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Changes in Acid Invertase and Fructanase Activities and Sugar Distribution in Asparagus Spears Harvested in Three Different Seasons

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Abstract: Changes in acid invertase (EC3.2.1.26), fructanase (EC3.2.1.7) activities and sugar distribution were investigated in the different portions of asparagus (*Asparagus officinalis.* L. cv. E414, HC, WC and S-235, grown in greenhouse) spears harvested in "Spring harvest (2nd and 3rd of March)", "Middle harvest (27th and 29th of May)" and "Summer harvest (27th of August)". The weight of "Middle harvest" asparagus was apt to be higher than "Spring harvest" and "Summer harvest". In soluble and cell wall-bound fractions, the acid invertase and fructanase activities of the two spears increased in the "Middle harvest" concurrent with the most rapid increased in reducing sugar content but with a lower sucrose and fructane contents. In the top portion of 'HC', substrate of sucrose was negatively correlated with acid invertase activity in soluble fraction and accounted well for the relation between the substrate and the activity. Due to the existence of fructane in the spear, fructose content was considered to be higher than glucose content. The best harvest time was in the "Middle harvest" since the total sugar content and fresh weight were adequate.

Key words: Asparagus, fructanase, invertase, "Spring harvest", "Middle harvest"

Introduction

Carbohydrates are the major biochemical components of edible asparagus spears and fructans composed of fructose residue are deposited in asparagus root. The quality is mainly determined by the amount of carbohydrates in the spear. In the growing tender shoot, sucrose is translocated to the apical portion from tuberous root (Hurst and Clark, 1993; Hurst *et al*., 1993) and reducing sugars are transferred from the roots to spears during the growing period. Invertase simply splits sucrose into glucose and fructose (Copeland, 1990) to provide substrates for growth (King *et al*., 1997), whereas fructanase breaks down fructan into glucose and fructose or fructose and sucrose (Barman, 1969) in prepara-tion for sprouting (Kim and Sakiyama, 1989). Appeldoorn *et al.* (1997) indicated that significant changes in the pathway of sucrose degradation can be associated with the development of tender shoot. Kim and Sakiyama (1989) reported that fructan started to increase from August and showed the highest content before dormancy. Therefore, the analysis of carbohydrate metabolic enzymes is important. However, there has been no study conducted regarding the changes in invertase and fructanase activities with sugar and fructane accumulation in asparagus spears during its development at three different seasons where atmospheric or soil temperature is changed. Asparagus spear is produced in the spring and production is largely dependent upon carbohydrate reserves accumulated during the previous season. Asparagus plants need low temperature (or domant period) to produce and to store carbohydrates for initiation of bud, spear and fern growth the following season (Shelton and Lacy, 1980). On the other hand, high temperature re-duces spear yield (Yen *et al.,* 1996). In Shikoku Island, few asparagus cultivars are grown. From those, four cultivars (E414 HC, WC and S-235) have been selected for this study. 'E414' is an early, higher-yielding cultivar adapted to somewhat warmer conditions, while 'WC' is adapted to moderate cooler conditions and mainly grown in Japan. 'HC' and 'S-235' are just a new cultivars which are in the experimental phase. Therefore, we determined the changes of the invertase and fructanase activities and sugar and fructane accumulations in the different portions of the spear to identify suitable cutivars and different harvesting period for asparagus production.

Materials and Methods

Plant materials: Four green asparagus cultivars, E 414, HC, WC and S-235 were grown in the greenhouse at the Agricultural Research Station, Miki-Cho branch Kagawa, Japan.Air temperature of "Spring harvest (2nd-3rd of March)", "Middle harvest (27th-29th May)" and "Summer harvest (27th of August 1998)" was maintained at an average day time temperature of 13, 22 and 26°C and night time of 7, 17 and 20°C, respectively. In the fourth year, asparagus spears (ca. 25 cm length) were harvested randomly.
After weighing, spears were immediately stored at -30°C until required.

Enzyme extraction: 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% polyvinylpolypyrrolidone (PVPP) and 1 g sea sand.

Invertase and fructanase: The sample was then homogenized using a cooled mortar and pestle with 10 ml of 0.2 M citrate-phosphate buffer (C-P buffer) at pH 5.0. The resulting homogenate was then filtered through four layers of cotton cloth and the filtrate was centrifuge at 11,000 × g for 10 min. The total supernatant was dialyzed with 0.2 M C-P buffer (pH 5.0), diluted 40 times for 12 hr and the inner solution was designated as soluble fraction. The residual tissue was re-extracted in 10 ml of 0.2 M NaCl C-P buffer for 24 hr with occasional stirring. The supernatant was described above and the dialyzed solution was designated as cell wall bound fraction.

