Changes in Invertase Activities and Reducing Sugar Content in Sweetpotato Stored at Different Temperatures†

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Cured sweetpotato roots were stored at different temperatures (4.5, 15.6, and 24 °C) for 7 weeks and assayed for invertase activities and reducing sugar levels during two separate years. Invertase activities and reducing sugar concentration significantly increased in the roots kept at low temperature. Of the three types of invertases assayed, acid invertase specific activity was the highest. Acid invertase was the most influential in determining reducing sugar levels in stored sweetpotato. Cultivar differences were found in invertase specific activities and reducing sugar concentration. Reducing sugar content was highly correlated to acid and total invertase activity, regardless of cultivar.

Keywords: *Ipomoea batatas; enzymes; postharvest physiology; ultrafiltration*

INTRODUCTION

Sucrose, glucose, and fructose are the principal sugars present in fresh sweetpotato roots (Picha, 1986; Morris and Mann, 1955). There are numerous reports on sugar changes in sweetpotato during storage (Lewthwaite et al., 1997; Takahata et al., 1996; Ajlouni and Hamdy, 1988; Truong et al., 1986; Picha, 1986; Morris et al., 1955), because the composition and concentration of free sugars are important components of eating quality. Sugar-metabolizing enzymes such as invertases, sucrose-synthase (SS), and sucrose phosphate synthetase (SPS) likely influence sugar levels in sweetpotato roots during storage. Several studies have been conducted on sugar-metabolizing enzymes in sweetpotato. Previous invertase studies focused on changes in acid and alkaline invertase activities induced by mechanical injury (Matsushita and Uritani, 1974) and during root initiation and development (Takahata et al., 1996; Acock and Garner Jr., 1987). One study reported on changes in sucrose concentration and SS and SPS activities in sweetpotato roots during storage (Takahata et al., 1995). Ajlouni and Hamdy (1988) reported the effects of combined γ-irradiation and storage period on the activities of starch phosphorylase, SS, SPS, β-amylase, and phosphoglucomutase.

The purpose of this research was to report changes in invertase activities and reducing sugar (fructose and glucose) contents in sweetpotato roots stored at various temperatures.

MATERIALS AND METHODS

Plant Materials. Two cultivars (Beauregard and Hernandez) of sweetpotatoes (*Ipomoea batatas* L. Lam) were obtained from the Louisiana Sweet Potato Research Station, Chase, LA, in 1997 and 1998. Fresh roots were cured at 32 °C and 90% relative humidity (RH) for 10 days immediately after harvest. The cured roots were placed in 15.6 °C storage (90% RH) for 7 weeks followed by analyses of invertase activity and concentrations of fructose, glucose, and sucrose. These roots served as the controls for the subsequent temperature treatments. Additional cured roots were randomly chosen and transferred to three different temperature treatments (4.5, 15.6, and 24 °C) for 7 more weeks followed by analyses of invertase and sugar concentrations. In addition, a subsample from the 4.5 °C storage treatment was analyzed after transfer to 15.6 °C storage for 1 week following the 7-week period.

Invertase Buffer and Extract Preparation. The extraction buffer consisted of 50 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid [(HEPES)−KOH, pH 7.4] containing 1% (w/v) insoluble polyvinylpyrrolidone (PVP-40), 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM phenylmethanesulfonyl fluoride (PMSF), 5 mM dithiothreitol (DTT), 0.1% Triton X-100, and 1% glycerol. Each extraction consisted of a composite sample of 10 g of internal flesh tissue from four different roots. Crude extracts were prepared as described (Huang et al., 1998). The resultant crude extracts were vacuum-filtered five times through two layers of Whatman No. 2 filter paper and twice through 0.45 and 0.2 μm Nalgene filterwares (Nalgene Co., Rochester, NY). The filtrate was further cleaned and concentrated in Ultrafree-15 centrifugal filter devices (Millipore Corp., Bedford, MA). The main purpose of this procedure was to remove glucose and fructose from the invertase preparations (Figure 1). Specifically, the filtrate obtained from the Nalgene filterwares was concentrated in a Biomax-100 microfilter, and the Biomax-100 filtrate was concentrated in a Biomax-5 microfilter. The combined concentrates were reconsituted to ≈8 mL using 50 mM HEPES/KOH buffer (pH 7.0) containing 2 mM EDTA and centrifuged for 15 min at 650*g*. Three additional cycles of reconstitution and concentration were performed to ensure that >99% of hexose was removed from the enzyme samples (Figure 1). The final concentrated fractions were used for invertase assays.

