

Variation of Fructooligosaccharide Contents, Invertase, 1-SST, 1-FFT and 6G-FFT Activities in Green Asparagus Spears Stored under Different Temperature Regimes

Noureddine Benkeblia^{1*} • Norio Shiomi²

¹ Department of Life Sciences, The University of The West Indies, Mona Campus, Kingston 7, Jamaica

² Department of Food and Nutrition Sciences, Graduate School of Dairy Science Research, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

Corresponding author: * noureddine.benkeblia@uwimona.edu.jm

ABSTRACT

Fresh spears of asparagus were stored in the dark at 4, 10 or 20°C for 14 days. During storage contents of glucose, fructose, sucrose, 1-kestose, neokestose and nystose, and activities of invertase, 1-KHE, 1-SST, 1-FFT and 6G-FFT were determined. Invertase activity decreased after two days at 4 and 10°C, while it increased after two days at 20°C and then also decreased progressively. 1-KHE activity also varied slightly at 4 and 10°C, while at 20°C, 1-KHE increased after two days, before decreasing after four days and remaining quite stable during the last 10 days of storage. 1-SST increased during the first four days at the three temperatures. During the last 10 days, 1-SST activity decreased progressively at 4°C but remained stable at 10 and 20°C. 1-FFT activity varied slightly but was high at 4°C and low at 20°C. The activity of 6G-FFT was similar to 1-FFT although its level was higher. Glucose was stable during the first week and decreased during the second week at 10 and 20°C while it increased at 4°C. Fructose decrease progressively at 10 and 20°C but increased considerably at 4°C. Sucrose content decreased progressively at 10 and 20°C but increased significantly at 4°C during the second week of storage. 1-kestose and neokestose showed a slight increase during the first days but decreased progressively during the ten last days of storage. Nystose content decreased progressively and the decrease was almost linear during storage although the final content at 20°C was lower. It was concluded that sugars contents of asparagus spears declined rapidly during the first four days of storage. This decline explains well the short shelf-life of the spears and the rapid loss of their quality attributes after few days.

Keywords: enzyme activity, oligosaccharide, saccharide, spear, storage, temperature

Abbreviations: 1-SST, sucrose:sucrose 1-fructosyltransferase; 1-FFT, fructan:fructan 1-fructosyltransferase; 6G-FFT, fructan:fructan 6^G-fructosyltransferase; 1-KHE, 1-kestose hydrolyzing enzyme; sFOS, short fructooligosaccharide

INTRODUCTION

Unlike most vegetable crops, spear of asparagus, a hardy perennial plant, is commonly consumed and appreciated because it is low in calories and provides substantial amounts of reducing sugars and very low amount of fructooligosaccharides (FOS), and these fructosyl polymers are well known for health benefits (Lipton 1990). The chemical characteristics of the spears are markedly affected by temperature (Alam *et al.* 1998), growth conditions (Lill *et al.* 1990), and harvest date (Hurst *et al.* 1993). Carbohydrates are major constituents of asparagus spears and contribute considerably to their quality (Lipton 1990). The main carbohydrates in asparagus spears are glucose, fructose and sucrose (Hurst *et al.* 1993; Alam *et al.* 1998) while the presence of FOS has never been reported. In asparagus carbohydrates are translocated from storage roots to spears, where they are hydrolyzed into hexoses and used during the catabolic activities of the spears (Hurst *et al.* 1993). Thus, soluble carbohydrate levels decline particularly during the first hours after harvest, and this decline triggers deterioration in the spears (Irving and Hurst 1993; King *et al.* 1993). While little FOS are detected in aerial parts of asparagus, it also remains still unclear the reason for the difference of FOS content between the top and the bottom parts of the spears. On the other hand, the role of FOS in spears tissues is also unknown. It is not clear if their presence results from simple translocation from roots to spears to provide energetic substrates, or whether they are translocated to play specific roles such as osmoregulation, or to partially com-

pensate for the rapid decline of sucrose in tips.

Numerous investigations have been carried out on structures (Shiomi *et al.* 1976, 1978; Shiomi 1981; Shiomi *et al.* 1991; Shiomi 1993), enzymology (Shiomi 1989) and molecular biology (Ueno *et al.* 2005) of FOS in roots of asparagus and such research works were recently reviewed by Shiomi *et al.* (2007a), but there have been no reports on their presence in aerial tissues. Although many studies have focused on the carbohydrate content of edible spears after harvesting, few studies have focused on soluble and reducing sugars, including soluble invertase, while no investigations have been carried out on FOS and their metabolizing enzymes in asparagus spears, except for the work of Shiomi *et al.* (2007b), who reported the variation of saccharides and FOS in spears portions under different temperature regimes.

From a technological point of view, freshly harvested spears deteriorate rapidly at ambient temperatures (Lipton 1990), and have a short shelf-life, which is strongly related to respiratory activity (Brash *et al.* 1995). This postharvest deterioration is accompanied by biochemical changes, particularly in spear tips where soluble carbohydrates, such as sucrose, decline rapidly (King *et al.* 1988).

The purpose of this investigation was to assess qualitatively and quantitatively the variation of the short chain FOS and the hydrolyzing and synthesizing carbohydrate enzymes of green asparagus spears during storage under different temperature regimes.

MATERIALS AND METHODS

Plant material

Fresh spears of asparagus (cv. 'HLA-7') were obtained from an experimental field of Yotei farmers cooperation, Hokkaido, Japan. The spears of 20-22 cm in length were manually harvested, sorted for absence of defects, slightly rinsed with tap water and left for 2 h at room temperature to drain residual water.

Storage conditions

Immediately after water drain and three hours after harvest, asparagus spears were stored in the dark at three different temperature regimes. For the low temperature regimes, spears were placed in a refrigerated chamber (Eyelatron, model FLI 301 NH, Rikakikai Co. Ltd, Tokyo) at $4 \pm 0.1^\circ\text{C}$ and $85 \pm 1\%$ relative humidity (RH), and $10 \pm 0.1^\circ\text{C}$ and $85 \pm 1\%$ RH. For the high temperature regime, 20°C , spears were placed in a ventilated room with an average RH of 60%. The relative humidities are set according to the storage practices recommended for vegetables.

