The accumulation of saccharides and fructooligosaccharides (FOS) in the individual leaf-bases of onion (*Allium cepa* L.) was investigated during growth and bulb development. Saccharides and FOS were analysed by means of high performance anion exchange chromatography-pulsed amperometric detection (HPAEC-PAD). The glucose content was the highest, while the content of saccharides (glucose, fructose and sucrose) increased during June, July and August and decreased slightly during September. The trisaccharides all accumulated to a similar extent, although the neokestose [3b, 6\(^{-}\)-D-fructofuranosylsucrose] content was higher than that of 1-kestose [3a, 1\(^{-}\)-D-fructofuranosylsucrose]. Tetra-, penta- and high-DP (degree of polymerization) FOS also showed a similar pattern, though the contents of 4b [6\(^{3}\)- (1-\(\beta\)-D-fructofuranosyl)sucrose] and 5b [6\(^{5}\) (1-\(\beta\)-D-fructofuranosyl)sucrose] were higher compared with that of other tetra- [4a, 1\(^{-}\) (1-\(\beta\)-D-fructofuranosyl)sucrose and 4c, 1\(^{-}\), 6\(^{2}\)-di-\(\beta\)-D-fructofuranosyl sucrose] and penta-saccharides [5a, 1\(^{5}\) (1-\(\beta\)-D-fructofuranosyl)sucrose]. Total FOS accumulated to a greater extent in the inner (youngest) leaf-bases than in the outer (oldest) leaf-bases, and their content was high during August. The total carbohydrates content was 6.71, 7.25, 8.10 and 6.30 g 100 g\(^{-1}\) FW during June, July, August and September, respectively. During bulb formation, a balance was observed between the glucose, fructose, sucrose and FOS contents, with an average ratio of 20:10:10:60 of total carbohydrates, respectively.

**Key words:** saccharides, fructooligosaccharides, leaf-bases, accumulation, growth, *Allium cepa*

**Introduction**

About 80% of bulb dry matter consists of non-structural carbohydrates (Darbyshire and Henry, 1981). The most predominant of these non-structural carbohydrates is glucose, followed by fructose, sucrose and low-molecular-weight fructans, while starch and raffinose are absent (Darbyshire and Henry, 1981; Benkeblia et al., 2002).
The fructooligosaccharides (FOS), polyfructosylsucroses of varying molecular size, are the main carbohydrate reserve of onion. They accumulate during bulbing and are then catabolized during regrowth and the sprout development of the bulbs (Darbyshire, 1978). FOS may have functions other than carbon storage: they have been implicated in protecting plants against water deficit by drought or low temperature (Hendry, 1993; Hendry and Wallace, 1993; Vijn and Smeekens, 1999), or acting as osmoregulators (Hendry, 1993; Livingston and Henson, 1998; Hincha et al., 2000), and they have an important impact on the storability of onion bulbs (Rutherford and Whittle, 1984).

During the growth and bulbing of the onion plant, the leaf scales thicken and form the characteristics of the bulb. At the onset of bulbing, the leaf sheaths swell, bladeless bulb scales are initiated and these swell to form the central storage tissues and accumulation sites of FOS. Bulb formation and subsequent growth are influenced by photoperiod and temperature (Brewster, 1977), and the bulbing process is initiated mainly by long days (Lercari, 1982; Lancaster et al., 1996; Tei et al., 1996) and high temperatures (Lancaster et al., 1996; Kato, 1964). Clearly, many factors other than photoperiod and temperature affect bulbing, e.g. cultural conditions (Brewster, 1995) and climate change (Wurr et al., 1998).