Invertase Assays. Neutral (pH 7.0) and alkaline (pH 7.6) invertase activities were determined by continuous assay (Huang et al., 1998) in 50 mM *N*-(2-acetamido)iminodiacetic acid (ADA; pH 7.0) and 50 mM (HEPES)−KOH (pH 7.6), respectively. Each buffer contained 10 mM $MgCl_2$ and 20 mM KCl to meet the specific requirements of the auxiliary enzymes

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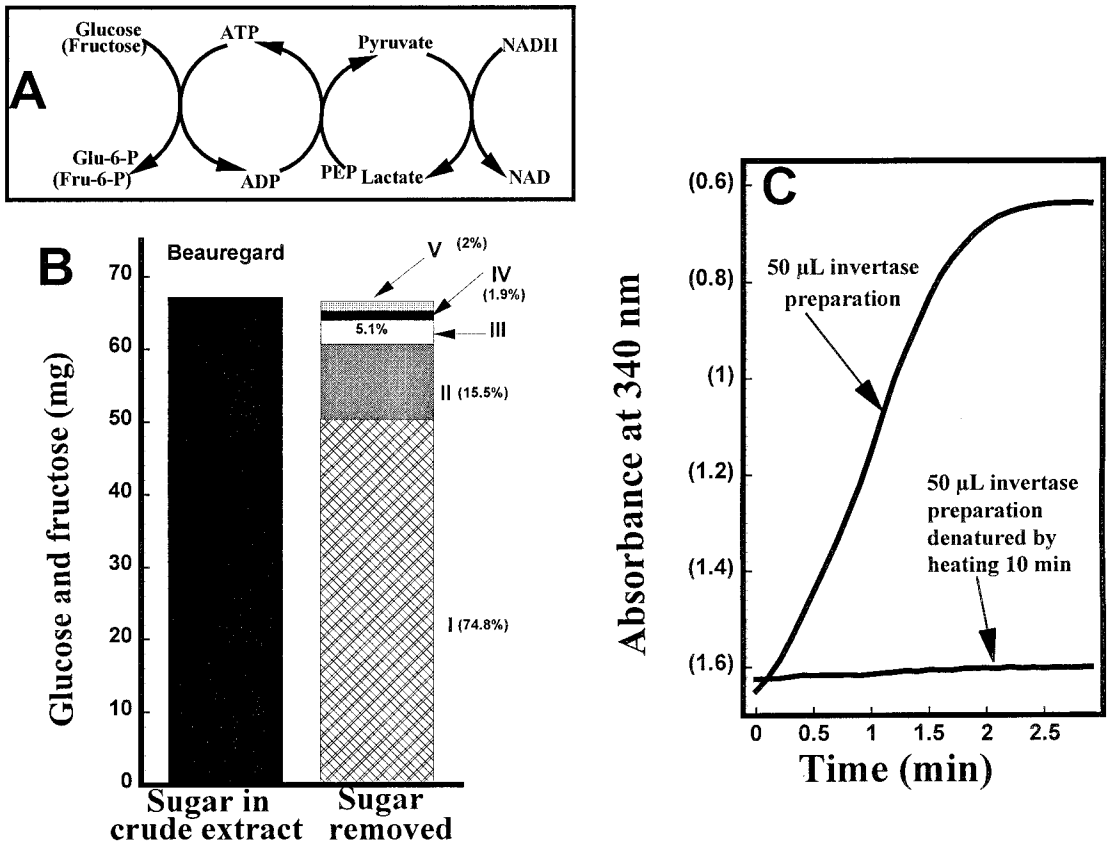

