Effect of Temperature on Invertase, Invertase Inhibitor, and Sugars in Potato Tubers

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Summary. The accumulation of reducing sugars in potato tubers exposed to low temperatures occurs with concomitant formation of the enzyme invertase. During the initial period of cold treatment when reducing sugars increase rapidly, invertase formation proceeds until the level of enzyme exceeds that of an endogenous macromolecular invertase inhibitor, resulting in a basal invertase activity. As the rate of sugar accumulation decreases and the sugar level becomes nearly constant, total invertase decreases, the basal activity disappears, and a low excess of inhibitor develops. On transfer of cold-stored tubers to warmer temperatures, sugars and invertase decrease sharply and a large excess of inhibitor develops. These changes in sugars, invertase and inhibitor occur reversibly when the tubers are subjected to alternating temperatures.

In spite of the importance of temperature as an environmental variable for plants, very little is known about its physiological and biochemical effects (5). One aspect of the effects of temperature on plants that has received considerable attention is the composition changes related to the development of frost resistance. A commonly observed change is the accumulation of reducing sugars which appear to function as protective agents against frost damage (9). However, the starch-sugars conversions produced by low temperatures are not restricted to hardy plants, but also occur in unhardy materials such as the potato tuber.

The accumulation of reducing sugars in potato tubers stored at low temperatures, and their subsequent disappearance when the tubers are returned to higher temperatures, was reported as early as 1882 (7). More recently there has been renewed interest in these reactions because of the undesirable effects of reducing sugars on the processing quality of potatoes (2, 10). Whereas the effect of temperature on the sugar balance in potato tubers has been well documented, the biochemical pathway of the starch-hexoses conversion is essentially unknown. This transformation has been attributed to a temperature induced shift in the starch-sugar equilibrium (4). However, the evidence on hand suggests a sucrose intermediate (11) and consequently the participation of the enzyme invertase. This is supported by a report of a relation between hexose content and invertase activity (3). Previous studies on potato invertase have been hindered by the presence of an endogenous inhibitor and the earlier reports, no doubt, dealt with excess or basal invertase activity which occurs only under certain circumstances. We have now developed a simple procedure for selectively destroying the inhibitor in extracts of potato tubers (8). This method allows the measurement of not only the basal activity but also total invertase.

In the earlier report (8) preliminary evidence for the effect of temperature on the level of invertase in potato tubers was presented. The present paper describes the results of detailed studies on the levels of invertase, invertase inhibitor and sugars in relation to storage temperature. The results demonstrate a high dependence of reducing hexoses and invertase on temperature and suggest a direct role for invertase in hexose accumulation in tubers at low temperatures.

Materials and Methods

Potatoes were grown in 1965 at the Research Farm of the Red River Valley Potato Growers' Association, Grand Forks, North Dakota. Two varieties, Red Pontiac and Kennebec, were studied. Two weeks before harvest the vines on the plants were removed by cutting. After harvest, the tubers were stored at 18° for 2 weeks before placing into cold storage at 4°. Cold-stored samples were transferred to 18° storage at frequent intervals.

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Analysis for Sugars. Six tubers were taken from each lot of potatoes, washed and cut in halves. One set of the halves was saved for invertase and inhibitor assays. The other set was sliced and 50 g were added to 211 ml of boiling 95 % ethanol. After 20 minutes, the sample was cooled, blended and filtered. The residue was re-extracted with 100 ml of 80 % ethanol and the filtrates were combined. The solution was deionized with Amberlite IR-120(H^+), followed by neutralization, and then with Dowex-1 (formate). The solution was made to volume and analyzed for reducing sugars by the arsenomolybdate method (1) and for total sugars by the anthrone method (1). Sugar levels are expressed as percent on a fresh weight basis. The alcohol extract was evaporated under reduced pressure and the sugars were determined qualitatively by paper chromatography. The chromatograms were irrigated with butanol:acetic acid:water (4:1:5) using the descending technique. Sugars were located by developing the sheets in diphenylamine aniline reagent followed by heating.