The biochemical pathway of FOS synthesis in liliaceous plants was well described by Shiomi (1989) and Fujishima et al. (2005). Moreover, a substantial literature exists on the variation of FOS in onion bulbs during the post-harvest life of the bulbs. In spite of this abundance of investigations focused on this aspect, few or none described the content of different FOS during the bulbing of onion, especially across different scales of the bulb tissues. A recent investigation reported the carbohydrate chemistry of the onion leaf bases (Ng et al., 1998), and Darbyshire and Henry (1978) investigated the non-structural carbohydrate content of individual leaf-bases of onion and noted that total fructan concentration decreased from the youngest (inner) to the oldest (outer) leaf-bases of the bulb. These results are in agreement with the results of Jaime et al. (2001). However, values for the variation of FOS during bulbing, as well as their variation across leaf bases, are not readily available in the current literature. Thus, the objective of this study was to investigate variation in the accumulation of saccharides and different FOS in onion leaf-bases during the bulbing period.

Materials and methods

Plant materials and growth conditions

Onion bulbs (Allium cepa var. Sapporo H1, summer cultivar) were cultivated in the University Farm, Ebetsu, Hokkaido, Japan, where the average daylength in June, July, August and September is 15, 15, 14 and 13 h, the average temperature 12, 16, 20 and 18°C and the average rainfall 122, 160, 198 and 173 mm, respectively. These climatic data are the averages of the past five years (from 2000 to 2004). The seeds were sown on March 3 and the seedlings were transplanted on May 8. The bulbs were sampled on June 13 (3 leaf-bases), July 14 (6 leaf-bases) and August 13 (8 leaf-bases), with the final sampling at harvest on September 8. The leaves were

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numbered from 1 (outer, older ones) to 8 (inner, younger ones). After separation, leaf tissue samples were stored at –40°C until use.

**Fructooligosaccharide extraction**

Fructooligosaccharides (FOS) were extracted by the method of Shiomi (1992). Leaf tissue (10 g) was 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. Then the homogenate was filtered and the residue was extracted three times with aqueous ethanol and once with water under 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 a vacuum at 35°C using a Büchi rotavapor (Büch Laboratoriums-Technik, Flawil, Switzerland). The concentrated sugars were collected in one ml of water, passed through a 0.45 µm filter and analysed by high performance anion exchange chromatography (HPAEC, Dionex, Sunnyvale, CA, USA). All processes were run in triplicate.

**Fructooligosaccharide analysis**

FOS were separated on an HPLC-carbohydrate column PA1, Carbo Pack 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 Na acetate 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 min, 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 min, 25 mM. 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 µC. Fructooligosaccharides were expressed in g per 100 g fresh weight (g 100 g–1 FW). System I was used for the separation of oligosaccharides, and system II for that of oligo- and polysaccharides.

Glucose, fructose and sucrose standards were purchased from Nacalai Tesque Inc. (Kyoto, Japan). 1-Kestose [3a, 1-β-D-fructofuranosylsucrose, 1-kestotriose] and nystose [4a, 1- (1-β-D-fructofuranosyl)sucrose, 1,1-kestotetraose] were previously prepared in the laboratory as described by Takeda et al. (1994). Neokestose [3b, 6'-β-D-fructofuranosylsucrose, 6G-kestotriose], 4b [6' (1-β-D-fructofuranosyl)sucrose, 1, 6G-kestotetraose], 4c [1'- (1-β-D-fructofuranosyl) sucrose, 1, 1-kestotetraose], 5a [1'- (1-β-D-fructofuranosyl) sucrose, 1, 1,6G-kestopentaose], 5c [1'- (1-β-D-fructofuranosyl)2-6G-β-D-fructofuranosyl sucrose, 1,1 and 6G-kestopentaose], 5d [1'- (1-β-D-fructofuranosyl)6'- (1-β-D-fructofuranosyl) sucrose, 1 and 1,6G-kestopentaose], 6a [1'- (1-β-D-fructofuranosyl)6'- (1-β-D-fructofuranosyl) sucrose, 1 and 1,6G-kestopentaose], 6b [1'- (1-β-D-fructofuranosyl)6'- (1-β-D-fructofuranosyl) sucrose, 1,1,1-kestopentaose], 6c [m = 3, n = 1 (1,1,1 and 6G-kestopentaose); 6d; m = 1, n = 3 (1 and 1,1,6G-kestopentaose); 6d; m = 2, n = 2 (1,1 and 1,6G-kestopentaose)] and DP (degree of polymerisation) up to 12 were obtained from asparagus roots as described in previous papers (Shiomi et al., 1976; 1979; Shiomi, 1981). The standards 6a [1' (1-β-D-fructofuranosyl)sucrose, 1,1,1-kestopentaose] and 7a [1' (1-β-D-fructofuranosyl)sucrose, 1,1,1,1-kestopentaose] were prepared from Jerusalem artichoke tubers in our laboratory. Because the nomenclature of fructans is not simple due to the very complex structures, the nomenclatures for FOS proposed by Lewis (1993) and Waterhouse and Chatterton (1993) were also used. All isolated and synthesized standards were of high grade purity (≥ 99.8%).