Saccharides and fructooligosaccharides extraction

Saccharides and fructooligosaccharides (FOS) were extracted by the method of Shiomi (1992). Asparagus spear was chopped and 10 g tissues were weighted and homogenized in 80 mL of aqueous ethanol (70%) using a small amount of calcium carbonate (0.5 g L^{-1}). The homogenate was boiled under reflux in a water bath for 10 min. Homogenate was filtered and the residue was extracted three times with aqueous ethanol and one time with water in the same conditions. The filtrates were combined and made up to 500 mL with distilled water. An aliquot of the filtrate (10 mL) was concentrated to dryness under vacuum at 35°C using a Büchi rotavapor (Büchi labortechnik AG, Flawil Switzerland). This dry concentrate was redissolved in one mL of water, filtered through a $0.45 \mu\text{m}$ filter and analyzed by high performance anion exchange chromatography (HPAEC Dionex, Sunnyvale, CA, USA). All processes were run in triplicate.

Saccharides and fructooligosaccharides analysis

Glucose, fructose, sucrose, and FOS (1-kestose, neokestose and nystose) were separated on an HPLC-carbohydrate column PA1, Carbo Pack (Sunnyvale, CA, USA) with a Dionex Bio LC series HPLC (Sunnyvale, CA, USA) and pulsed amperometric detector (PAD). The gradient was established by mixing eluent A (150 mM NaOH) with eluent B (500 mM acetate-Na in 150 mM NaOH) in two ways. System I: 0–1 min, 25 mM; 1–2 min, 25–50 mM; 2–20 min, 50–200 mM, 20–22 min, 500 mM; 22–30, 25 mM. System II: 0–1 min, 25 mM; 1–2 min, 25–50 mM; 2–14 min, 50–500 mM, 14–22 min, 500 mM; 22–30, 25 mM. The established gradient of mixing eluent A with eluent B was: 0–1 min, 95% A–5% B; 1–2 min, 80% A–20% B; 2–20 min, 60% A–40% B; 20–22 min, 100% B, 22–30 min, 95% A–5% B. The flow rate through the column was 1.0 mL min^{-1} . The applied PAD potentials for E1 (500 ms), E2 (100 ms) and E3 (50 ms) were 0.01, 0.60 and -0.60 V respectively, and the output range was $1 \mu\text{C}$. Samples ($25 \mu\text{L}$) were injected using an auto-sampler (Intelligent auto-sampler, model AS-4000, HITACHI Ltd, Tokyo, Japan). FOS are estimated by comparison with standards peaks and expressed in mg per gram fresh weight (mg g^{-1} fresh weight).

Glucose, fructose and sucrose standards were purchased from Nacal Tesque Inc. (Kyoto, Japan). 1-Kestose [**3a**, $1^{\text{F}}\text{-}\beta\text{-D-fructofuranosylsucrose}$, 1-kestotriose] and nystose [**4a**, $1^{\text{F}}(1\text{-}\beta\text{-D-fructofuranosyl})_2\text{ sucrose}$, 1,1-kestotetraose] were previously prepared in the laboratory as described by Takeda *et al.* (1994). Neokestose [**3b**, $6^{\text{G}}\text{-}\beta\text{-D-fructofuranosylsucrose}$, 6G-kestotriose] was obtained from asparagus roots as described previously by Shiomi *et al.* (1976).

Enzymes extraction

All operations of enzymes extraction were carried out on ice. The chopped asparagus tissues (50 g) were homogenized in 100 mL of

ice-cold phosphate buffer (0.01 M, pH 7.0) using a blender (model IFM-140, Iwatani Int. Corp., Tokyo, Japan). The homogenate was squeezed through three layers of cheesecloth, filled up to 30 mL with distilled water and centrifuged at $15,000 \times g$ at 0°C for 30 min. The supernatant was then collected and dialyzed for 48 h against the same phosphate buffer using cellulose tube. The dialysate was concentrated, to a final volume of 5 mL by centrifugation ($3000 \times g$) using an Amicon Centriprep YM 10 filter (Amicon Bioseparation, Millipore, Bedford, Mass., USA). This enzyme extract was used for enzyme assays.

1-SST and invertase activity essays

One unit of SST activity was defined as the amount of enzyme which catalyzed fructosyl transfer from sucrose to sucrose to produce $1 \mu\text{M}$ of 1-kestose (**3a**) in 1 min under the conditions described below. A mixture of enzyme extract (400 μL), 0.4 M sucrose solution (200 μL), McIlvaine buffer pH 5.5 (200 μL) and trace amount ($\sim 50 \mu\text{L}$) of toluene, was incubated at 30°C for 3 h. The reaction was stopped by heating in a boiling water bath for 3 min, and the mixture was analyzed by HPAEC-PAD as described above.

One unit of invertase activity was defined as the amount of enzyme which hydrolyzed $1 \mu\text{M}$ of sucrose in 1 min. The experimental conditions were those described for SST measurements. Invertase activity was calculated from the amount of fructose released and analyzed by HPAEC as described above.

1-FFT, 6G-FFT and 1-KHE activity essays

One unit of 1-FFT activity was defined as the amount of the enzyme which catalyzed the fructosyl transfer from 1-kestose to another 1-kestose to synthesis $1 \mu\text{M}$ of nystose (**4a**) in 1 min under the conditions described below. A mixture of enzyme (400 μL), 0.4 M 1-kestose solution (200 μL) and McIlvaine buffer (pH 5.5, 200 μL) was incubated at 30°C for 3 h. The reaction was stopped by heating in boiling water. The activity of 1-FFT was calculated from the amount of nystose formed.

One unit of 6G-FFT activity was defined as the amount of the enzyme which catalyzed the fructosyl transfer from 1-kestose to another 1-kestose to synthesis $1 \mu\text{M}$ of $1^{\text{F}}, 6^{\text{G}}\text{-di-}\beta\text{-D-fructofuranosyl sucrose}$, [1,6G-kestotetraose] in 1 min. The activity of 6G-FFT was tested using the mixture for 1-FFT assay, and calculated from the amount of 1,6G-kestotetraose formed.

One unit of 1-KHE activity was defined as the amount of enzyme which hydrolyzed $1 \mu\text{M}$ of 1-kestose in 1 min. The experimental conditions were those described for 1-FFT measurements. 1-Kestose hydrolyzing activity was calculated from the amount of fructose released and analyzed by HPAEC as described above.