Enzyme assay: The standard assay medium for acid invertase consisted of 0.2 ml of 0.2 M C-P buffer (pH 5.0), 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 a distilled water instead of sucrose. The assay mix-ture was incubated at 45°C for 15 min. After the reaction mixture was neutralized with 0.1 N NaOH or 0.1 N HCl, a coloring Somogyi's copper reagent (Smogyi,1952) was added and the mixture heated for 10 min in boiling water. The amount of reducing sugars was estimated by the method of Smogyi (1952). Soluble protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. The enzyme activity was expressed as the amount of glucose produced per min per mg of protein. In the case of fructanase, 0.1 ml of 1% fructane was substituted for 0.1 ml of 0.5 M sucrose and the other conditions were the same as described above. The enzyme activity was expressed as the amount of fructose per mg of protein.

Determination of sucrose, glucose and fructose by high performance liquid chromato- graphy (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 wascentrifuged at 11,000 × g for 10 min at 2°C. The mixture was filtered through cellulose nitrate membrane filter (0.45 μm pore size). Soluble sugar were analyzed by HPLC using stainless steel column (10.7 mm ID × 30 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 60?. An RI monitor (Hitachi L-3300) was used. Sucrose, fructose and glucose were identified by their retention times and were quantified according to standards.

Determination of fructane: Three g of sliced sample was homogenized in a Potter's homogenizer with 10 ml of water. The homogenate was centrifuged at 3,000 rpm for 10 min and the supernatant was discarded. The residue was rinsed and centrifuged with 5 ml of water twice to remove soluble sugar. The residue was added with 100 ml of 0.75 N HCl and the mixture was boiled in a water bath for 1.5 hr under a reflux condenser.

The solution was neutralized and made up to 250 ml with water and filtered. The reducing sugar was determined by the Somogyi method and the amount of fructane was given as the fructose value multiplied by 0.3 (Kamada, 1982).

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

Results

Sample weight: The average weight of asparagus spears is presented in Fig. 1. The weight of"Middle harvest"asparagus was higher than "Spring harvest" and "Summer harvest", but there was no significant difference at among the three harvests. The highest weight was obtained by 'HC' while 'S235' got the lowest throughout the experimental period.

Acid invertase activity in soluble fraction: Acid invertase activity in the soluble fraction is shown in Fig. 2A and B. In the four cultivars, the acid invertase showed a higher activity in the top portion than in the bottom portion. In both portions, the invertase activity was highest at " Middle harvest" and the lowest was during the "Summer harvest". The invertase activity of 'E 414' was highest in both portions throughout the experimental period , whereas 'HC' obtained the lowest in both portions.

Acid invertase activity in cell wall-bound fraction: Figure 2C and D shows the acid Invertase activity in the cell wall-bound fraction. Invertase showed a significantly higher activity in the bottom portion than in the top portion of the four cultivars. In both portions, the invertase activity was the highest at the "Middle harvest" and the lowest was observed during the "Summer harvest". The invertase activity of 'HC' was highest in both portions throughout the experimental period, whereas 'S235' was lowest in the top portion. On the other hand, 'WC' was lowest in the bottom portion.

Fructanase activity in soluble fraction: *Fructanase* activity in the soluble fraction is shown in Fig. 3A and B. In the four cultivars, the fructanase showed a higher activity in the bottom portion than in the top portion. In both portions, the fructanase activity was highest at "Middle harvest" asparagus and "Summer harvest" was lowest. in the top portion, S235 and HC cultivars obtained the highest and lowest fructanase activity, respectively. On the other hand, 'HC' and 'WC' got the highest and lowest fructanase activity in the bottom portion.

Fructanase activity in cell wall-bound soluble fraction: Figure 3C and D shows the fructanase activity in the cell wall-bound fraction. Fructanase activity showed a signifi-cantly higher activity in the bottom portion than in the top portion for all cultivars. In both portions, the fructanase activity was highest at the "Middle harvest" and the lowest was observed during the "Summer harvest". The highest and lowest activities in the top portion were 'HC' and 'WC', respectively, whereas in the bottom portion 'HC' and 'WC', respectively.