**Statistical analysis**

All determinations were carried out in triplicate (three bulbs were sampled per assay date) and expressed on a fresh weight (FW) basis. The experiment was repeated twice and the data averaged. Data analyses were performed using Graph Pad InStat 3.06 (GraphPad Software, Inc. San Diego, CA, USA) and LSD (at P < 0.05) was calculated. Total carbohydrate means were compared by Student’s t-test (at P < 0.05) using the same software.

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Results

The content of mono- and disaccharides is shown in Figure 1. At the three leaf-bases stage, glucose ranged from 1.20 to 1.50 g 100 g⁻¹ FW. Afterwards, glucose increased during July and August and ranged from 1.40 to 2.20 and from 1.30 to 1.70 g 100 g⁻¹ FW, respectively. During September, the glucose content across the leaf-bases decreased slightly and ranged from 0.80 to 1.50 g. The fructose content was low during June (0.30 to 0.70 g) and increased four to six times (1.10 to 1.80 g) during July, then decreased and remained stable during August and September, ranging from 0.40 to 1.00 g 100 g⁻¹ FW.

Surprisingly, the sucrose content varied slightly during bulb formation and did not accumulate in the leaf-bases. The sucrose content in June, July, August and September varied from 0.60 to 0.85 g, 0.55 to 1.20 g, 0.70 to 1.10 g, and 0.50 to 0.75 g 100 g⁻¹ FW, respectively.

The content of tri-FOS isomers showed a different pattern, as illustrated in Figure 2. 1-Kestose (3a) was higher during June and ranged from 0.40 to 0.60 g 100 g⁻¹ FW, while during July, August and September it showed a steady state, ranging from 0.20 to 0.35 g 100 g⁻¹ FW. The neokestose (3b) content was high during June, ranging from 0.70 to 0.90 g, then decreased during July. In August, the content increased to the levels observed during June. Afterwards, neokestose decreased in September to values of 0.20 to 0.30 g 100 g⁻¹ FW. Total trisaccharides were higher during June and August, but were lower during July and September. It was also noted that the inner leaf-bases (youngest ones) showed high levels of trisaccharides, especially during August.

The nystose (4a) content was highest in June, decreasing to, 0.10 to 0.15 g in July, 0.15 to 0.23 g in August, and 0.10 to 0.15 g 100 g⁻¹ FW in September (Fig. 3). The tetra-isomer 4b content was higher, varying from 0.50 to 0.60 g during June, while it decreased to 0.12 to 0.30 g 100 g⁻¹ FW during July. During August, 4b increased again, ranging from 0.23 to 0.55 g 100 g⁻¹ FW, while decreasing during September to 0.10 to 0.25 g 100 g⁻¹ FW. Isomer 4c showed a similar pattern to 4b, with values of 0.40 to 0.55 g, 0.10 to 0.21 g, 0.23 to 0.55 g, and 0.10 to 0.17 g 100 g⁻¹ FW during June, July, August and September, respectively. The total tetra-saccharides showed a similar pattern, and their content was high during June and August, ranging from 1.20 to 1.45 g and 0.57 to 1.13 g 100 g⁻¹ FW, respectively, while decreasing to about half during July and September, with values of 0.28 to 0.53 g and 0.34 to 0.55 g 100 g⁻¹ FW, respectively. Nevertheless, a similar content was noted in the inner leaf-bases, especially for isomers 4b and 4c, during August and September.