Statistical analysis

The analyses were run in triplicate and experiment was conducted in duplicate. The data were analyzed statistically by comparison of means by one-way ANOVA test and determination of least significant difference (*LSD* at $P = 0.05$) using GraphPad InStat 3.06 (GraphPad Software Inc, San Diego, CA, USA).

RESULTS

Variation of hydrolyzing enzymes

During storage, variation of invertase activity was similar at 4 and 10°C although this activity was slightly higher at 10°C (Fig. 1A). This activity decreased, sharply from 1.27 to 0.82 and 0.42 U g^{-1} fresh weight after four day of storage, then progressively to 0.31 and 0.18 U g^{-1} fresh weight after 14 days, respectively. At 20°C , invertase activity increased to 1.53 U g^{-1} fresh weight after two days, and then decreased sharply to 0.56 U g^{-1} fresh weight after four days and progressively to 0.36 U g^{-1} fresh weight after 14 days. The increase observed during the first two days would be mainly due to the harvesting stress (cutting) causing an increase of the respiration rate. Thus, demand of substrate (glucose) becomes higher and this would trigger the in-

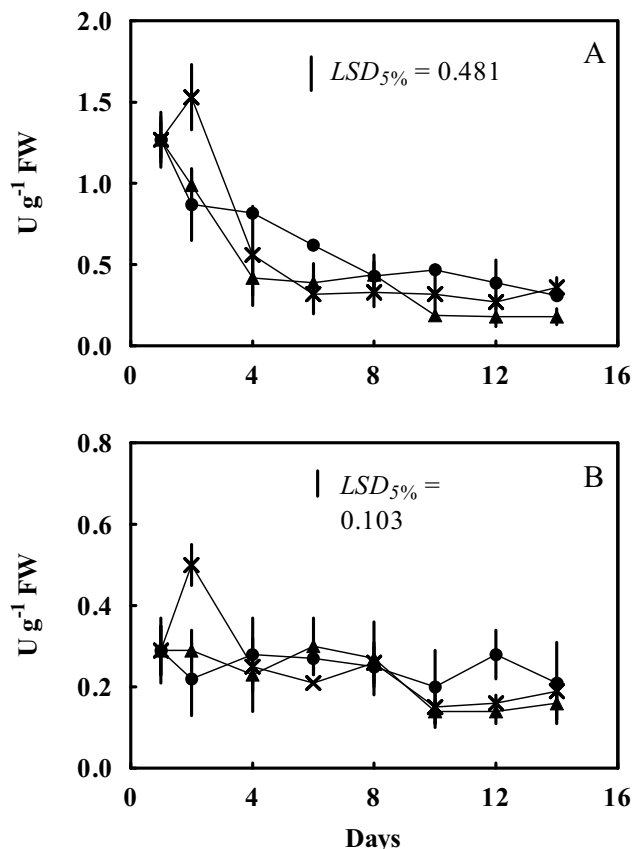


Fig. 1 Variation of invertase (A) and 1-kestose-hydrolyzing (1-KH) activities (B) in green asparagus spears stored at (●) 4, (▲) 10 and (×) 20°C. Vertical bars represent \pm SD of $n = 6$ and LSD was calculated at $P = 0.05$.

crease in invertase activity. On the other hand, the progressive decrease would be caused by the high activity of the elongation zone of the spears and the high demand for substrate which lead to a gradual exhausting of the substrate and senescence of the spears which appear first in the tip zone.

The activity of 1-KHE enzyme was very similar to that of invertase activity (**Fig. 1B**). At 4 and 10°C, activity varied slightly although the final values were low when compared to those observed at the beginning of storage and a slight increase was observed during the last two days of storage at 4°C. At 20°C, activity increased from 0.29 to 0.50 $U\ g^{-1}$ fresh weight after two days, before decreasing to 0.25 $U\ g^{-1}$ fresh weight after four days and remained quite stable during the ten last days of storage.

Variation of synthesizing enzymes

The activity of 1-SST, which is the first enzyme involved in FOS biosynthesis, increased from 0.03 to 0.04, 0.05 and 0.04 $U\ g^{-1}$ fresh weight during the first four days at 4, 10 and 20°C, respectively. During the last 10 days, 1-SST activity decreased progressively at 4°C while it remained quite stable at 10 and 20°C (**Fig. 2A**). Surprisingly, the pattern of 1-SST was close to that of 1-kestose hydrolyzing activity, and this suggests that a balance in released 1-kestose is maintained in the spears in order to avoid accumulation of the reaction products of 1-KHE i.e., sucrose and fructose. Thus, it seems that 1-kestose plays a close metabolic role to sucrose by maintaining a specific osmotic pressure along the spear. On the other hand, these patterns demonstrate well the high catabolic activities occurring during the first four days when the spear needs large amount of substrate resulting from the harvesting stress and the high respiration rate of the fresh spears.

The observed activity of the synthesizing enzyme 1-FFT varied slightly during the storage period. After 14 days the final activity was 50% less than these observed at the

