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Changes in Carbohydrate Content and the Activities of Acid invertase, Sucrose Synthase and Sucrose Phosphate Synthase in Asparagus Spears During Storage

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Abstract: We held asparagus *(Asparagus officinalis* L.) spears at 25°C for up to 5 days after harvest and examined changes in soluble carbohydrates and the activities of enzymes concerned with carbohydrate breakdown in both top and bottom portions of the spears. The acid invertase in soluble fraction showed a higher activity than that in cell wall bound fraction and the top portion of the spear showed a significantly higher soluble acid invertase activity than the bottom portion. But the activity of cell wall bound acid invetase was higher in bottom portion. In both the top and bottom portions soluble acid invertase activity increased during first day of storage and after that it started to decrease gradually up to five days. In case of cell wall bound acid invertase the activity increased during first two days and after that it also started to decrease. Sucrose synthase activity, found higher in bottom portion than top, started to decline from the first day of storage and continued up to five days. But the activity declined rapidly on third day. In case of sucrose phosphate synthase there was no specific inclining or declining pattern of activity in any portion of the spear. Among the soluble sugars fructose and glucose were predomonant and fructose content was significantly higher than the glucose and sucrose. All the three sugars started to decrease from the first day and continued up to five days. Sucrose content was negatively correlated with invertase and sucrose synthase in both portions and accounted well for the relation between the substrate and enzyme activity. But sucrose phohphate synthase activity remained almost constant during the storage period and there was no significant correlation between sugar content and sucrose phosphate synthase activity.

Key words: *Asparagus officinalis,* invertase, storage, sucrose synthase, sucrose phosphate synthase, sugar content

Introduction

Carbohydrates are the major biochemical components of edible asparagus spears and the spear quality is mainly determined by the amount of carbohydrates present in it (Alam *et al.*, 1998). The postharvest deterioration of asparagus spear is accompanied by many physiological and biochemical changes (Lipton, 1990) where, amongst other things, the soluble carbohydrate levels rapidly decline (King *et al.,* 1988). In plant tissues, cleavage of the glycosidic bond in sucrose is by either sucrose synthase (SS) or invertase. SS requires UDP as a co-substrate and produces fructose and UDP-glucose, whereas invertase simply splits sucrose into glucose and fructose (Copeland, 1990). Sucrose synthase is a cytoplasmic enzyme that catalyses a reversible reaction and under normal conditions acts only in the breakdown of sucrose. There are two types of invertase in plants. Acid invertase, which has optimum activity about pH 5, is present in vacuoles and in the apoplast, bound to cell walls. Neutral (alkaline) invertase has optimum activity at pH 7-7.5 and is found in cytoplasm. Invertases catalyze an irreversible reaction (Hurst *et al.,* 1993). There is evidence that rapid decline of carbohydrate levels in the spear tip triggers or signals deterioration in the whole spear (King *et al.,* 1993) and the spears stored at 25°C usually have a shorter shelf life (Bhowmik *et al.,* 2000) It seems possible, therefore, that if we could control the postharvest decline of carbohydrate levels in asparagus spears we may be able to maintain the quality and also extend its shelf life. Whilst previous authors have studied the changes in sucrose metabolizing enzymes in asparagus spears (Alam *et al.*,1998; Hurst *et al.,* 1993), none have done so during storage. That is why , as the first step towards understanding and therefore controlling the rapid decline of carbohydrate levels in asparagus spears during storage, we examined changes in soluble carbohydrates and the activities of enzymes concerned with carbohydrate breakdown in both top and bottom portions of the spears.

Materials and Methods

Plant material and storage: Green asparagus spears *(Asparagus officinalis* L. cv. Welcome) harvested from a commercial crop in Miki-cho, Ikenobe, Kagawa, Japan were obtained directly from a packing house. The cultivar 'Welcome' was selected due to its adaption to moderate cooler condition and availabilty throughout the country. Spears were hand harvested and trimmed to approximately 25 cm length. The spears which were straight, undamaged, with closed bracts and with no obvious signs of disease were put in plastic bags and held at 25°C for up to 5 days. At harvest (initial) and subsequent 24 h intervals the spears were weighed and frozen at -30°C for sugar and enzyme analysis.

Extraction buffers: A 0.2 M citrate-phosphate (C-P) buffer at pH 5.0 for soluble acid invertase and 0.2 M NaC1C-P buffer at the same pH for cell wall-bound acid invertase were used. On the other hand, 0.2 M potassium-phosphate (K-P) buffer at pH 7.8 containing 10 mM ascorbate, 15 mM $Mgcl_2$, 1 mM EDTA and 1 mM dithiothreitol (DTT) was used for the extraction of sucrose synthase (SS) and sucrose phosphate synthase (SPS).

