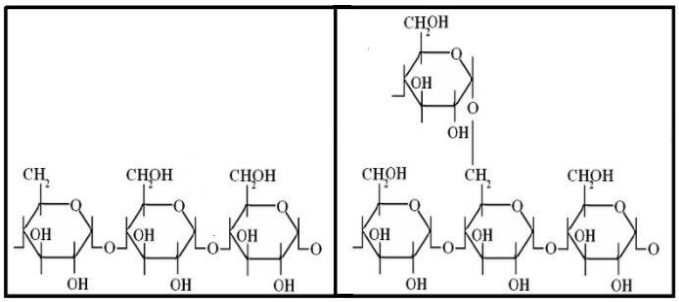

Figure 1a: Amylose **Figure 1b:** Amylopectin

The presence of starch can be measured by its reaction with iodine. Starch and iodine form a dark-blue complex with an absorbance maximum at 600 nm [1].

This application note describes a simple procedure to extract soluble starch from fresh potatoes. The amount of soluble starch extracted is then measured with the Photopette® at 600 nm.

MATERIALS AND APPARATUS

Instruments:

- Photopette® OD600 or Cell with 600 nm
- Bench centrifuge

Reagents:

- Iodine Reagent
- Starch reference solution made from soluble starch powder (Sigma, S9765).

Material:

- Fresh Potato

METHOD

Before performing experiments please refer to the risk-assessment and refer to the Photopette® User Manual for operating and safety precautions [2].

EXPERIMENTAL PROCEDURE

Preparation

Iodine Reagent: The preparation of the Iodine reagent is made following the CLEAPSS Recipe. The reagent is prepared by adding 3 g of KI to 2.54 g I_2 and toping up with water to 100 mL. Subsequently, the stock solution is diluted 10 times and stored in the dark.

Starch Reference Solution: A 0.1% starch reference solution is made using soluble starch powder (Sigma, S9765). Weight 50 mg of soluble starch powder and add 10 mL of room temperature di water to the starch powder. Then top up to ~40 mL with hot di water. Heat the starch solution to 90 °C until the starch is dissolved. Cool down the solution to room temperature and transfer to a 50 mL volumetric flask. Finally, top up to 50 mL with room temperature di water.

Starch Isolation from Potatoes: The sample preparation was adapted from reference [3]. Take a slice of fresh peeled potato of about 0.3 to 0.5 g. (Record the actual weight of the potato slice as this will be taken into the calculation for later). Grind the potato with mortar and pestle with 2 mL of added di water. Grind the potato as fine as possible to maximise the amount of starch extracted. Transfer the blended potato to a 15 mL reaction tube. Top up the potato juice to a final volume of 10 mL with cold water and shake. Centrifuge the potato juice at 5500 rpm for 10 min. Carefully pipette out the supernatant without disrupting the pellet. Discard the supernatant. Starch is present in the pellet. Wash the pellet again by topping up to a final volume of 10 mL with cold di water and centrifuge at 5500 rpm for 10 min again. Discard the supernatant. Quantitatively transfer the starch pellet into a 100 mL glass beaker and top up to ~30 ml with di water. Put a stirrer bead into the beaker and heat up the solution under stirring. Turn off the heat when the temperature reaches close to 100 °C. Cool down the solution to about 50 °C. Filter the hot starch suspension into a 50 mL volumetric flask to remove any solid particles. Wash the filter with hot water. Cool down to room temperature and fill up to 50 mL with di water. Dilute the sample solution 1:10 before measurements.

Experiment

Measurements: Turn on the Photopette® device and connect to the Photopette® iOS/Andriod app. Select "600 nm" as the wavelength. Select dataset and set additional settings (if needed) before selecting 'Start Measurement'. Please follow the video-tutorials available at www.tipbiosystems.com to get familiar with the measurement process. Place a CuveTip® firmly on the device probe and insert into the blank sample to perform auto-zero measurement. Ensure that there is no air-bubble trapped in the CuveTip® cavity as presence of air-bubbles can disrupt the optical-path, and can result in erratic values. After the auto-zero measurement in di-water the starch samples or starch standard solutions are measured using the same CuveTip.

Add 30 µL of iodine reagent solution to 1 mL of the diluted starch sample or the starch standard dilutions. Vortex and measure the absorbance at 600 nm with Photopette®. For blank solution, add 30 µL of iodine reagent to 1 mL of di water. For exact results, no sample shall be transferred to the next sample.