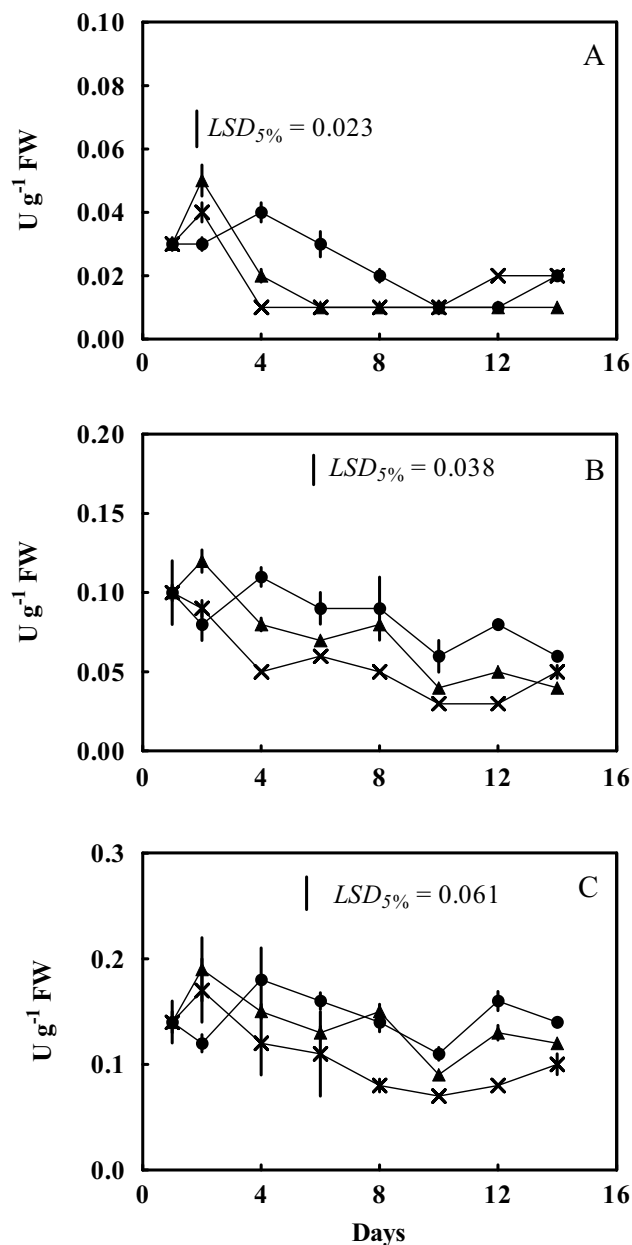


Fig. 2 Variation of 1-SST (A), 1-FFT (B) and 6G-FFT (C) activities in green asparagus spears stored at (●) 4, (▲) 10 and (×) 20°C. Vertical bars represent \pm SD of $n = 6$ and LSD was calculated at $P = 0.05$.

beginning of storage (**Fig. 2B**). However, it was observed that this activity was high at 4°C while the lowest activity was observed at 20°C. The low activity observed at 20°C is probably due to the high catabolism of 1-kestose and less availability of substrate mainly sucrose which is used as precursor of short chain FOS biosynthesis as shown by the activities of the 1-FFT and the 6G-FFT. However, at low temperature we also noted that catabolic activities (invertase and 1-KHE) are slightly lower than those observed at 20°C, and thus availability of substrate, mainly sucrose and fructose, would be higher allowing the biosynthesis of nystose. This would explain the regular activities of the synthesizing enzymes – 1-FFT and 6G-FFT – in comparison with degrading enzymes – invertase and 1-KHE (**Figs. 1, 2**).

The activity of 6G-FFT was similar to 1-FFT, however, its level was higher (**Fig. 2C**). The highest activity was observed at 4°C, while the lowest activity was observed at 20°C. Similarly, we noted a slight decrease if the activity, however, after 14 days storage, the activity decreased by one third only.

The activity ratio was estimated, and the values found were similar and seems temperature independent (**Fig. 3A**). At 4°C, the ratio increased progressively from 1.4 to 2.3,

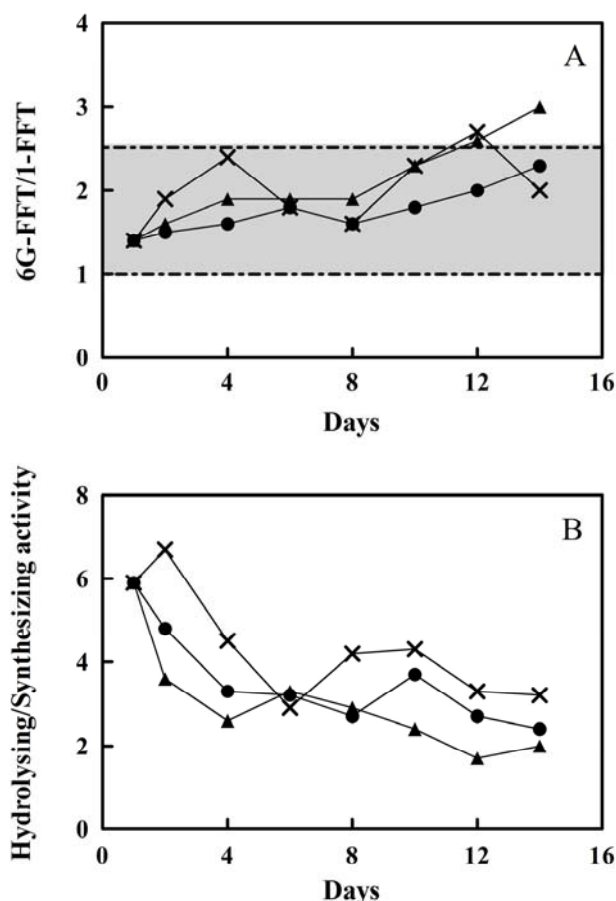


Fig. 3 Variation of the 6G-FFT to 1-FFT ratio (A) and the hydrolyzing [invertase + 1-KHE] to synthesizing [1-SST + 1-FFT + 6G-FFT] activities (B) in green asparagus spears stored at (●) 4, (▲) 10 and (×) 20°C.

while at 10°C this ratio increased from 1.4 to 3.0 after 14 days. At 20°C the 6G-FFT/1-FFT ratio increased from 1.4 to 2.7 after 10 days, and then decreased to 2.3 four days later. This ratio demonstrate well the high activity of the 6G-FFT compared to 1-FFT and this explain well why the content of 1-kestose is too high compared with the content of neokestose and nystose. It also demonstrates well the high demand of substrate at high temperature while at low temperature more substrate is accumulated. As previously reported (Shiomi 1989; Shiomi *et al.* 2007a, 2007b), 6G-FFT catalyses preferably the elongation chain to produce inulin-type FOS rather than inulin-neoseris types even though both are produced. Likewise, the hydrolysis activity uses the most available substrate – nystose – producing 1-kestose and fructose.

On the other hand, total hydrolyzing to synthesizing activities were estimated and showed by **Fig. 3B**. Total hydrolysis activities were five to six times higher during the first two days after harvesting indicating high carbohydrate catabolism. Afterwards, the ratio decreased regularly and reached 2.4, 2.0 and 3.2 after 14 days at 4, 10 and 20°C, respectively. The observed decrease is mainly due to the exhausting of substrate causing the rapid decline in the shelf-life of the spears.