Enzyme extraction for acid invertase: Each spear was cut into two equal halves (designated as top portion and bottom portion) just before extraction. Approximately 10 g of sample from each portion was mixed with 1% of polyvinylpolypyrrolidone (PVPP) and 1 g sea sand. The sample was then homogenized using a cooled mortar and pestle with 10 ml of 0.2 M C-P buffer (pH 5.0). The resulting homogenate was then filtered through four layers of cotton cloth and the filtrate was centrifuged at 11000xg for 10 min. The total supernatant was dialyzed with 40 times diluted 0.2 M C-P buffer (pH 5.0) for 12 h and the inner solution was designated as the soluble fraction. The residual tissues were re-extracted in 10 ml 0.2 M NaCI C-P buffer for about 24 h with occasional stirring. The supernatant was dialyzed as described above and the dialyzed solution was designated as cell wall-bound

fraction. All extraction procedures were carried out at 0-4°C followed immediately by the enzyme assays.

Enzyme assays for acid invertase: The standard assay medium for acid invertase consisted of 0.2 ml of 0.2 M C-P buffer (pH 5.01, 0.1 ml of 0.5 M sucrose, 0.1 ml of water and 0.1 ml of crude enzyme solution. The blank experiment contained distilled water instead of sucrose. The assay mixture was incubated at 45°C for 15 min. After the reaction, assay mixture was neutralized with 0.1 N NaoH or 0.1 N HCl, a coloring Sornogyi's copper reagent was added and the mixture was heated for 10 min in boiling water. The amount of reducing sugars was estimated by the method of Sornogyi. Soluble protein content was determined by the method of Lowry by using bovine serum albumin as the standard. The enzyme activity was expressed as the amount of glucose produced per min per mg of protein.

Enzyme extraction for SS and SPS: Approximately 10 g of sample from each portion was mixed with 1% of polyvinylpolypyrrolidone (PVPP) and 1 g sea sand. The sample was then homogenized with 10 ml of 0.2 M K-P buffer (pH 7.8) containing 10 mM ascorbate, 15 mM $MgCl_2$, 1 mM EDTA and 1 mM dithiothreitol (OTT) using a cooled mortar and pestle. The resulting homogenate was then filtered through four layers of cotton cloth and the filtrate was centrifuged at 11000xg for 20 min. The total supernatant was dialyzed with 40 times diluted 0.2 M K-P buffer (pH 7.8) for 16 h and the inner solution was used as the crude enzyme. All extraction procedures were carried out at 0-4°C.

Enzyme assays for SS and SPS: SS and SPS activities were assayed at 37°C by the method described by Hubbard (Hubbard and Pharr, 1992) with slight modifications. Reaction mixtures (70 µl) contained 50 mM Hepes-NaOH buffer (pH 7.5), 15 mM $MgCl_2$, 25 mM fructose-6-phosphate, 25 mM glucose-6-phosphate and 25 mM UDP glucose. The mixtures were incubated for 30 min at 37°C and the reaction was terminated with the addition of 70 µl of 30 % KOH. Enzyme blanks were terminated with KOH at 0 min. Tubes were kept at 100°C for 10 min to destroy any unreacted fructose or fructose-6-phosphate. After cooling, 2 ml of anthrone reagent (150 mg anthrone with 100 ml of 15% H_2SO_4) was added and incubated in a 40°C water bath for 15 min. After cooling color development was measured at 620 nm. SS was assayed as above but with 25 mM fructose instead of fructose-6phosphate and in the absence of glucose-6-phosphate. The soluble protein content was determined by the method of Lowry by using bovine serum albumin as the standard. The enzyme activity was measured as p-mole of sucrose or sucrose-phosphate produced per min per mg protein.

Determination of sucrose, glucose and fructose contents by high performance liquid chromatography(HPLC): About 7.5 g of asparagus sample (for each portion) was mixed with 1 g sea sand and homogenized in a cooled mortar and pestle. Twenty five ml of distilled water was added to the homogenate and centrifuged at 11000 x g for 10 min. The mixture was filtered through a cellulose nitrate membrane filter (0.45 AZ m pore size). Soluble sugars were analyzed by HPLC using a stainless steel column (10.7 mm IDx30 cm) packed with silica gel (gel pack C610). The mobile phase (filtered water) was pumped through the column at a flow rate of 1.0 ml/min. The pressure was adjusted to 14-15 kg/cm^2 and the temperature to 60°C. ARI monitor (Hitachi L-3300) was used. Sucrose, glucose and fructose were identified by their retention times and were quantified according to standards.