Invertase Assay. The remaining tuber halves were peeled and passed through a juicerator (Acme Juicer Manufacturing Company, Lemoyne, Pennsylvania). Two ml of 0.8M sodium sulfite (pH 6.0) were added to each 100 ml of extract to prevent enzymatic darkening. About 50 ml of each extract was then dialyzed overnight against 4 liters of 0.1M NaCl. The dialyzed extract was diluted with 1 volume 0.2M sodium acetate, pH 4.75, and warmed to 37°. Forty ml of the diluted extract were placed in a 100 ml VirTis homogenizer jar and homogenized at medium speed for 10 minutes while maintaining the temperature at 37°. Use of the VirTis homogenizer simplified the blending procedure originally employed (8). Properly diluted portions of the original and blended extracts were assayed for basal and total invertase, respectively, according to the procedure previously described (8). A unit of invertase is defined as that amount of enzyme which catalyzes the formation of 1 μmole hexose per hour at 37° and pH 4.75.

Invertase Inhibitor Assay. The excess inhibitor was measured in the dialyzed unblended extracts as described previously (8).

Protein. Protein was measured by the biuret method (6). Specific activities of invertase and inhibitor are expressed as units per mg protein.

Results

Invertase and Sugars in Tubers During Growth. Potato tubers were obtained at weekly intervals from the time they were 2.5 cm in diameter until harvest. A total of 11 samples of each variety were analyzed for sugars and invertase. Throughout the growth period, the total sugar content in both varieties varied from 0.6 to 1.0 % with no definite pattern in the small fluctuations. A de-

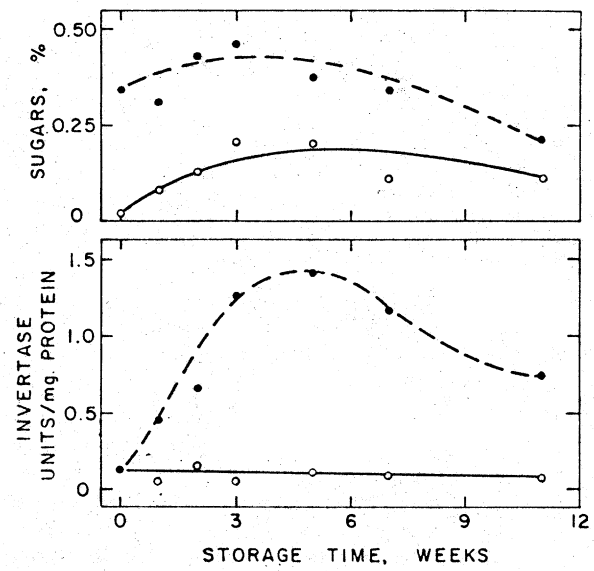

FIG. 1. Changes in sugars and invertase in Pontiac tubers stored at 18°. ●, total sugars, total invertase; ○, reducing sugars, basal invertase.