First, a standard curve is prepared. Standard starch solutions with concentrations between 0.05 mg/mL to 1 mg/mL were prepared by serial dilutions of the starch standard solution. Add 30 µL of iodine reagent solution to 1 mL of the starch sample solution, mix well and measure absorbance at 600 nm with Photopette®. The measurement results are tabulated in Table 1 below.

Concentrations of starch standard solutions (mg/mL)	Average Absorbance at 600 nm (AU)	SD
0.05	0.37	0.025
0.10	0.61	0.011
0.15	0.88	0.016
0.20	1.17	0.026
0.25	1.41	0.085
0.30	1.61	0.041
0.35	1.80	0.048
0.40	1.83	0.039
0.50	1.87	0.046
0.55	1.93	0.040
0.60	2.02	0.010
0.85	2.05	0.101
1.00	2.08	0.021

Table 1: Absorbance values of the prepared starch standard solutions.

DETERMINING CONCENTRATION OF AN UNKNOWN SAMPLE

The starch extract is then measured in a similar way and the average of measurements of 2 or 3 repeats is calculated. Following a similar approach, the starch concentration for other vegetables (yam, sweet potatoes, rice) can be measured. Using Photopette®, the amount of soluble starch extracted can be calculated by substituting the absorbance value into the equation of the standard curve.

DISCUSSIONS

The data was used for the generation of a standard curve and to determine experimental parameters such as limit of detection, linear range and the upper measurement limit. The absorbance values in Table 1 were plotted in Figure 2.

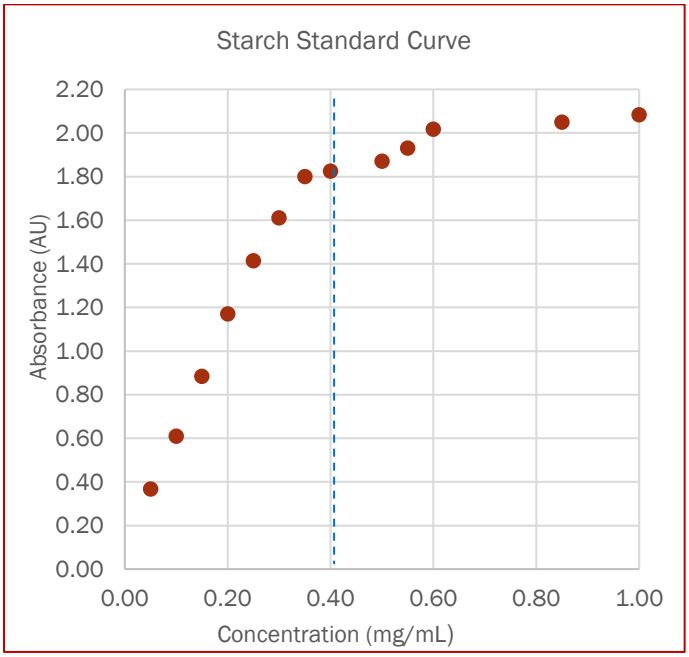

Figure 2: Absorbance as a function of starch concentration using starch standards.

EXPERIMENTAL PAPRAMETERS

Upper measurement-limit and linear Range

Figure 2 shows that the readings start to saturate beyond 0.40 mg/mL (as indicated by the blue dotted line). Therefore, it is not recommended to include data beyond 0.4 mg/mL in the standard curve as the measurement accuracy will be reduced. Regression analysis indicates a linear range between 0 and 0.3 mg/mL starch solution.

STANDARD CURVE

A standard curve was plotted in Figure 3 for the data within the range of 0.05 mg/mL to 0.3 mg/mL. A linear regression was performed on the data using Microsoft Excel® software, and a linear fit was obtained. The equation of the standard curve of Figure 3 has an R-squared value of 0.9948. The slope of the standard curve obtained was 4.881 mL AU/mg. Photopette® users may download a pre-configured worksheet for the starch analysis from our online resource section. The worksheet is compatible with Microsoft Excel® and similar worksheet software, and will aid users in performing the calculations and generating the standard curve.