Saccharides and FOS variation

Sugar analysis by HPAEC-PAD confirmed that glucose, fructose and sucrose constitute the major part of the total soluble sugars in green asparagus. Glucose, fructose and sucrose averaged 39.94, 47.75 and 9.61%, respectively, while low DP fructooligosaccharides (FOS), 1-kestose, neokestose and nystose averaged 2.02, 0.17 and 0.49%, respectively.

Glucose content showed little variation during the first eight days of storage at the three temperatures (**Fig. 4A**).

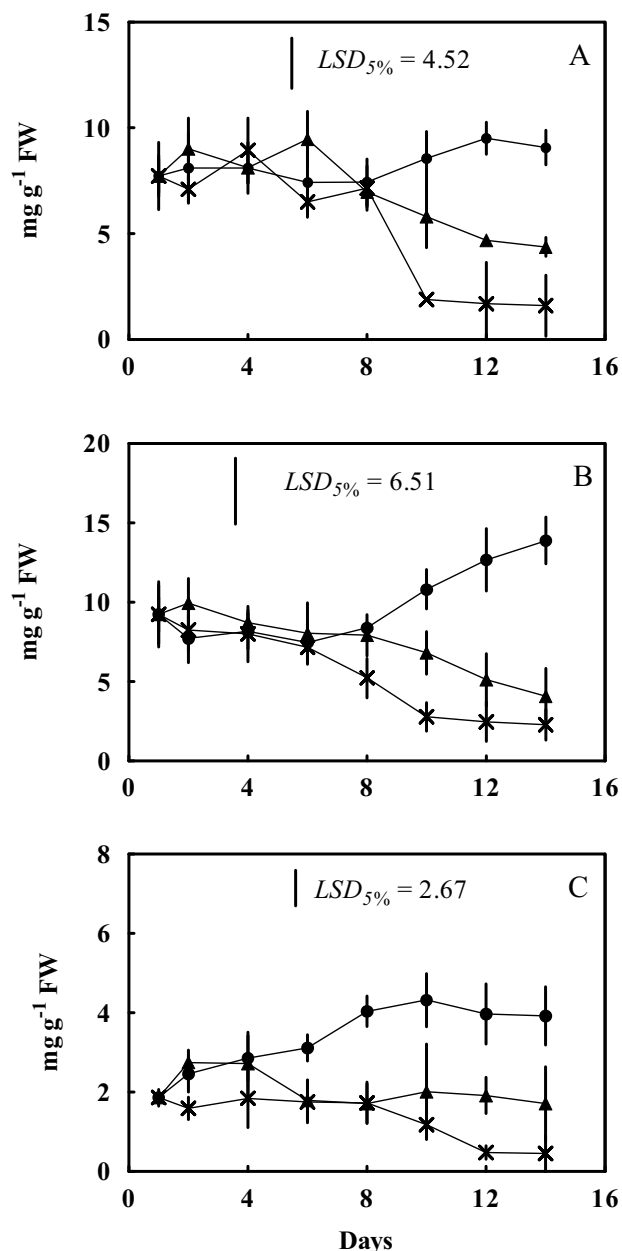


Fig. 4 Variation of glucose (A), fructose (B) and sucrose (C) contents in green asparagus spears stored at (●) 4, (▲) 10 and (×) 20°C. Vertical bars represent ± SD of n = 6 and LSD was calculated at $P = 0.05$.

During the first week, glucose content remained quite stable, but decreased abruptly from day eight to day 10 at 20°C from 7.16 to 1.60 mg g⁻¹ fresh weight and remained stable during the last four days of storage. This decrease is due to the high metabolic rate including respiration rate and the rapid and early exhausting of this substrate at this higher temperature. At 10°C, glucose content decreased progressively during the second week of storage from 6.97 to 4.36 mg g⁻¹ fresh weight, while it increased from 7.44 to 9.09 mg g⁻¹ fresh weight at 4°C during the same last period of storage.

Fructose content showed a similar pattern to glucose even though the decrease observed during the second week of storage at 10 and 20°C was progressive. On the other hand, at 4°C fructose content increased considerably from 7.44 to 9.06 mg g⁻¹ fresh weight and this increase is due to the low metabolic activity at low temperature and high accumulation of substrate because of the low demand (**Fig. 4B**).

Surprisingly, sucrose content exhibited a specific pattern with a steady state observed at 10°C and a slight decrease observed at 20°C during the last four days of storage. On the other hand, its content increased significantly from