Figure 1. Illustration for removal of glucose and fructose from the crude extracts. Invertase preparations were made from crude extracts by use of Ultrafree-15 centrifugal filter devices. (A) Schematic diagram of reaction pathway used to determine hexose in crude extracts and filtrates. Experimental conditions were identical to those for neutral invertase assay (see Materials and Methods), except sucrose was excluded from the assay mixture. (B) The solid bar indicates the amount of hexoses present in 13 mL of Beauregard crude extract before removal of hexose by ultrafiltration, and the layered bar on the right shows the percentage of hexose removed at each step: (I) The 13 mL crude extract was concentrated in a Biomax-100 filter, the Biomax-100 filtrate was concentrated in a Biomax-5 filter, and the Biomax-5 filtrate was collected; (II−V) the combined concentrates of Biomax-100 and Biomax-5 filters were reconstituted and concentrated four times. The Biomax-5 filtrates collected at each step were subject to hexose analyses. (C) The efficiency evaluation for hexose removal from the invertase extract followed the procedure described for neutral invertase assay. The two lines are the nondenatured and heat-denatured (horizontal line) invertase preparations, respectively.

for metal ions. The assay mixture (3 mL) contained 100 μmol of sucrose, 1.53 μmol of adenosine 5′-triphosphate (ATP), 1.2 μmol of phosphoenolpyruvate (PEP), 0.9 μmol of reduced β-nicotinamide adenine dinucleotide (NADH), 8 units of hexokinase (HK), 8 units of pyruvate kinase (PK), and 8 units of lactate dehydrogenase (LDH). The invertase preparation (40 or 80 μL) was added to initiate the reaction.

Acid invertase assay followed the procedure of Robyt and White (1987) with a slight modification. Ten microliters of enzyme concentrate was added to 990 μL of 50 mM incubation buffer (25 mM citrate/25 mM ADA, pH 4.6) and incubated for 1 min. The reaction was terminated by adding 47 μL of 3 M KOH. The medium was brought to neutral pH with 31.4 μL of 2.4 M HCl, followed by boiling for 5 min in a water bath. The amount of glucose produced was measured by spectrophotometry as described for the neutral invertase assay. Controls were prepared by omitting the invertase preparation to correct for sucrose hydrolysis due to nonenzymatic reactions.

Invertase activity was expressed as micromoles of glucose per gram of root fresh weight per minute.

Sugar Determination. Sugar extraction followed previously described procedures, and glucose and fructose were quantitated by high-performance liquid chromatography (Picha, 1986). Reducing sugar content was expressed as amount of sugar (milligrams) per gram of fresh weight.

Data Analysis. The data were analyzed by the general linear model procedure, and mean separations were accomplished by LSD (SAS Institute, Cary, NC).

RESULTS

Differences between Cultivars for Invertase Activities and Reducing Sugar Contents. *1997.* Invertase activities closely followed the pattern of reducing sugar concentration. Invertase activities and reducing sugar contents were highest at 4.5 °C and lowest at 24 °C. Acid invertase had the highest value among the different invertases. Cv. Hernandez showed significantly higher acid and alkaline invertase activities than cv. Beauregard at all temperatures. Acid invertase activity was from 35.6% (4.5 °C) to 123.0% (15.6 °C) higher in Hernandez than in Beauregard (Table 1), and alkaline invertase activity was from 21.6% (4.5 °C) to 75.2% (control) higher (Table 1). Hernandez also had higher neutral invertase activity at 15.6 (control), 24, and 4.5 °C but lower activity in the 4.5/15.6 °C treatment, relative to Beauregard. Coincidentally, Hernandez had higher levels of reducing sugars than Beauregard at all storage temperatures (Table 2). Fructose ranged from 18 to 93.9% higher in Hernandez, glucose ranged from 27.7 to 130.9% higher, and total reducing sugar ranged from 23.5 to 114.7% higher. The difference in reducing sugar concentration between cultivars was greatest at 15.6 °C and least at 4.5 °C.

Table 1. Effect of Storage Temperature and Cultivar on Invertase Activities in Sweetpotato Roots[a]