Soluble sugar contents: Fig. 4A and D and Fig. 5A and B shows the glucose, fructose and sucrose content in the top and bottom portions respectively. In the four cultivars, glucose, fructose and sucrose showed a higher content in the bottom portion than in the top portion. The highest and lowest glucose content in the top portion were 'WC' and 'E414', respectively, whereas those in the bottom portion 'E414' and 'S235', respectively. The highest and lowest fructose content in both portions were 'WC' and 'E414', respectively. There was no significant difference between cultivars on the sucrose content. In both portions, glucose and fructose content was highest at the "Middle harvest" and the lowest was observed during the "Summer harvest". On the other hand, the sucrose content was highest at the "Summer harvest" and lowest at the "Middle harvest". The highest and lowest sucrose content in the top portion were 'E414' and 'HC', respectively, while in the bottom portion was 'WC' throughout the experimental period. Fructane content is shown in Fig. 5C and D. There was no signifi- cant difference between the top and the bottom portions in the four cultivars. In both portions, the fructane was highest at "Spring harvest" and lowest was during the "Middle harvest". The highest and lowest fructane content in the top portion were 'E414' and 'WC', respectively, wheile in the bottom portion was 'S235' throughout the experimental period.

Table 1: Coefficients (r) between carbohydrate and related enzyme activities in four different asparagus cultivars during development

Characters	Cultivars	AI (SF)	AI (CWBF)	F(SF)	F(CWBF)
Glc	E414-T	0.912**	0.509		
	E414-B	0.993**	0.993**		
Fru	E414-T	0.942**	0.793**		
	E414-B	0.962**	0.832**		
Fructane	E414-B			0.223	-0.744*
Fru	HC-T	0.368	0.805**		
Suc	HC-T	-0.720*	0.134		
Fructane	HC-T			0.340	-0.876**
Glc	S235-T	0.893**	0.893**		
	S235-B	0.719*	0.719*		
Fru	S235-T	0.744*	0.982**		
	S235-B	0.831**	0.854**		
Fructane	S235-B			0.315	-0.772*
Glc	WC-T	0.912**	0.912**		
Flu	WC-T	0.826**	0.858**		
	WC-B	0.831**	0.598		
Fructane	WC-T			0.145	-0.834**

E414, S233, HC and WC = Asparagus cultivars,
T = top portion, B = bottom portion, AI = acid invertase, F = fructanase, SF = soluble fraction, CWBF = cell wall-bound fraction; *,** = denote significant at $p<0.05$ and $p>0.01$, respectively.
Every omitted combinations for example Gic. HC-T or HC-B were not significantly different between SF and CWBF

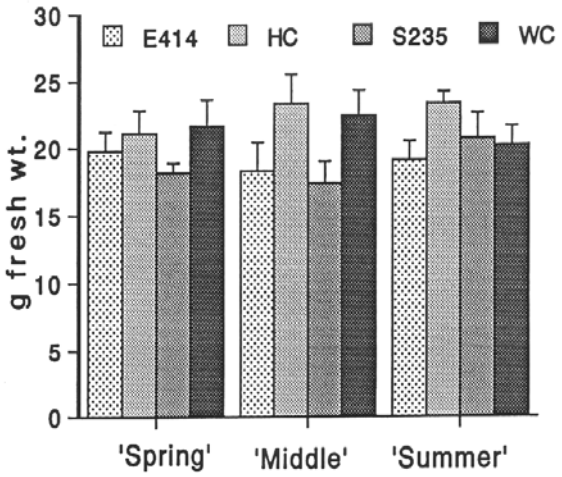

Fig. 1: Changes in fresh weight of different asparagus cultivars during the three harvest season. Data are means of three replicate composites of three spears each. Bars represent the standard error.

Correlation coefficients (r) between enzyme activities and sugar content: Table 1 shows the correlation coefficients (r) between enzyme activities and sugar content. In the soluble fraction, a significant negative correlation between the acid invertase activity and sucrose content was observed in the top portion of 'HC' cultivar but there was no correlation found between the acid invertase and the cell wall-bound fraction. There was a significant positive correlation between acid invertase activities and glucose or fructose content in both portions of 'E-414' and 'S-235' of the soluble fraction. On the other hand, in the soluble fraction a significant positive correlation between acid invertase activi-ties and glucose or fructose in both portions of 'E-414'. In the cell wall-bound fraction, there was a significant positive correlation between