Statistics: A randomized complete block design was adopted with three replications. The level of significance was calculated from the F value of ANOVA. The relationship between sugars and enzyme activities were described with linear correlation analysis.

Results

Acid invertase activities: In both the top and bottom portions soluble acid invertase activity increased during first day of storage and after that it started to decrease gradually up to five days (Fig. 1). The top portion of the spear showed a significantly higher soluble acid invertase activity than the bottom portion. On the other hand the activity of cell wall bound acid invertase was higher in bottom portion than top. The activity of cell wall bound acid invertase in both top and bottom portions also decreased up to five days, after an initial increase on day one and two (Fig. 2). A highly significant negative correlation was found between acid invertase activity and sucrose content.

Table 1: Correlation coefficients (r) among enzyme activities and sugar contents in asparagus spears held at 25°C

Sugar Contents	Portion	Enzyme activity			
		Acid invertase		SS	SPS
		(SF)	(CWBF)		
Sucrose	Top		-0.706**	-0.604**	0.013
	Bottom	-0.796**	-0.743**	-0.607**	0.088
Glucose	Top	0.476**	0.516**	0.186	-0.194
	Bottom	0.634**	0.673**	0.336	-0.029
Fructose	Top	0.509*	0,628**	0,478*	-0.223
	Bottom	0.570**	0.584**	0.438*	0.242

SF = Soluble fraction CWFB = Cell Walk-hound fraction
SS = Sucrose synthase SPS = Sucrose phosphate synthase
* and ** denote significant at $p<0.05$ and $p<0.01$ respectively

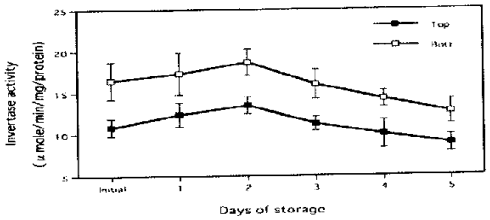

Fig. 1: Changes in soluble acid invertase activity in the top and bottom portions of asparagus spears held at 25°C. Each point represents the mean of three replications. Vertical bars indicate SE

Sucrose synthase activity: SS activity is shown in Fig. 3. Like cell wall bound acid invertase, sucrose synthase activity was also found higher in bottom portion than top. In both the top and bottom portions sucrose synthase activity started to decline from the first day of storage and continued up to five days having a rapid decline on third day. A highly significant negative correlation was also found between SS activity and sucrose content.

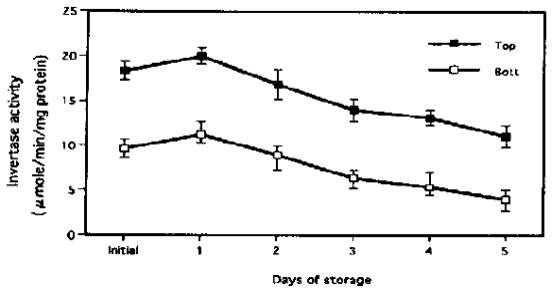

Fig. 2: Changes in cell wall bound acid invertase activity in the top and bottom portions of asparagus spears held at 25°C. Each point represent the mean of three replications. Vertical bars indicate SE

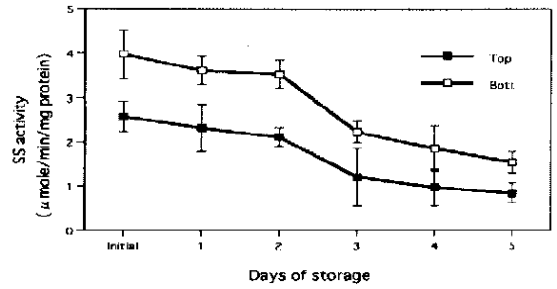

Fig. 3: Changes in sucrose synthase activity in the top and bottom portions of asparagus spears held at 25°C. Each point represent the mean of three replications. Vertical bars indicate SE