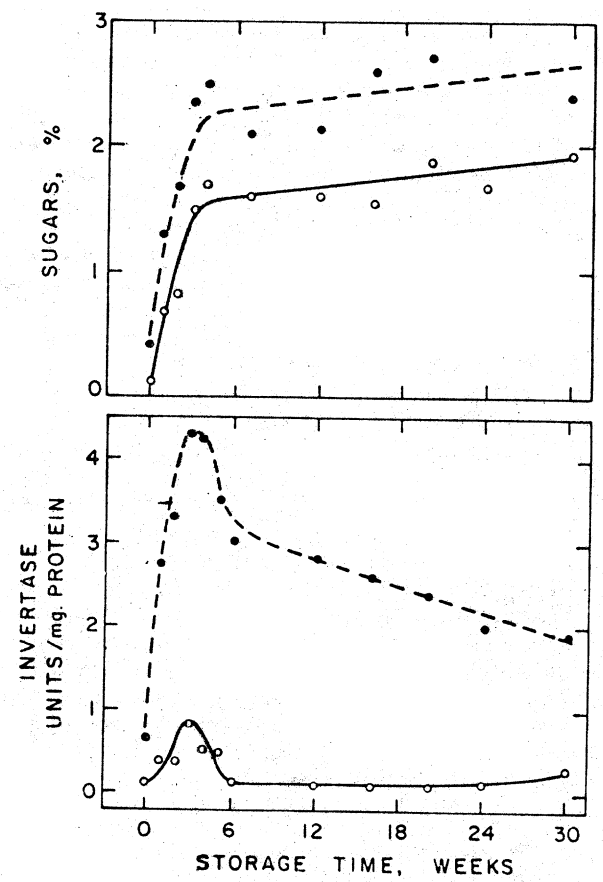

FIG. 2. Changes in sugars and invertase in Pontiac tubers stored at 4°. ●, total sugars, total invertase; ○, reducing sugars, basal invertase.

crease in sugar content was observed after the vines were removed. The predominant sugar was sucrose. The reducing sugar content was generally very low (0.1 %) and usually consisted solely of glucose. Fructose was not detectable chromatographically in most samples.

Basal invertase activity was practically negligible (0.06 to 0.22 unit/mg protein) in most samples. Total invertase was also low (0.10 to 0.30 unit/mg protein) in the tubers during the growth season. The number of samples analyzed for invertase inhibitor was limited, but it was found that an excess of inhibitor is present in mature tubers at harvest time.

Invertase and Sugars in Tubers Stored at 18°. Potato tubers stored continuously at 18° after harvest remained in good condition for about 7 weeks and then slowly deteriorated. Analysis of Pontiac tubers during 11 weeks at this temperature yielded the data presented in figure 1. Total sugars increased slightly after several weeks and then decreased. Reducing sugars increased significantly to a maximum after about 3 weeks but the level was very low. Basal invertase activity did not develop, but total invertase increased to about 1.4 units/mg protein after 5 weeks and then decreased slowly. Similar changes were observed for Kennebec tubers.

Invertase and Sugars in Tubers Stored at 4°. In contrast to the small variations in sugars and development of a low level of invertase in tubers stored continuously at 18°, marked changes occurred in tubers after transfer to cold storage (fig 2). Immediately after the tubers were placed in storage at 4°, reducing and total sugars increased rapidly. The rate of sugar accumulation decreased after 3 weeks of cold storage although the sugars continued to increase slowly on continued cold storage. The sugars consisted mainly of glucose and fructose which occurred at approximately equal levels. Sucrose was also always present, but at lower levels. Other sugars were occasionally detected chromatographically and one of these appeared to be raffinose.

The changes in invertase in tubers at low storage temperature were also pronounced. Total invertase increased rapidly to a maximum after 3 weeks. The activity decreased quite sharply during the next 3 weeks and slowly on continued cold storage. The maximum total invertase attained at 4° was about 3 times higher than that in tubers at 18° (fig 1). Whereas a basal invertase activity did not develop at 18°, a significant level developed in tubers at 4°. The basal activity increased to a maximum after 3 weeks and then rapidly decreased to a negligible level. After the basal activity decreased to zero, an excess of inhibitor developed in the tubers and persisted until after 24 weeks. On still longer cold storage, the inhibitor disappeared and a low level of basal invertase developed.

Effect of Warm Temperatures on Invertase, Inhibitor and Sugars in Cold-stored Tubers. It is well known that the sugar content of cold-stored potatoes can be reduced by placing the tubers at a warm temperature for several weeks. We reported earlier (8) that in addition to the changes in sugars, the level of invertase decreased and an excess of inhibitor developed when the tubers are transferred from cold to warm storage. These preliminary results were confirmed in the current studies when a large number of samples, after various durations of cold storage, were transferred to 18° and analyzed at regular intervals. In all the samples studied, total invertase decreased rapidly after the change in temperature was made. Basal invertase, if present, decreased to zero and an excess of inhibitor always developed. Large decreases in reducing and total sugars accompanied the changes in invertase and inhibitor. Most of the changes occurred during the first 2 weeks at 18° with further small changes on continued warm storage.