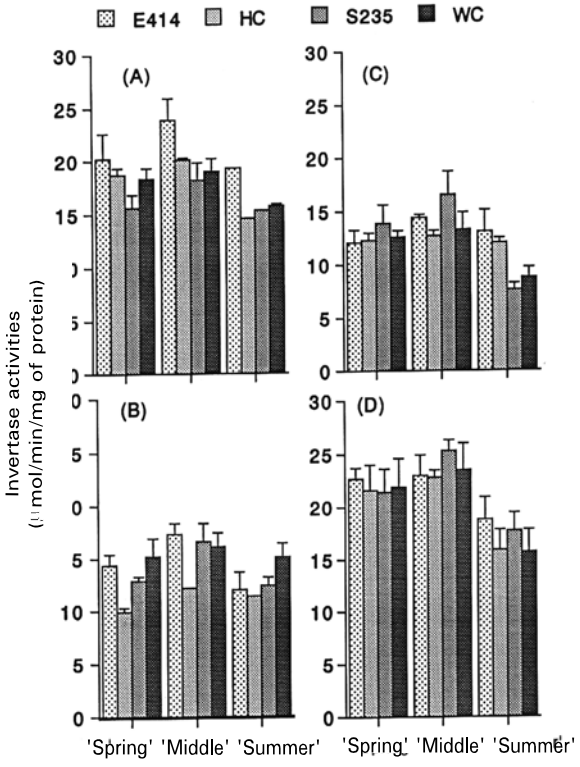

Fig. 2: Changes in soluble acid invertase activities in top portion (A) and bottom portion (B) and in cell wall-bound activities in top portion © and bottom portion (D) of different asparagus cultivars during three harvest season. Bars represent thestandard error

fructanase and fructane in the top portion of 'E-414' and 'WC'.

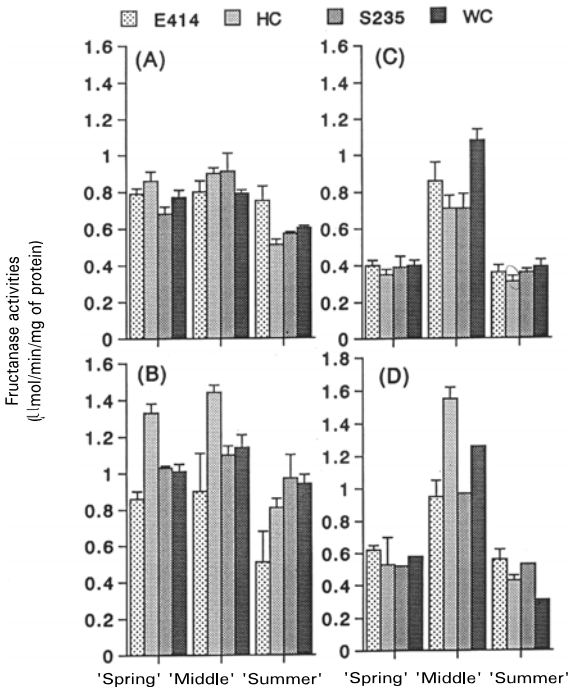

Fig. 3: Changes in soluble fructanase activities in top portion (A) and bottom portion (B) and in cell wall-bound fructanase activities in top portion (C) and bottom portion (D) of different asparagus cultivars during three harvest season. Bars represent the standard error

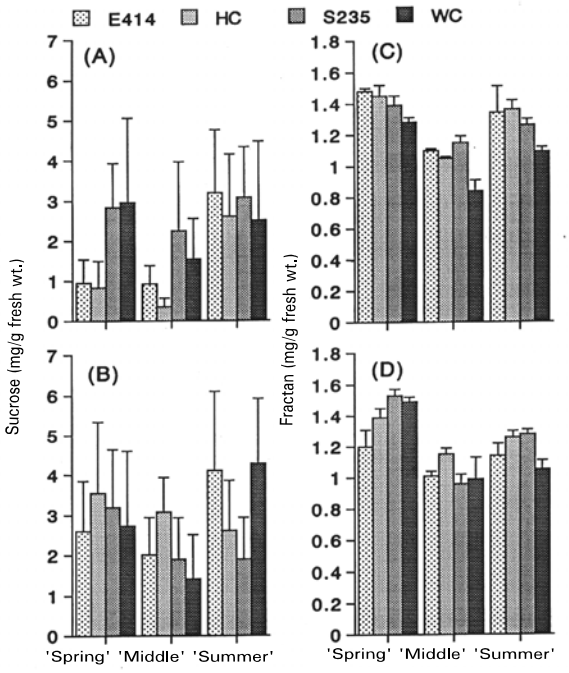

Fig. 5: Changes in sucrose content in top portion (A) and bottom portion (B) and in fructans content in top portion (C) and bottom portion (D) of different asparagus cultivars during three harvest season. Bars represent the standard error