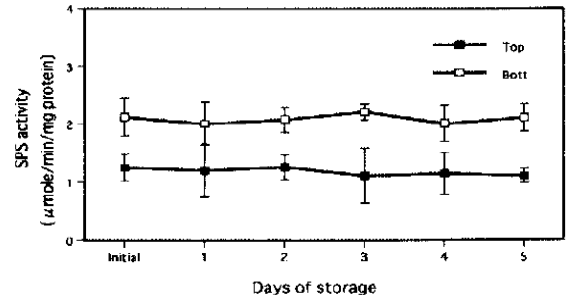

Fig. 4: Changes in sucrose phosphate synthase ctivity in the top and bottom portions of asparagus spears held at 25°C.Each point represent the mean of three replications. Vertical bars indicate SE

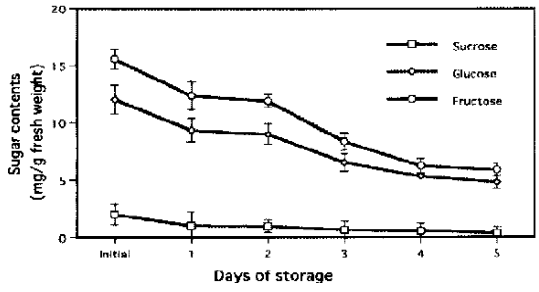

Fig. 5: Changes in soluble sugar contents in the top portions of asparagus spears held at 25°C. Each point represent the mean of three replications. Vertical bars indicate SE

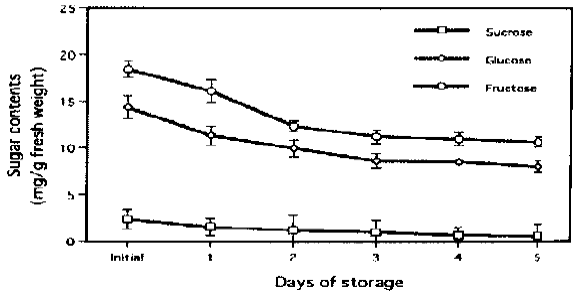

Fig. 6: Changes in soluble sugar contents in the bottom portion of asparagus spears held at 25°C. Each point represents the mean of three replications. Vertical bars indicate SE

Sucrose phosphate synthase activity: Figure 4 shows the SPS activity. There was no specific inclining or declining pattern of SPS activity in any portions of the spears, but in case of top portion it showed a wavy pattern with a slight increase on third day. SPS showed higher activity in the bottom portion than in the top portion. There was no significant correlation between soluble sugar content and SPS activity.

Soluble sugars: Among three sugars, the level of fructose always remained higher than that of glucose and sucrose in the top as well as bottom portions of the spears. Actually fructose and glucose were the predominant sugars, sucrose was present in trace amount. All the three sugars started to decrease from the first day and continued up to five days. Sucrose content was negatively correlated with invertase and sucrose synthase in both the top and bottom portions (Fig. 5, 6).

Correlation coefficients (r) between activity and sugar content: Table 1 shows the correlation coefficients (r) between the enzyme activity and sugar content. In both the soluble and cell wall bound fraction, a significant negative correlation between the acid invertase activity and sucrose content was detected. Sucrose synthase showed the same correlation. But There was no significant correlation between soluble sugar content and SPS activity.

Discussion

Fructose and glucose were the major soluble carbohydrates in asparagus spears during storage. Sucrose was found in trace amount. All the three sugars started to decrease from the first day and continued up to five days. This result differs from earlier work (Hurst *et al.*, 1993) where sucrose was the most abundant sugar. Given that the sucrose level drops very rapidly after harvest, it is likely that the discrepancies are due to processing delay and little bit high storage temperature (25°C) which one we selected to simulate retail display in the super market during spring and summer season. Soluble acid invertase activity was highest in the top portion because of the presence of rapidly growing tissues in that region. Acid invertases are commonly found in rapidly growing and expanding zonees of fruit (Hubbard and Pharr, 1992), stems (Sehtiya *et al.,* 1991), leaves (Nielsen, 1992) and roots (Getz, 1991), where they are said to provide hexoses for cell wall synthesis and to play a major role in maintaining the source sink gradient in sucrose concentration by rapidly hydrolysing incoming sucrose. The differences in enzyme activity between top and bottom portions were because of the diversity of

tissue maturity in asparagus spears. The top contains rapidly dividing meristematic cells whereas the bottom comprises more mature lignifying tissue (Culpepper and Moon, 1939; Lipton, 1990). Initially the acid invertase activity increased in storage and thereafter it gradually declined. The activities of sucrose synthase and sucrose phosphate synthase were generally low compared with acid invertase. But overall enzyme activities declined up to five days of storage.

Our enzyme activity data support the view that the sucrose synthase pathway is important in asparagus spears during storage and the differences in sugar composition are associated with differences in enzyme activities.

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