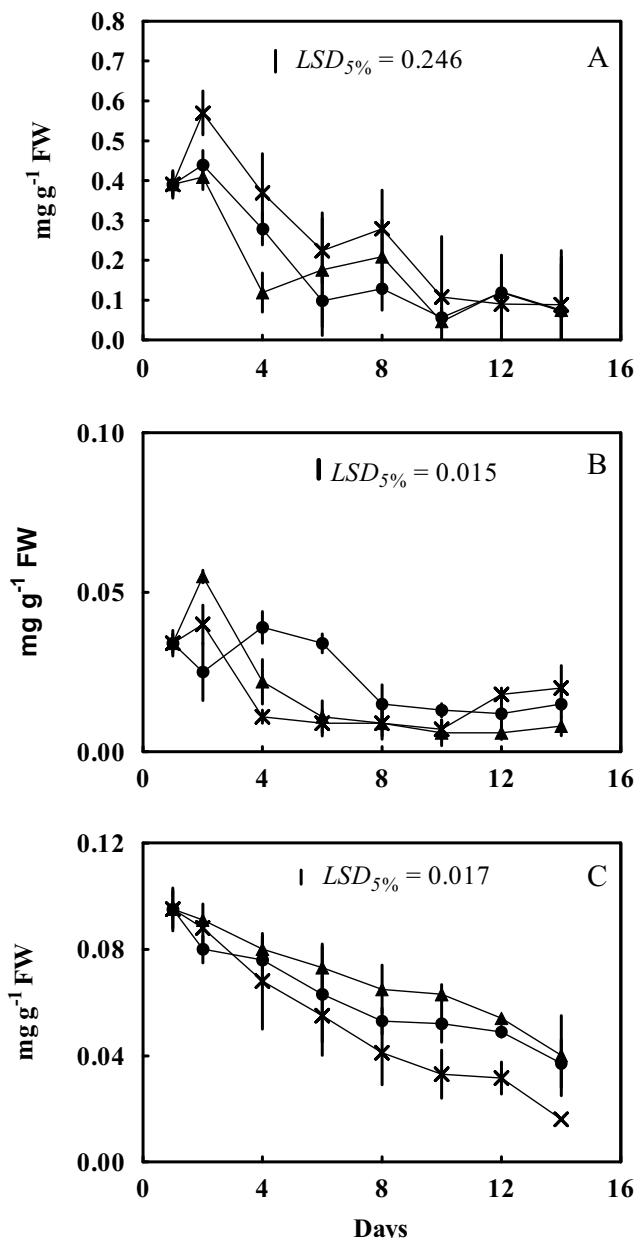


Fig. 5 Variation of 1-kestose (A), neokestose (B) and nystose (C) contents in green asparagus spears stored at (●) 4, (▲) 10 and (×) 20°C. Vertical bars represent \pm SD of $n = 6$ and LSD was calculated at $P = 0.05$.

2.86 to 4.32 mg g⁻¹ fresh weight at 4°C from day 6 to day 10 and then remained stable (**Fig. 4C**).

The general pattern of saccharide contents variation showed that an increase was observed during the last week at 4°C, while a decrease at 10 and 20°C, and this indicate that hydrolyzing and synthesizing activities occurs simultaneously but quantity of substrate utilized under this low temperature is lower causing a slight accumulation of fructose mainly.

The content of 1-kestose showed similar pattern and no significant difference was observed under the three temperatures of storage (**Fig. 5A**). 1-kestose content increased slightly during the first two days and then decreased progressively from 0.44, 0.41 and 0.57 mg g⁻¹ fresh weight to 0.057, 0.047 and 0.11 mg g⁻¹ fresh weight at 4, 10 and 20°C during eight days, respectively. During the last four days, 1-kestose content remained stable and no significant difference was observed between the temperature regimes. However, we noted that 1-kestose content was high at 20°C in comparison with 4 and 10°C. We also noted that 1-kestose content was positively correlated to fructose released particularly at 20°C.

The variation of neokestose was close to that of 1-kes-

tose although its content was lower (**Fig. 5B**). At 10 and 20°C, neokestose content increased slightly after two days from 0.034 to 0.055 and 0.044 mg g⁻¹ fresh weight, respectively. Afterwards, the content decreased progressively to 0.09 and 0.06 mg g⁻¹ fresh weight, respectively, although a slight increase was observed at 20°C during the last four days. At 4°C, neokestose content increased to 0.034 mg g⁻¹ fresh weight after six days and then decreased to 0.015 mg g⁻¹ fresh weight and remained quit stable during the last week of storage.

Nystose content variation was more regular and almost linear during storage (**Fig. 5C**). Nystose decreased progressively from 0.095 to 0.037, 0.040 and 0.016 mg g⁻¹ fresh weight at 4, 10 and 20°C, respectively. This decrease demonstrates well the exhausting of the carbon reserve of the spears producing lower polymerized FOS (1-kestose and neokestose) and fructose which was higher as shown above.

DISCUSSION

Although extensive literature reports on the presence of fructooligosaccharides in asparagus roots exists, to date, there is no referenced data on the presence of short chain fructooligosaccharides (sFOS) in asparagus spears except for the work of Shiomi *et al.* (2007b), who reported the variation of saccharides, fructooligosaccharides and their related metabolizing enzymes in different portions of spears stored under different temperatures regimes. Because the carbon source is exported during the sprouting of the root, the slight presence of sFOS could be due to their transport through the sucrose gradient. The study by Shiomi *et al.* (2007b) explained why no sFOS are found in the tips of spears. This suggestion is well supported by the activity of 1-SST which is weak during the last days of storage, while the activities of 1-FFT and 6G-FFT are slightly higher. In fact, there is probably no initiation of FOS biosynthesis at this stage but only an elongation of pre-existent substrate (1-kestose), while this initiation starts from sucrose and 1-SST.

Although these enzymes act simultaneously on identical substrates, the 6G-FFT to 1-FFT ratio was not reported previously except by the study of Ueno *et al.* (2005) who reported similar ratio of native proteins, but the ratio of the cloned proteins was very high and another catalyzing reaction was also observed. Moreover, the high hydrolyzing activities to synthesizing activities ratio observed demonstrates the high carbohydrate catabolic activities occurring in the spears after harvest, and the rapid decline in the shelf-life of the spears.

The high activity of invertase activity observed during the first four days of storage, particularly at 20°C, is the result of high metabolism and respiration rate. The difference observed among the temperature regimes is the consequence of substrate and energy demands, which are positively correlated to temperature. Similar results were reported by Bhowmik *et al.* (2001), who investigated the variation of saccharides in different portions of asparagus spears and noted that soluble invertase activity was two times higher in the top portions than in the bottom portions. A similar observation of neutral invertase in the middle portions, which was slightly higher than in this study, was reported by Hurst *et al.* (1993), and previous studies also reported that invertase decreased during storage.

Surprisingly, the activity of kestose-hydrolase was not known and was highlighted only recently in onion (1-kestose hydrolase) (Benkeblia *et al.* 2005) and wheat (6-kestose hydrolase) (Van den Ende *et al.* 2005), while it has never been reported in other fructan-containing plants. This recent finding seems to be due to the lack of studies of these two enzymes in plants, particularly liliaceous.

Similarly, the activities of FOS synthesizing-enzymes were also not investigated. While these activities were reported to be high during root growth sprouting, they seemed to play a role in the harvested and storage stages of crops. The predominant hypothesis suggests that the noticed bio-

synthetic activities during postharvest would regulate sFOS and sucrose levels by maintaining a balance of substrates, mainly sucrose, because their high accumulation increases osmotic pressure and then would inhibit the hydrolysis process. Indeed a continuous metabolic activity of FOS occurs in the vacuole regardless of the plant's state (Frehner *et al.* 1984), but this metabolism would be diverted to synthesis or hydrolysis depending on either the state or the stage of the plants.

