A Research Note

ALPHA-AMYLASE IN SWEET POTATOES. A Comparison Between the Amyloclastic and Chromogenic Starch Methods of Analysis

INTRODUCTION

ALPHA-AMYLASE ACTIVITY is frequently determined by an amyloclastic method which is based on the rate of enzymatic hydrolysis of a soluble starch substrate. This method depends on the fact that as sample α-amylase fragments the substrate, there is a progressive decrease of the blue color formed when iodine is added. A typical amyloclastic procedure is that of the AOAC (1960) which with certain modifications (Briggs, 1961) is used extensively. However, the AOAC or similar procedures are not ideal due to technical deficiencies such as difficulty in reproducible preparation of the starch substrate and considerable operator attention for each assay.

Recently insoluble derivatives of starch have been developed which can serve as substrate for α-amylase assay (Rinderknecht et al., 1967). These chromogenic substrates, prepared by reacting starch with organic dyes, are hydrolysed by α-amylase causing a release of color into the solution. The amount of color released is proportional to enzyme activity in the sample. Although measurement of α-amylase activity with chromogenic starch substrates has received considerable attention in medical research (Hall et al., 1970), little has been published on the use of this type substrate in food or agricultural areas. Recently, however, an assay for α-amylase in malt was reported which employed chromogenic starches as substrate (Barwald et al., 1970).

During our investigation of α-amylase levels in sweet potato varieties, it became evident that a simpler more rapid procedure was needed. Consequently, we decided to develop a chromogenic starch method applicable to sweet potatoes and compare it with the AOAC procedure.

MATERIALS & METHODS

SWEET POTATOES were obtained from a root maintenance collection of the N.C. Agricultural Experiment Station at Clayton, N.C. The roots were harvested and cured at 90–95°F and 85% relative humidity for 7 days. After curing, they were stored at 60°F until used. The varieties used were Centennial, Porto Rico, Pelican Processor, Australian Canner and Jewel.

The roots were hand peeled, grated and juice equivalent to 23–25% of the weight was obtained by squeezing the grated roots in a Carver Press. The juice was centrifuged at 3000G for 10 min (5°C) and the supernatant collected. Toluene was added as a preservative. Amylase assays were run at 40°C and 60°C on the juice or dilutions thereof. The 60°C assay was run on several varieties at two different times while the 40°C assay was run on the same varieties at one time period only.

Alpha-amylase assays

Chromogenic starch method. Amylopectin Azure (Calbiochem) was the substrate used. A 2% slurry of substrate was prepared using 0.02M phosphate buffer (pH 6.0) containing 0.3% NaCl. The substrate was placed in a water bath at the reaction temperature for 15 min. Juice samples or dilutions (0.3 ml) were put in test tubes and placed in the water bath for 0.5 min. The substrate was swirled until a homogenous suspension was obtained and then 2.7 ml was rapidly pipetted into the sample tube.

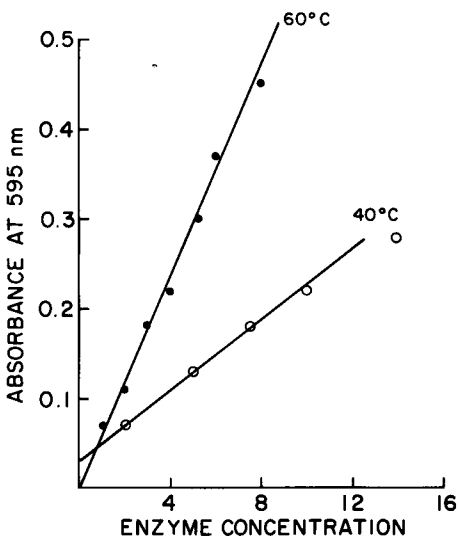

Fig. 1—Effect of enzyme concentration on the hydrolysis of amylopectin azure substrate (2% pH 6.0). Juice from Porto Rico variety sweet potatoes incubated 15 min at 40°C and 60°C. The enzyme concentration shown is a dilution factor times 100 of a stock juice sample.

The tube was shaken and a timer started. Other samples were treated the same way with the reaction being started at 1 min intervals. After exactly 15 min, the reaction of the first tube was stopped by adding 1.2 ml of 5% v/v trichloracetic acid followed by vigorous mixing. Following the same sequence and time interval, other samples were assayed. Blanks were prepared by adding trichloracetic acid solution to the juice sample prior to substrate addition. Several blanks were run with each batch of samples. After stopping the reaction, the tubes were centrifuged for 3 min in a clinical centrifuge and filtered through Whatman #2 paper. Absorbance was then measured at 595 nm. The calculations are as follows:

$$\text{APA Amylase Units per ml} = \frac{As_{595} - As_{595}\ (\text{blank})}{As_{595}\ \text{of 0.1M } CuSO_4} \times \text{Dilution factor.}$$

The 0.1M copper sulfate solution is used as an arbitrary standard so that results using different spectrophotometers can be compared (Hall et al., 1970).