	cv. Beauregard		cv. Hernandez	
treatment	1997	1998	1997	1998
		Acid Invertase		
4.5 °C	16.2 ± 0.9 a	9.1 ± 1.2 a	22.0 ± 0.9 a	16.7 ± 3.6 a
4.5/15.6 °C[b]	11.8 ± 0.4 b	8.8 ± 1.9 a	20.4 ± 0.8 b	14.7 ± 2.1 a
15.6 °C	9.4 ± 0.7 c	6.3 ± 1.2 b	20.9 ± 0.4 b	8.9 ± 2.1 b
24 °C	7.8 ± 0.9 d	5.1 ± 1.1 b	12.6 ± 0.6 c	8.1 ± 2.2 b
control[c]	10.9 ± 0.5 b	5.4 ± 0.9 b	17.4 ± 0.4 d	6.9 ± 0.8 b
		Neutral Invertase		
4.5 °C	7.2 ± 1.1 b	2.8 ± 0.2 a	9.0 ± 1.2 a	3.4 ± 0.5 a
4.5/15.6 °C[b]	9.4 ± 1.8 a	2.5 ± 0.6 a	7.3 ± 1.1 b	2.6 ± 0.6 b
15.6 °C	6.1 ± 1.2 bc	2.1 ± 0.4 b	6.4 ± 1.6 b	2.6 ± 0.5 b
24 °C	5.2 ± 1.1 c	2.0 ± 0.3 b	7.3 ± 1.5 b	1.9 ± 0.3 c
control[c]	5.7 ± 0.3 c	1.9 ± 0.3 b	6.4 ± 0.3 b	1.4 ± 0.2 c
		Alkaline Invertase		
4.5 °C	8.9 ± 1.1 a	3.2 ± 0.6 a	10.8 ± 1.7 a	4.0 ± 1.1 a
4.5/15.6 °C[b]	9.1 ± 1.8 a	2.9 ± 0.6 ab	11.6 ± 1.9 a	3.2 ± 0.6 b
15.6 °C	7.2 ± 1.6 b	2.4 ± 0.4 bc	10.2 ± 2.3 ab	2.7 ± 0.7 bc
24 °C	6.8 ± 1.6 b	2.3 ± 0.3 c	9.1 ± 2.3 b	2.2 ± 0.5 cd
control[c]	2.8 ± 0.3 c	2.1 ± 0.4 c	4.9 ± 0.3 c	1.8 ± 0.3 d

[a] Results are reported as μmol of glucose·g^{-1} of fresh weight·min^{-1}. Significance at $P \leq 0.05$. [b] One week at 15.6 °C following 7 weeks of 4.5 °C storage. [c] Samples prior to temperature treatments.

Table 2. Effect of Storage Temperature and Cultivar on Reducing Sugar Levels in Sweetpotato Roots[a]

	fructose		glucose		fructose + glucose	
treatment	1997	1998	1997	1998	1997	1998
			Cv. Beauregard			
4.5 °C	14.8 ± 2.3 a	14.1 ± 0.2 a	18.9 ± 2.5 a	18.2 ± 0.4 a	33.7 ± 4.8 a	32.2 ± 0.5 a
4.5/15.6 °C[b]	10.9 ± 0.8 b	15.0 ± 1.8 a	14.0 ± 1.2 b	17.2 ± 2.6 a	24.8 ± 1.1 b	32.2 ± 4.3 a
15.6 °C	7.6 ± 0.7 c	7.7 ± 1.0 b	9.9 ± 1.8 c	12.5 ± 1.2 b	17.5 ± 2.3 c	20.3 ± 2.1 b
24 °C	6.3 ± 1.0 c	5.0 ± 0.6 b	13.1 ± 2.3 bc	11.4 ± 1.0 b	19.4 ± 2.9 c	16.3 ± 1.5 b
control[c]	7.9 ± 1.3 c	7.0 ± 0.8 c	10.3 ± 1.9 c	10.9 ± 1.9 b	18.2 ± 2.3 c	18.0 ± 2.6 b
			Cv. Hernandez			
4.5 °C	17.4 ± 2.6 a	16.2 ± 0.5 a	24.2 ± 2.4 a	21.3 ± 0.6 a	41.6 ± 6.7 a	37.5 ± 1.0 a
4.5/15.6 °C[b]	17.5 ± 2.2 a	15.6 ± 2.0 a	24.8 ± 1.9 a	18.9 ± 2.8 a	42.3 ± 4.0 a	34.5 ± 7.4 a
15.6 °C	14.8 ± 1.6 ab	12.7 ± 1.1 b	22.8 ± 1.9 ab	17.1 ± 1.5 b	37.6 ± 3.3 ab	29.8 ± 2.1 b
24 °C	10.5 ± 1.2 c	7.7 ± 0.8 c	23.3 ± 2.5 a	15.8 ± 1.6 c	33.8 ± 3.3 b	23.5 ± 2.3 c
control[c]	12.6 ± 1.8 bc	9.0 ± 1.5 c	18.8 ± 2.8 b	12.6 ± 1.5 c	31.4 ± 4.5 b	21.6 ± 3.0 c

[a] Results are reported as mg of sugar·g^{-1} of fresh weight. Significance at $P \leq 0.05$. [b] One week at 15.6 °C following 7 weeks of 4.5 °C storage. [c] Samples prior to temperature treatments.