The carbohydrate content of asparagus roots and stems during growing has been subject of an extensive literature, while few one reported their content and variation during storage. Little has also been reported on the carbohydrate content of asparagus spears, nevertheless no data on the presence of FOS are available to our knowledge.

Previous studies have reported that glucose, fructose and sucrose constitute the major part of carbohydrate in asparagus spears, although small amounts of 1-kestose and nystose have been detected (Benkeblia *et al.* 2008), and they decreased during storage particularly during the first three days (King *et al.* 1993; Villanueva-Suárez *et al.* 2003). Bhowmik *et al.* (2002) also noted that total sugar content is high and was higher in the bottom portions than in the top portions. Hurst *et al.* (1993) also reported sugar content of different portion and noted that glucose, fructose and sucrose levels in the top portions of green asparagus were higher in short spears than in long spears, while their levels were higher in the bottom portions than in top portions. This gradient from the bottom to the top was previously reported by Lill *et al.* (1990). The low contents of reducing sugars observed in the top portions is the result of intense and extensive metabolic changes and spears elongation, which are linked to the high respiration rate during the first days of storage (Irving and Hurst 1993; Papadopoulou *et al.* 2001) and the length development of the spears (Paull and Chen 1999). McKenzie *et al.* (2004) also reported that glucose content decreased after one day of storage but increased later, while sucrose content did not change significantly during four days storage of asparagus spears. Similar results have been reported by Renquist *et al.* (2005) who reported an abrupt decrease by 3-folds of glucose and sucrose content of asparagus tips during the first two days of storage of the spears at 20°C under either dry or wet air, while fructose decreased to the same level after only 8 days under similar storage conditions.

On the other hand, the low content observed in the top portion results from the low level of sucrose during growth stage than during young or mature stages of plants (Ross *et al.* 1994). Guo *et al.* (2001) found that sucrose content was slightly lower during developmental stages (10 and 20 cm in length). The authors also reported that hexoses contents in lower portions were higher than those in the top portions and tips. This pattern is strongly linked to the central role of sucrose in carbohydrate metabolism and its relationship with the main substrate of invertase, 1-SST and 1-FFT. This pattern also demonstrates the role of these enzymes in maintaining osmotic pressure in the spear because as demonstrated by the increase of fructose, 1-kestose and neo-kestose observed at 4 and 10°C. On the other hand, water loss would contribute to increase the content of fructose, and even other fructooligosaccharides, however, hydrolysis of the sFOS and sucrose is behind accumulation of large amount of fructose while glucose is actively used in the respiration process particularly during the first days of storage when respiration rate increases by one-fold as reported by Zhang *et al.* (2008). Nevertheless, the role of sucrose is more complex, and its cleavage is vital not only for the allocation of crucial carbon resources but also for the initiation of hexose-based sugar signals in importing structures. Because only the invertase and reversible sucrose synthase (SS) reactions catalyze known pathways of sucrose breakdown *in vivo*, the regulation of these reactions and its consequences has therefore become a central issue in plant carbon metabolism (Koch 2004).

From the results we conclude that sugars contents of

asparagus spears declined during storage and this decline was particularly evident during the first four days of storage. The exhausting of carbohydrates is not caused by the high catabolic activity (respiration) only, but also other physiological processes such as water loss and lignification (King *et al.* 1990; Hennion *et al.* 1992; An *et al.* 2006). Enzyme activities, both hydrolyzing and synthesizing, showed a balance by maintaining stable levels of glucose and sucrose which are considered as indicator molecules in plants providing information on carbohydrate status. There is evidence that decline in the sugar contents of the spears triggers their deterioration by the loss of the turgor. This process is visible at first in the top portion which is typically the first part of the spear to show signs of quality deterioration.

In conclusion, by controlling, or at least reducing the hydrolyzing activities of the enzymes, particularly invertase, the shelf-life of asparagus spears could be maintained for longer. Moreover, it seems likely that higher contents of 1-kestose and nystose would help the activities of synthesizing enzymes to increase, and invertase activity to decrease. In the near future, additional work will be undertaken to show how these sFOS might influence carbohydrate metabolism in asparagus spears.

REFERENCES

- Alam AKMS, Matsui T, Ikeuchi T (1998) Changes in acid invertase activity and sugar distribution in asparagus spears harvested in autumn. *Japanese Journal of Tropical Science* **42**, 257-262
- An J, Zhang M, Lu Q (2006) Effects of pretreated ozone and modified atmosphere packaging on the quality of fresh-cut green asparagus. *International Agrophysics* **20**, 113-119
- Benkeblia N, Onodera S, Shiomi N (2005) Variation of 1-fructo-exohydrolase (1-FEH) and 1-kestose hydrolyzing (1-KH) activities, and fructo-oligosaccharides (FOS) status in onion bulbs. Influence of temperature and storage time. *Journal of the Science of Food and Agriculture* **85**, 225-234
- Benkeblia N, Yoshida N, Ooi Y, Nagamine T, Onodera S, Shiomi N (2008) Variations of carbohydrate content and invertase activity in green and white asparagus spears - Effects of spear length and portion. *Acta Horticulturae* **776**, 459-464
- Bhowmik PK, Matsui T, Kawada K, Suzuki H (2001) Seasonal changes of asparagus spears in relation to enzyme activities and carbohydrate content. *Scientia Horticulturae* **88**, 1-9
- Bhowmik PK, Matsui T, Ikeuchi T, Suzuki H (2002) Changes in storage quality and shelf life of green asparagus over an extended harvest season. *Postharvest Biology and Technology* **26**, 323-328
- Brash DW, Chris CM, Wright S, Bycroft BL (1995) Shelf-life of stored asparagus is strongly related to postharvest respiratory activity. *Postharvest Biology and Technology* **5**, 77-81
- Frehner M, Keller F, Wiemken A (1984) Localization of fructan metabolism in the vacuoles isolated from protoplasts of Jerusalem artichoke tubers (*Helianthus tuberosus* L.). *Journal of Plant Physiology* **116**, 197-208
- Guo J, Jermy A, Turnbull MH (2001) Carbon metabolism in developing spears of two asparagus (*Asparagus officinalis*) cultivars with contrasting yield. *Australian Journal of Plant Physiology* **28**, 1013-1021
- Hennion S, Little CHA, Hartmann C (1992) Activities of enzymes involved in lignification during the postharvest storage of etiolated asparagus spears. *Physiologia Plantarum* **86**, 474-478
- Hurst PL, Hyndman LM, Hannan PJ (1993) Sucrose synthase, invertases, and sugars in growing asparagus spears. *New Zealand Journal of Crop and Horticultural Sciences* **21**, 331-336
- Irving DE, Hurst PL (1993) Respiration, soluble carbohydrates and enzymes of carbohydrate metabolism in tips of harvested asparagus spears. *Plant Science* **94**, 89-97
- King GA, Henderson KG, O'Donoghue EM, Martin W, Lill RE (1988) Flavour and metabolic changes in asparagus during storage. *Scientia Horticulturae* **36**, 183-190
- King GA, Woollard DC, Irving DE, Borst WM (1990) Physiological changes in asparagus spear tips after harvest. *Physiologia Plantarum* **80**, 393-400
- King GA, Hurst PL, Irving DE, Lill RE (1993) Recent advances in the post-harvest physiology, storage and handling of green asparagus. *Postharvest News and Information* **4**, 85N-89N
- Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology* **7**, 235-246
- Lill RE, King GA, O'Donoghue EM (1990) Physiological changes in asparagus spears immediately after harvest. *Scientia Horticulturae* **44**, 191-199
- Lipton WJ (1990) Postharvest biology of fresh asparagus. In: Janick J (Ed) *Horticultural Reviews* (Vol 12), Timber Press, Portland, pp 69-149
- McKenzie MJ, Greer LA, Heyes JA, Hurst PL (2004) Sugar metabolism and