In the case of Kennebec tubers, the levels of total and reducing sugars after 6 weeks at 18° were usually about 0.6 % and 0.4 %, respectively. Total invertase decreased to about 2 units/mg protein during this time. The levels of sugars were usually higher and of invertase usually lower in Pontiac tubers. The duration of cold storage prior to the warm treatment did not have a great effect on the residual sugars and invertase. In contrast, the level of excess invertase inhibitor was found to be highly dependent on the duration of

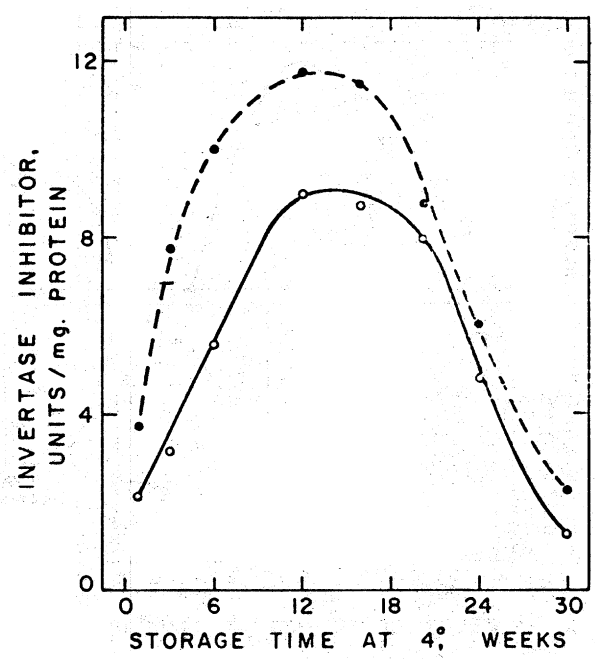

FIG. 3. Effect of duration of cold storage on the development of excess inhibitor in tubers on transfer to 18°, ●, Pontiac tubers; ○, Kennebec tubers.

cold storage (fig 3). Highest levels of inhibitor were found in tubers stored at 4° for 12 to 16 weeks followed by warm treatment for 6 weeks. The level of inhibitor that developed in the tubers decreased rapidly as cold storage exceeded 16 weeks.

Effect of Alternating Temperatures. We have thus far described the effects of constant high or low temperature and low temperature followed by high temperature on sugars and the invertase system in potato tubers. However, variations in these components are obtained when low and high temperatures are alternated several times (fig 4). In this experiment, Kennebec tubers were stored at 4° for 6 weeks, transferred to 18° for 6 weeks, back to 4° for 8 weeks, and finally back to 18°. The most striking feature of the results is the relationship between temperature and the levels of sugars, invertase and inhibitor. Each time the sample was transferred from cold to warm temperature, sugars decreased, invertase decreased, and the inhibitor increased. When the tubers were transferred from warm to cold temperature, the opposite effects were observed, i.e., sugars increased, invertase increased, basal invertase developed and excess inhibitor disappeared.

A number of specific details in the fluctuations with temperature should be pointed out. The level of sugars accumulated during the second storage period at 4° was lower than that during the first period at this temperature. Basal and total invertase increased at slower rates during the second cold treatment, but total invertase exceeded the level attained the first time at 4°. The sugars remained at a higher level during the second period at 18° than that during the first warm treatment. In contrast, the level of inhibitor was considerably lower after the second period at 18° compared to that after the first storage at 18°. It is difficult to evaluate the significance of these differences because of the relatively long time involved. However, the results suggest that alternating temperatures lead to relatively low sugar levels at 4° and relatively high levels at 18° as the number of temperature changes increases.