1998. Invertase activities also paralleled reducing sugar concentration of 1998, but less consistently than in 1997. Acid invertase was also the predominant invertase in 1998 among the three different invertases (Table 1). Cv. Hernandez had higher acid invertase activity than cv. Beauregard at all storage temperatures. Little or no differences in neutral and alkaline invertase activities existed between the two cultivars. Both fructose and glucose concentrations were generally higher in Hernandez than in Beauregard. For both cultivars, all three invertases were lower in activity in 1998. Hernandez had a low reducing sugar content in 1998. For Beauregard, there was only a slight difference in reducing sugar content between both years, except at 4.5/15.6 °C.

The effect of temperature on reducing sugar levels followed the changes in invertase activity. A good correlation was obtained between reducing sugar content and acid invertase activity ($R^2 = 0.85$ in 1997, $R^2 = 0.78$ in 1998) (Figure 2A) and between reducing sugar content and total invertase activity ($R^2 = 0.89$ in 1997, $R^2 = 0.82$ in 1998) (Figure 2B) for both cultivars at the different storage temperatures. A weaker positive correlation also existed between neutral invertase activity and reducing sugar content ($R^2 = 0.24$ in 1997, $R^2 = 0.75$ in 1998) and between alkaline invertase activity and reducing sugar content ($R^2 = 0.56$ in 1997, $R^2 = 0.77$ in 1998) for both cultivars.

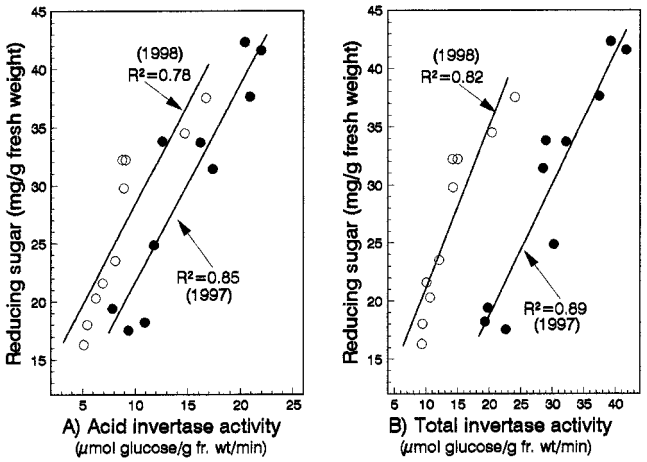

Figure 2. Correlation between reducing sugar content and acid and total invertase activities in two sweetpotato cultivars stored under different temperatures.

DISCUSSION

Enzyme activity is determined by measuring the amount of product produced or the amount of substrate

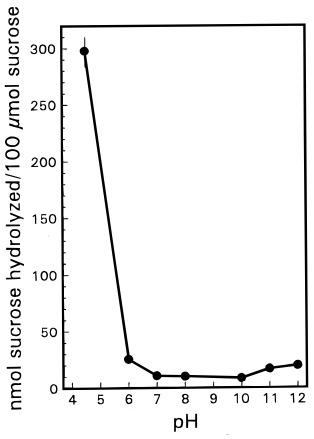

Figure 3. Nonenzymatic hydrolysis of sucrose by heating in boiling water at various pH values. Each data point is the mean of six separate measurements. Vertical bars represent the standard error, and where not apparent, fall within the dots. Conditions were identical to denaturation of enzyme activity in the discontinuous assay of acid invertase, except the invertase preparation was omitted from the incubation buffer.

