SUCROSE METABOLISM IN POSTHARVEST SUGARBEET ROOTS: ACTIVITIES AND PROPERTIES OF THE MAJOR SUCROLYTIC ENZYMES

Karen Klotz[a] and Fernando Finger[b]
[a]USDA/ARS/ Northern Crop Science Laboratory, Fargo, ND 58105
[b]Departamento de Fitotecnia, Universidade Federal de Viçosa, 36571-000 Viçosa, MG, Brazil

Introduction

Sucrose is lost during postharvest storage and processing due to the continuing metabolic activity of sugarbeet roots and the presence of endogenous enzymes capable of degrading sucrose. Until frozen, sugarbeet roots actively degrade sucrose. This metabolism is necessary to heal wounds that occur during harvest and for maintenance of healthy root tissue. Postharvest sucrose metabolism, however, is costly for the sugarbeet industry. It has been estimated that 100 to 250 g of sucrose is lost per day per ton of roots during storage (Bugbee 1993, van der Poel *et al.* 1998). With a harvest of approximately 14 million tons of sugarbeet roots in the Red River Valley of Minnesota and North Dakota, this translates into the loss of many thousands of tons of sucrose each storage season. The enzymes of sugarbeet sucrose metabolism are also involved in the sucrose loss that occurs when stored roots thaw and during the initial stages of processing. In both cases, cell rupture caused by a freeze-thaw cycle or slicing during the first steps of processing, eliminates the cellular compartmentalization that separates sucrose from the enzymes that degrade it.

Sucrose catabolism occurs primarily by the action of three enzyme activities. Acid invertase, alkaline invertase and sucrose synthase catalyze the conversion of sucrose to the invert sugars, glucose and fructose, and uridine 5'-diphosphate glucose, a metabolically active form of glucose. The role of these enzymes in postharvest sucrose loss is unknown, although it has been suggested that sucrose synthase and/or acid invertase are involved (Sakalo & Tyltu 1997, Wyse 1974). In this study, the activities of the major sucrose degrading enzymes were determined in postharvest sugarbeet roots after prolonged storage or storage under unfavorable conditions. The capacity of sucrose synthase and acid invertase to degrade sucrose under typical storage and processing conditions was also determined.

Materials and Methods

Sugarbeet hybrid VDH66156 was planted on May 19 and hand harvested September 16. Roots were washed prior to storage at 6, 12 or 21^{o}C and 95 to 99% relative humidity. Ten roots were sampled for each data point. Representative longitudinal sections were removed from each root, rapidly frozen in $N_{2(l)}$ and lyophilized. Soluble proteins were extracted by homogenization of lyophilized tissue in 100 mM HEPES, pH 7.2, 10 mM Na_2SO_3, 5 mM DTT and 1 mM $MgCl_2$. Homogenates were centrifuged to remove cell debris and dialyzed against 10 mM HEPES, pH 7.2, 1 mM DTT and 1 mM $MgCl_2$ to remove sugars. Protein extracts were assayed for acid and alkaline invertase activity by the method of Goldstein and Lampen (1975) and for sucrose synthase activity by the method of Somogyi (1952).

Soluble acid invertase was partially purified from lyophilized 5-6 week old root tissue by homogenization in 50 mM HEPES, pH 7.5, 5 mM ß-mercaptoethanol**,** 1 mM EDTA, 1 mM $MgCl_2$, 1 mM benzamidine and 100 µM PMSF. Homogenate was filtered through cheesecloth and centrifuged to remove cell debris. Acid invertase was precipitated by $(NH_4)_2SO_4$ at 61-80% saturation and dialyzed against 10 mM HEPES, pH 7.5 and 1 mM ß-mercaptoethanol before use. Sucrose synthase isoforms were partially purified from 6 and 16 week old roots for sucrose synthase I and sucrose synthase II, respectively. Lyophilized tissue was homogenized with 50 mM HEPES, pH 7.2, 5 mM ß-mercaptoethanol**,** 10 mM Na_2SO_3 and 1 mM $MgCl_2$. Homogenate was filtered through cheesecloth and centrifuged. Sucrose synthase isoforms were precipitated by $(NH_4)_2SO_4$ at 20-45% saturation and dialyzed against 10 mM HEPES, pH 7.2 and 1 mM ß-mercaptoethanol**.** Dialyzed fractions were purified over a cibachron blue column eluted with 0.5 M NaCl. Sucrose synthase II was further purified by passage over a Q-sepharose column eluted with a 0.2 to 0.6 M NaCl gradient. Sucrose synthase isoforms were dialyzed as described above after passage over each column.