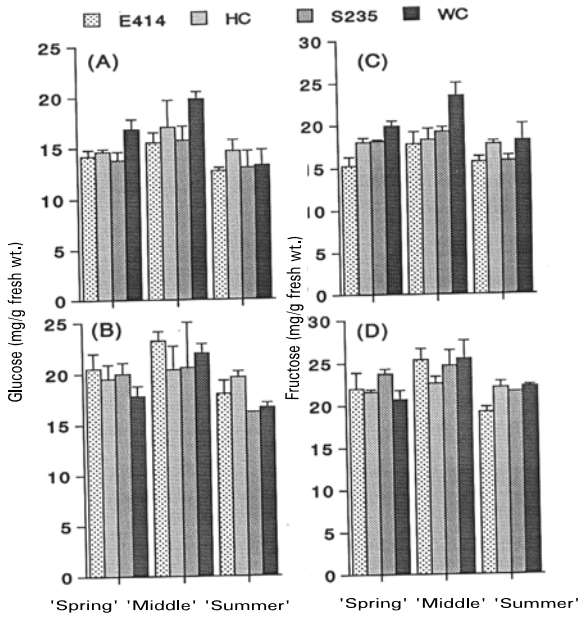

Fig. 4: Changes in glucose content in top portion (A) and bottom portion (B) and in fructose content in top portion (C) and bottom portion (D) of different asparagus cultivars during three harvest season. Bars represent the standard error

Discussion

The spear weight of"Middle harvest" asparagus was highest and "Spring harvest" was higher than that of "Summer harvest". This finding coincided with Alam (1999) report. The study demonstrated that acid invertase activity in the soluble fraction was highest in the top portion of the"Middle harvest" asparagus spear. Hurst *et al.* (1993), reported that the highest soluble acid invertase activity was found in the rapidly expanding region of the spear. Acid invertase are commonly found in rapidly growing or expanding zones of fruit (Hurst *et al.,* 1993) stem (Sehtiya *et al.,* 1991), leaves (Nielsen,1992) and roots (Getz, 1991). In the soluble fraction, there was a significant negative correlation between the acid invertase activity and sucrose content the top portion of 'HC' (Table 1), suggest-ing that the decrease in the acid invertase activity of the soluble fraction (Fig. 2) was associated with the increase in the sucrose content (Fig. 5). Hurst *et al.* (1993) reported that sucrose is the major sugar translocated from the roots to the growing spear. Invertase shows a high affinity for sucrose (Avigad, 1982) and simply splits it into glucose and fructose. In both fractions, there was a significant positive correlation between acid invertase and glucose/fructose in the top or bottom portion in most of the cultivars tested except for the cultivar 'HC'. In this study it was observed that the acid invertase activity of the soluble fraction was higher in the top portion than in the bottom portion, whereas the cell wall-bound fraction was lower in the top portion than in the bottom portion. On the other hand, in both fractions fructanase activity was highest in the bottom portion of the spear and was higher in the cell wall-bound than in the soluble fraction. Shiomi (1981) reported that the fructan in asparagus roots is the major storage sugar translocated from root to the growing

spear during the spear formation period in May and slightly decreased during stem elongation period in July and then reached the lowest in August. The fructan content was lowest in both portions of the spear at "Middle harvest". The trend of the fructan content in the spear coincided with that in the root root but it increases during "Summer harvest". In the cell wall-bound fraction, there was a significant negative correlation between the fructanase activity and fructane content in the top portion of 'E414', 'S-235' and 'WC' (Table 1), suggesting that the decrease in fructanase activity was associated with the increase in fructane content.

Although fructane is present in the root as the storage sugar, it contained 3.47-4.67 mg/1 g fresh weight (Fig. 5) as fructose in the top portion and 3.97-4.70 mg in the bottom portion of the spear. The contribution of the fractane to fructose was ca. 22% in the top portion and ca. 19% in the bottom portion, respectively (Fig. 4). Most of fructose is considered to produce by invertrase. When comparing fructose produced by fructanase with that produced by invertase, the rate of fructanase contribution in soluble fraction was estimated to be about 4% of total production in the top portion and about 7% of that in the bottom portion, whereas that in cell wall-bound fraction was about 3.8% of the total production in the top portion and about 3.6% in the bottom portion, respectively.