utilized per unit of reaction time by a given amount of enzyme under high or saturated concentrations of substrates (Robyt et al., 1987). Therefore, enzyme activity is essentially the measurement of the initial reaction velocity under optimal maximum velocity conditions. The initial velocity typically remains for only a few seconds to several minutes for most enzymes (Allison and Purich, 1983). In the case of invertase reaction, once the invertase preparation is brought into contact with a sucrose solution, hydrolysis of sucrose to glucose and fructose is almost instantaneous (Huang et al., 1998). However, incubation time usually varies from 15 to 60 min in discontinuous procedures of acid invertase assay (Darnell et al., 1994; Hubbard et al., 1989; Zhu et al., 1997). Obviously, invertase activities obtained from these procedures might not reflect the initial reaction velocity. Therefore, a discontinuous procedure was optimized for pH and linear with time and enzyme concentration.

To ensure that the results of the acid invertase assay are valid, the following factors need to be considered: (1) the reaction has to be timely terminated at the end of the incubation period; (2) neutral and alkaline invertase activities must be eliminated during the thermal equilibrium period (which was 55 s in this case) when thermal denaturation is used; and (3) the nonenzymatic degradation of sucrose that occurs in acidic medium during heating has to be accounted for (Figure 3). Therefore, a double-denaturation procedure was employed to account for these factors. First, invertase activities were interrupted at the extreme pH with the addition of KOH solution to the reaction medium. Then the medium was adjusted back to neutral pH with an appropriate amount of HCl solution, followed by boiling for 5 min.

Acid invertase is compartmentalized and stored in vacuoles, whereas neutral invertase is a cytosolic enzyme (apRees, 1988; Preiss, 1982). Free sugars in sweetpotato roots are mainly present in vacuoles (Takahata et al., 1996). Although activities of the three types of invertases varied between both years, acid invertase activity was always higher than neutral and alkaline invertase activities (Table 1) and highly correlated with reducing sugar content (Figure 2A). This indicates that acid invertase is a critical enzyme in the regulation of reducing sugar levels in sweetpotato.

Previous reports of invertase activity in sweetpotato showed large differences and inconsistencies in results (Takahata et al., 1996; Matsushita and Uritani, 1974; Acock et al., 1987). According to Matsushita and Uritani (1974), acid invertase activity does not exist in fresh tissue, but it is inducible by mechanical injury. However, this is inconsistent with the present results. Our data indicated that acid invertase activity was higher than neutral or alkaline invertase activity. Compared on a fresh weight basis (micromoles of glucose per gram of fresh weight per minute), acid invertase activity in this study was 210−920-fold higher and neutral invertase activity was 40−60-fold higher than reported by Takahata et al. (1996). They reported a weak relationship between glucose content and acid invertase activity and a negative relationship between glucose content and neutral invertase activity. However, our results showed a strong correlation between reducing sugar content and acid invertase activity and a weak correlation between reducing sugar content and neutral and alkaline invertase activities. Several factors may account for the inconsistency in results. First of all, our assays were performed by specific coupling reactions instead of the Somogyi−Nelson method. Enzyme activity was calculated on the basis of the slope at the linear phase of the reaction, instead of using the discontinuous method, in which the reaction is incubated for a fixed length of time (usually 30−60 min) before the amount of product formed is measured. Also, we used filterwares and ultrafree centrifugal filter devices, which had high rates of enzyme recovery compared to cotton gauze and Sephadex G-25 columns, which were used in prior enzyme preparations (Matsushita and Uritani, 1974; Takahata et al., 1996). We optimized the extraction procedures and extracted the samples twice. In addition, differences in invertase activities may be due to cultivar and differences in root physiological age.

Invertase activity has been reported to increase in cold-hardy wheat leaves (Roberts, 1975) and in potato tubers stored at low temperature (Sasaki et al., 1971). Sweetpotato roots stored at 4.5 °C for 7 weeks showed a significant increase in activities of three types of invertases, regardless of cultivar or type of invertase (Table 1). Reducing sugar content also increased at low temperature and followed a pattern similar to the change in invertase activity (Table 2).

In conclusion, storage temperature significantly influenced the activities of invertases. Acid invertase activity was greater than neutral and alkaline invertase activities at all temperatures for both cultivars. Acid invertase was also influenced the most by temperature. Our results strongly suggest invertase activities determine reducing sugar concentration in sweetpotato roots during storage, and acid invertase is the critical invertase in the regulation of reducing sugar level.

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