- compartmentation in asparagus and broccoli during controlled atmosphere storage. *Postharvest Biology and Technology* **32**, 45-56
- Papadopoulou PP, Siomos AS, Dogras CC** (2001) Metabolism of etiolated and green asparagus before and after harvest. *Journal of Horticultural Science and Biotechnology* **76**, 497-500
- Paul RE, Chen NJ** (1999) Heat treatment prevents postharvest geotropic curvature of asparagus spears (*Asparagus officinalis* L.). *Postharvest Biology and Technology* **16**, 37-41
- Renquist AR, Lill E, Borst WM, Bycroft BL, Corrigan VK, O'Donoghue EM** (2005) Postharvest life of asparagus (*Asparagus officinalis*) under warm conditions can be extended by controlled atmosphere or water feeding. *New Zealand Journal of Crop and Horticultural Sciences* **33**, 269-276
- Ross HA, Davies HV, Burch IR, Viola R, McRae D** (1994) Developmental changes in carbohydrate content and sucrose degrading enzymes in tuberising stolons of potato (*Solanum tuberosum*). *Physiologia Plantarum* **90**, 748-756
- Shiomi N** (1981) Two novel hexasaccharides from the roots of *Asparagus officinalis*. *Phytochemistry* **20**, 2581-2583
- Shiomi N** (1989) Properties of fructosyltransferases involved in the synthesis of fructan in liliaceous plants. *Journal of Plant Physiology* **134**, 151-155
- Shiomi N** (1992) Content of carbohydrate and activities of fructosyltransferase and invertase in asparagus roots during fructo-oligosaccharide- and fructo-polysaccharide accumulating season. *New Phytologist* **122**, 421-432
- Shiomi N** (1993) Structure of fructo-polysaccharide (asparagosin) from roots of asparagus (*Asparagus officinalis* L.). *New Phytologist* **123**, 263-270
- Shiomi N, Yamada J, Izawa M** (1976) Isolation and identification of fructo-oligosaccharides in roots of asparagus (*Asparagus officinalis* L.). *Agricultural and Biological Chemistry* **40**, 567-575
- Shiomi N, Yamada J, Izawa M** (1979) A novel pentasaccharide in the roots of asparagus (*Asparagus officinalis* L.). *Agricultural and Biological Chemistry* **43**, 1375-1377
- Shiomi N, Onodera S, Chatterton NJ, Harrison PA** (1991) Separation of fructooligosaccharide isomers by anion-exchange chromatography. *Agricultural and Biological Chemistry* **55**, 1427-1428
- Shiomi N, Benkeblia N, Onodera S, Osaki M** (2007a) The metabolism of fructooligosaccharides in asparagus (*Asparagus officinalis* L.). In: Shiomi N, Benkeblia N, Onodera S (Eds) *Recent Advances in Fructooligosaccharides Research*, Research Signpost Publisher, Kerala, pp 213-230
- Shiomi N, Benkeblia N, Onodera S, Omori T, Takahashi N, Fujushima M, Yoshihira T, Kosaka S** (2007b) Saccharides and fructooligosaccharides contents, and invertase, 1-KHE, 1-SST, 1-FFT and 6G-FFT activities in green asparagus spears during storage: effect of temperatures and spear portion. *Journal of Applied Glycoscience* **54**, 187-194
- Takeda H, Sato K, Kinoshita S, Sasaki H** (1994) Production of 1-kestose by *Scopulariopsis brevicaulis*. *Journal of Fermentation and Bioengineering* **79**, 242-246
- Ueno K, Onodera S, Kawakami A, Yoshida M, Shiomi N** (2005) Molecular characterization and expression of a cDNA encoding fructan:fructan 6G-fructosyltransferase from asparagus (*Asparagus officinalis*). *New Phytologist* **165**, 813-824
- Van den Ende W, Yoshida M, Clerens S, Vergauwen R, Kawakami A** (2005) Cloning, characterization and functional analysis of novel 6-kestose exohydrolases (6-KEHs) from wheat (*Triticum aestivum*). *New Phytologist* **166**, 917-932
- Villanueva-Suárez MJ, Redondo-Cuenca A, Rodríguez-Sevilla MD, de la Heras-Martínez M** (2003) Characterization of nonstarch polysaccharides content from different edible organs of some vegetables, determined by GC and HPLC: Comparative study. *Journal of Agricultural and Food Chemistry* **51**, 5950-5955
- Zhang M, Zhanb ZG, Wangc SJ, Tang JM** (2008) Extending the shelf-life of asparagus spears with a compressed mix of argon and xenon gases. *LWT-Food Science and Technology* **41**, 686-691