Results and Discussion

The activities of the major sucrose degrading enzymes were determined in postharvest sugarbeet roots after prolonged storage or storage under unfavorable conditions. The purpose of these experiments was to determine the relative contribution of each enzyme activity to the total sucrose degrading activity of the root and to determine the effect of storage conditions on these activities. Sucrose synthase, alkaline invertase and acid invertase activities were measured in sugarbeet roots stored at 6, 12

and 21°C for zero to seventeen weeks (Figure 1). Sucrose synthase activity was the

A

B

C

Figure 1: Sucrolytic enzyme activity in sugarbeet roots stored at (**A**) 6°C, (**B**) 12°C or (**C**) 21°C. Field-grown, hand harvested roots were stored at 95-99% relative humidity and sucrose synthase activity (✍), alkaline invertase activity (✍), and soluble acid invertase (?) were measured after different durations of storage. Error bars = one standard deviation.

A **B** **C**

Figure 2: Temperature effect on activity of (**A**) sucrose synthase I, (**B**) sucrose synthase II, and (**C**) soluble acid invertase. Error bars = one standard deviation.

predominant sucrose degrading activity under all storage conditions and durations tested. Alkaline invertase activity was present at significantly lower levels than sucrose synthase activity. Acid invertase activity was barely detectable. Surprisingly few changes in enzyme activity were found even after prolonged storage (Fig. 1A) or storage at elevated temperatures (Fig. 1C). Only alkaline invertase activity exhibited a change in activity that was consistent over all temperature conditions studied. Alkaline invertase activity initially declined during storage. With subsequent storage, alkaline invertase activity increased gradually to a level similar to its activity at harvest. Although a slight increase in acid invertase was observed in sugarbeet roots stored at 6°C, this increase was not observed in roots stored at 12 or 21°C.

The effect of environmental conditions on sucrose degrading activity was also examined for sucrose synthase and acid invertase. The purpose of these experiments was to determine the capacity of these enzymes to degrade sucrose under the conditions typically encountered during storage and processing. Two sucrose synthase isoforms (sucrose synthase I and sucrose synthase II) contribute to sucrose synthase activity in postharvest sugarbeet roots. The environmental effects on the activity of these two isoforms were determined separately.

The effect of temperature on the activity of the two sucrose synthase isoforms and acid invertase is shown in Figure 2. The optimum temperatures for sucrose synthase I, sucrose synthase II and acid invertase activities were 50°, 45-50° and 35°C, respectively. Sucrose synthase II and acid invertase were completely and irreversibly inactivated at temperatures of 60°C or greater. Inactivation of sucrose synthase I required temperatures of 65°C or greater. A temperature of at least 65°C, therefore, is required to completely inactivate all three enzymes. These results imply that sucrose loss by these enzymes is possible

during sugarbeet processing. Although sucrose is extracted from sugarbeet root slices at 70 to 73°C (van der Poel

A

B

C

Figure 3: pH effect on activity of (A) sucrose synthase I, (B) sucrose synthase II, and (C) soluble acid invertase. Error bars = one standard deviation.

et al. 1998), temperatures that would complete inactivate sucrose synthase and acid invertase activities, sucrose degradation by these enzymes could occur in the time period between the slicing of the cold or frozen roots and the obtainment of optimum extraction temperatures. Of particular note is the heat stability of the two sucrose synthase isoforms. Not only was sucrose synthase found at high levels in postharvest sugarbeet roots (Fig. 1), but the activities of its two isoforms increased as temperature increased to 50°C. All three enzymes retained a portion of their activity at temperatures typical of storage. At 5°C, sucrose synthase I, sucrose synthase II and acid invertase retained, respectively, 8, 14 and 16% of their activity relative to their activity at 35°C. This suggests that both sucrose synthase isoforms and acid invertase are capable of degrading sucrose during postharvest storage.

The activities of the two sucrose synthase isoforms and acid invertase were also dependent on solution pH (Fig. 3). Sucrose synthase I was active in the pH range of 5.5 to 8.0; sucrose synthase II was active in the pH range of 5.5 to 7.5. Acid invertase exhibited a plateau of activity at pH 5.0 to 5.5 and its activity increased 7.5 fold with a decrease in pH from 5.0 to 3.0. Although the cause of the activity increase between pH 3.0 and 5.0 has not been determined, a similar pH response has been observed for an acid invertase in potato and is due to a decreased effectiveness of a specific acid invertase inhibitor (Pressey 1967). Solution pH during sugarbeet root extraction is typically in the range of 5.0 to 6.6 (van der Poel *et al*. 1998). At these pH values, sucrose degradation can occur by the action of sucrose synthase and/or acid invertase. Lower pH values have been observed during the processing of diseased roots and pH values as low as 4.5 have been reported (van der Poel *et al*. 1998). Sucrose loss due to acid invertase activity would be expected to be exacerbated by these conditions.

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