AOAC method. The method used was similar to that as described in AOAC (1960) except that 60°C and 40°C were used as the assay temperatures and all solutions were buffered to pH 6.0. Enzyme activities are expressed as dextrinizing units (DU) per ml of juice.

RESULTS & DISCUSSION

ALTHOUGH OPTIMUM reaction conditions for assay of sweet potato α-amylase have been described for the AOAC procedure (Ikemiya and Deobald, 1966) further studies were necessary before the chromogenic starch method could be applied. Time-reaction course studies showed that enzyme activity is linearly related to reaction time up through 20 min. Further studies using sweet potato juice as the enzyme source focused on the relationship between enzyme levels and the amount of blue color released (Fig. 1). At an assay temperature of 60°C, linear kinetics are observed up to 0.45 absorbance units. At 40°C, however, deviation is noted. Extrapolation to "zero" enzyme concentration gives an absorbance of about 0.03 units. In addition the linear portion at 40°C extends only to 0.225. Consequently, at this temperature a correction factor must be applied by

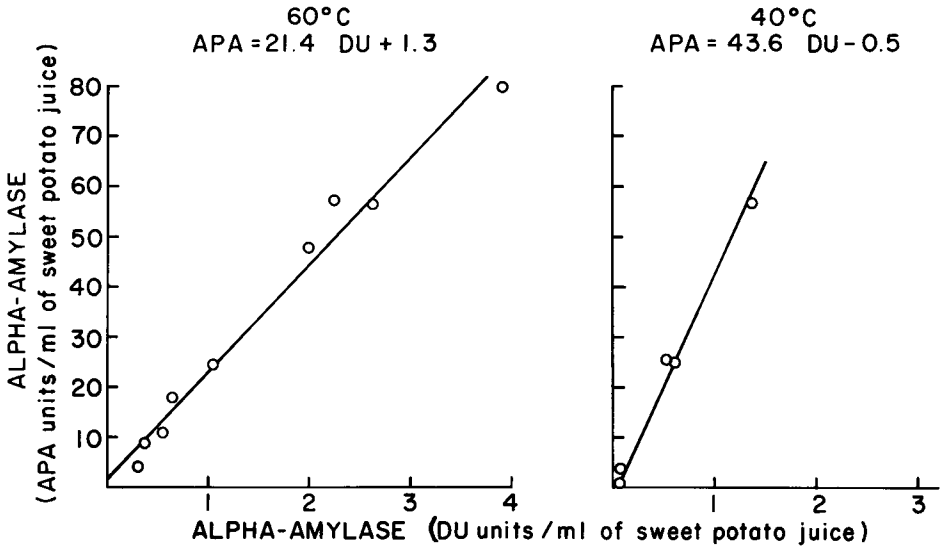

Fig. 2—Comparison of α-amylase activity in sweet potato varieties as determined by Blue Starch (APA Units) and AOAC (DU Units) methods. Assays run for 15 min at 40°C and 60°C. Regression equations are listed for each temperature.

subtracting the absorbance at "zero" enzyme concentration (about 0.03) from the actual absorbance of the sample. When this correction is applied, the method agrees well with the AOAC method which is linear at 40°C (Fig. 2). Samples having absorbancies greater than 0.450 at 60°C or 0.225 at 40°C must be diluted to bring them within these limits. The experiments described above indicate that the chromogenic starch method is valid for sweet potato α-amylase when performed within the prescribed limits.

Several sweet potato varieties were assayed for α-amylase by both the AOAC and chromogenic starch (blue starch) methods and the results compared in Figure 2. It should be noted that there is only slight deviation of the data points from the regression lines at both 40°C and 60°C.

Since sweet potatoes are a rich source of β-amylase, any method for assay of α-amylase must be free from interference by β-amylase. The AOAC method eliminates this problem by using β limit dextrin as the substrate. Our data show significant correlation for enzyme activities measured by the two methods (Fig. 2). This is convincing evidence that the blue starch method is not affected by β-amylase and that α-amylase activities from this newly developed method are comparable to those measured by the AOAC procedure. The chromogenic starch method shows adequate precision with a standard deviation of ± 4.7%.

The level of α-amylase is extremely important when sweet potatoes are processed into dehydrated flakes. Fresh-dug roots are starchy and give poor quality flakes unless considerable starch conversion occurs (Hoover, 1967). Since conversion times are governed by α-amylase activity, a knowledge of enzyme levels is of significant value in determining optimum processing conditions (Deobald et al., 1968). Presently the AOAC method is used for this determination. However, the simplicity, precision and sensitivity of the chromogenic starch method make it readily adaptable as a routine method for controlling conversion in flake production.

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