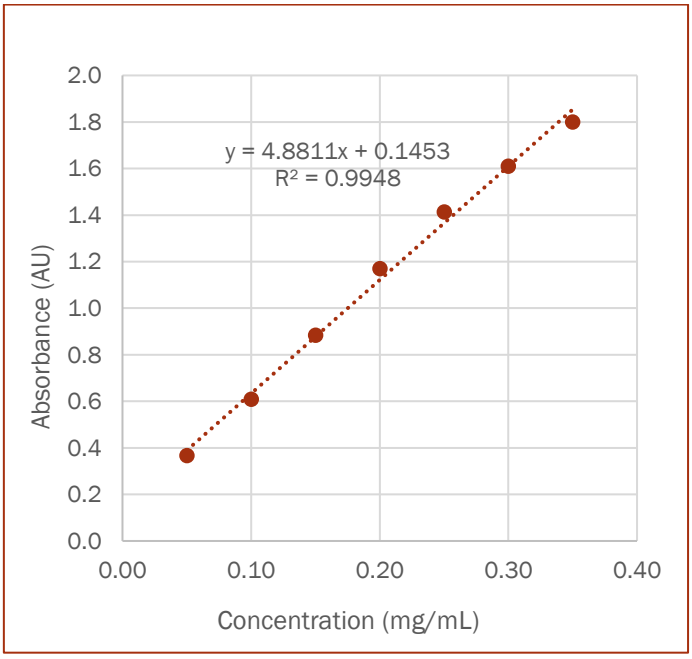

Figure 3: Standard curve for starch solutions using Photopette® at 600 nm.

LIMIT OF DETECTION

The Limit of Detection (LOD) for this measurement using Photopette® is determined by factoring in the standard-deviation for blank measurements as well as experimental data using the equation given below:

$$LOD = 3 \times SD_{blank} / Slope_{standard\ curve}.$$

Standard Deviation for blank measurements with 50 repeats using same CuveTip® was found to be 0.005 AU. Thus, the limit of detection for starch measurement at 600 nm wavelengths with Photopette® using the equation above was 0.003 mg/mL.

LOD = 3 x (0.005AU) /(4.8811 AU/mg/mL)
= 0.003 mg/mL

STARCH CONCENTRATION CALCULATION

The following example provides a calculation for the potato experiment. Weight of fresh potato 0.31 g; Average

absorbance value 0.92 AU. From the linear regression analysis of y=4.8811x+0.1453 we calculate the starch concentration by rearranging the formula to x=(y-0.1453)/4.8811. The starch concentration of the sample is 0.159 mg/mL. The dilution factor was 1:10 making it a concentration of 1.59 mg/mL. The total amount of starch in the 50 mL flask is therefore 79.5 mg. This amount was extracted from 0.42 g of fresh potatoes. The total amount of starch in 1 g potatoes is therefore 189 mg or 18.9% of the fresh weight.

LIMITATIONS

The amount of soluble starch extract largely depends on how fine the potato is grinded or blended. Any solid particles that were discarded after dissolving the starch in hot water may contain starch and hence may affect the quantification of soluble starch.

SUMMARY

This application notes provides a simple school experiment to determine soluble starch concentration in potatoes by measuring the absorbance of the starch-iodine complex at 600 nm. The experiment is easy to conduct, has low cost and low risks.

EDUCATIONAL LEARNING OUTCOMES

The experiment demonstrates the formation of a complex between starch and iodine that leads to a color change.

The different solubility of starch in cold and hot water is used to purify and isolate starch from plant sources such as potatoes.

The experiment gives an example how to generate a calibration curve and how to determine the limit of detection and to perform a linear regression of the data.

VARIOATIONS OF THE EXPERIMENT

Using the provided protocol starch from other plant sources such as rice, corn or grains can be isolated and the starch content of different plants can be compared. Alternative, starch in food products such as noodles or cake can be measured and compared with the nutrition information on the products.

By adding an amylase (e.g. from saliva) to a starch sample and measuring the starch concentration over a period of time the hydrolysis of starch can be demonstrated. This experiment demonstrates the enzymatic degradation of starch by hydrolysis. Alternatively, starch can be chemically hydrolysed by heat in the presence of acids or bases.

RISK ASSESMENT

There is no risk involved other than chemical risk of the iodine solution. Iodine is an oxidant and contract with skin and eyes must be avoided.

REFERENCES

[1] S. C. Alcazar-Alay and M. A. A. Meireles, "Physicochemical Properties, Modifications and Applications of Starches from Different Botanical Sources," *Food Sci. Technol, Campinas,* vol. 35, no. 2, pp. 215-236, May 2015.

[2] Tip Biosystems Pte Ltd, "Photopette® User Manual", *Singapore,* 2016.

[3] M. R. Kurt, D. L. James and D. F. Stickle, "Quantitative Assay for Starch" *Journal of Chemical Education,* vol. 81, no. 5, pp. 702-704, 2004.

Educational Questions:

- Is starch soluble in cold water?
- Is starch soluble in hot water
- How is the color with the iodine reagent generated?
- What happens if starch is hydrolyzed?
- What is the color of starch?