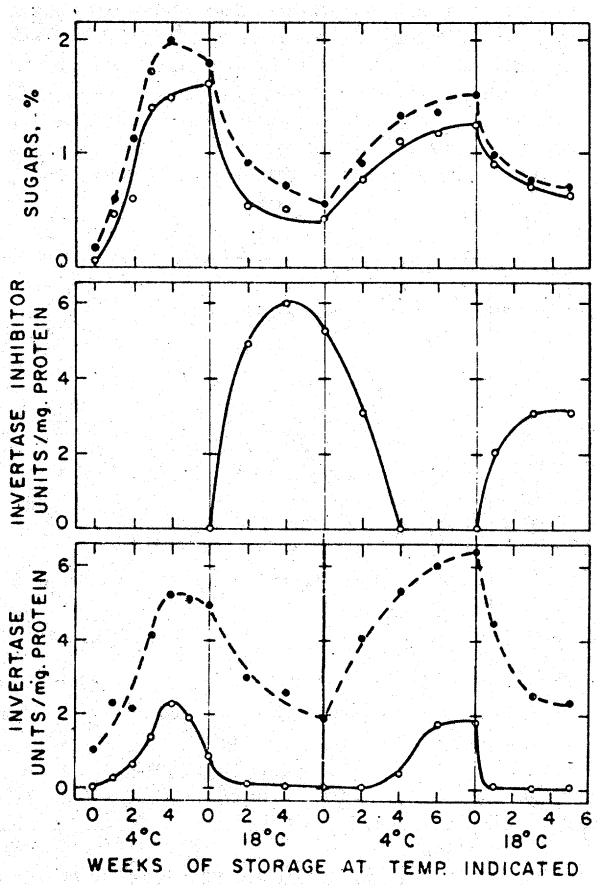

FIG. 4. Effect of alternating temperatures on sugars, invertase and excess invertase inhibitor in potato tubers. ●, total sugars, total invertase; ○, reducing sugars, basal invertase.

Discussion

The results of these experiments clearly demonstrate that temperature has a profound effect on not only the sugar balance in potato tubers but also on the invertase system consisting of the enzyme and a macromolecular inhibitor. Newly harvested mature tubers contain low levels of reducing sugars and total invertase activity. The basal invertase activity in the tubers is zero due to an excess of invertase inhibitor. Exposure of the tubers to low temperatures results in a rapid conversion of starch to hexoses accompanied by an equally rapid increase in invertase. Formation of invertase proceeds until its level exceeds that of the inhibitor and a basal invertase activity results. After a sufficient duration at a given temperature, a maximum in hexoses is attained, total invertase decreases markedly, the basal activity is depleted and an excess of inhibitor develops.

Transfer of cold-stored tubers to a higher temperature results in rapid decreases in sugars and total invertase and in the disappearance of basal invertase, if present. In addition, a high level of excess inhibitor develops. All of these changes are reversed if the tubers are transferred back to a low temperature. The results, therefore, establish that the level of the enzyme invertase in potato tubers fluctuates reversibly with changes in storage temperature. To the best of our knowledge, a similar variation of a plant enzyme with temperature has not been reported.

The data indicate a direct role for invertase, and hence a sucrose intermediate, in the pathway of hexose accumulation in tubers at low temperatures. Moreover, the existence of both an enzyme and its inhibitor and the nature of their variations

in relation to changes in hexoses are evidence that the invertase system exerts at least some regulatory effect on hexose accumulation. Usually, hexose accumulation occurs in the presence of basal invertase activity and high total invertase. An excess of inhibitor generally is associated with decreasing or constant sugar levels. However, in at least one instance (fig 4) hexoses were increasing when basal invertase activity was non-existent and an excess of inhibitor was present. Similarly, a high level of basal invertase activity does not always correspond with a rapid increase in hexoses. There are enough discrepancies, therefore, to suggest that other controlling factors, such as sucrose formation must be operative.

Acknowledgment

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