The equal amount of glucose and fructose are not observed in polysaccharide unaccumulat-ed fruits such as tomato and persimmon fruits. The fructose content was significantly higher than glucose and sucrose in green asparagus spear (Alam *et al*., 1998), indicating that the larger amount of fructose was due to the contents of fructane and the action of fructanase in green asparagus spears. The study indicates that in Shikoku Island where asparagus is grown in greenhoses, spear harvesting is preferable on "Middle harvest", because at this time the spear fresh weight was apt to remain higher and carbohydrate accumulation was adequate. Based on the result obtained, it is concluded that although the acid invertase was very important to produce the reducing sugars of asparagus spear, the fructanase was available to produce fructose in the spear.

References

Alam, A.K.M.S., 1999. Studies on physiological and biochemical changes during development of green asparagus as influenced by environmental factors. Ph.D. Thesis, Ehime United Graduate School of Agricultural Science, Ehime University, Japan.

Alam, A.K.M.S., T. Matsui and T. Ikeuchi, 1998. Changes in acid invertase activity and sugar distribution in asparagus spears harvested in autumn. Jpn. J. Trop. Agric., 42: 257-262.

Appeldoorn, N.J., S.M. de Bruijn, E.A. Koot-Gronsveld, R.G. Visser, D. Vreugdenhil and L.H. van der Plas, 1997. Developmental changes of enzymes involved in conversion of sucrose to hexose-phosphate during early tuberisation of potato. Planta, 202: 220-226.

Avigad, G., 1982. Sucrose and Other Disaccharides. In: Encyclopedia of Plant PhysiologyEncyclopedia of Plant Physiology Plant Carbohydrate I, Loewus, F.A. and W. Taner (Eds.). Vol. 13, Springer, Berlin, pp: 217-347.

Barman, T.E., 1969. Enzyme Handbook. Springer-Verlag, New York, Pages: 568.

Copeland, L., 1990. Enzymes of Sucrose Metabolism. In: Methods in Plant Biochemistry Enzymes of Primary Metabolism, Lea, P.J. (Ed.). Academic Press, London, pp: 73-86.

Getz, H.P., 1991. Activity of cell wall bound acid invertase of mature red beet root tissue. Plant Physiol. Biochem., 29: 585-593.

Hurst, P.L. and C.J. Clark, 1993. Postharvest changes in ammonium, amino acids and enzymes of amino acid metabolism in asparagus spear tips. J. Sci. Food Agric., 63: 465-471.

Hurst, P.L., L.M. Hyndman and P.J. Hannan, 1993. Sucrose synthase, invertase and sugars in growing asparagus spears. N. Z. J. Crop Hortic. Sci., 21: 331-336.

Kamada, Z., 1982. Carbohydrate Analysis. In: The Handbook of Food Analysis, Obara, T., T. Suzuki and H. Iwao (Eds.). Kenppakusha, Tokyo, pp: 223-226, (In Japanese).

Kim, Y.S. and R. Sakiyama, 1989. Changes in carbohydrates of asparagus storage roots on sprouting. J. Japanese Soc. Hortic. Sci., 58: 383-390.

King, S.P., J.E. Lunn and R.T. Furbank, 1997. Carbohydrate content and enzyme metabolism in developing canola siliques. Plant Physiol., 114: 153-160.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Nielsen, T.H., 1992. Differences in fructose-2,6-bisphosphate metabolism between sections of developing barley leaves. Physiol. Plant., 84: 577-583.

Sehtiya, H.L., J.P.S. Dendsay and A.K. Dhawan, 1991. Internodal invertases and stalk maturity in sugarcane. J. Agric. Sci., 116: 239-243.

Shelton, D.R. and M.L. Lacy, 1980. Effect of harvest duration on yield and on depletion of storage carbohydrates in asparagus roots. J. Am. Soc. Hortic. Sci., 105: 332-335.

Shiomi, N., 1981. Studies on fructosyltransferase of root asparagus. Memoirs Fac. Agric. Hokkaido Univ., 13: 242-315, (In Japanese).

Smogyi, M., 1952. Notes on sugar determination. J. Biol. Chem., 195: 19-23.

Yen, Y., M.A. Nichols and D.J. Woolley, 1996. Growth of asparagus spears and ferns at high temperatures. Acta Hortic., 415: 163-174.