The content of penta-saccharides is illustrated in Figure 4. During June, isomers 5a and 5b ranged from 0.12 to 0.16 g and 0.32 to 0.40 g 100 g⁻¹ FW, respectively. Afterwards, 5a decreased during July and August to 0.05 to 0.15 g 100 g⁻¹ FW, while 5b decreased during July but increased during August, ranging from 0.12 to 0.27 g 100 g⁻¹ FW. Isomers 5c + 5d showed a similar
pattern, ranging from 0.68 to 0.75 g, 0.10 to 0.18 g, 0.15 to 0.53 g and 0.10 to 0.14 g 100 g⁻¹ FW during June, July, August and September, respectively. Thus, the total penta-saccharides varied in the same manner, with values of 1.14 to 1.31 g, 0.25 to 0.45 g, 0.34 to 0.90 g and 0.30 to 0.54 g 100 g⁻¹ FW during June, July, August and September, respectively. The youngest (inner) leaf-bases also showed a higher amount of penta-saccharides than the oldest (outer) ones.

High-DP FOS (6 and higher) varied similarly and a difference between the inner and outer scales was also observed, as illustrated in Figure 5. Values varied from 0.20 to 0.30 g, 0.15 to 0.40 g, 0.20 to 0.35 g and 0.20 to 0.24 g 100 g⁻¹ FW during June, July, August and September, respectively.

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**Fig. 1.** Accumulation of glucose, fructose and sucrose across leaf-bases during bulbing of onion (LSD at $P < 0.05$) (Numbers 1 → 8 refer to inner (youngest) → outer (oldest) leaf-bases of the bulb)

**Fig. 2.** Accumulation of trisaccharides across leaf-bases during bulbing of onion (LSD at $P < 0.05$) (Numbers 1 → 8 refer to inner (youngest) → outer (oldest) leaf-bases of the bulb)
The total carbohydrate content showed a pattern close to that of high-DP FOS with a high level in June, ranging from 6.70 to 7.80 g 100 g\(^{-1}\) FW (Fig. 6), decreasing during July to 3.60 to 6.00 g, then increasing again to between 4.00 (outer leaf-bases) and 7.80 g (inner leaf-bases) during August. Afterwards, the total carbohydrates content decreased during September and ranged from 2.40 to 5.30 g 100 g\(^{-1}\) FW. The average carbohydrate content was 6.71, 7.25, 8.10 and 6.30 g 100 g\(^{-1}\) FW during June, July, August and September, respectively. Surprisingly, a balance between mono- and di-saccharides and high-DP FOS was observed during FOS accumulation, except for July, when the FOS content
was slightly lower compared to that in June, August and September. These balanced contents were approximately 20%, 10%, 10% and 60% for glucose, fructose, sucrose and FOS, respectively. On the other hand, mono-, di- and fructooligosaccharides were seen to decrease during September.

**Fig. 5.** Accumulation of DP 6 and higher (H) fructooligosaccharides across leaf-bases during bulbing of onion (LSD at $P < 0.05$) (Numbers 1 → 8 refer to inner (youngest) → outer (oldest) leaf-bases of the bulb)

**Fig. 6.** Total accumulated carbohydrates across leaf-bases, and total carbohydrate content accumulated during bulbing of onion (in second figure, bars with different letters are significantly different at $P < 0.05$) (Top figure: Numbers 1 → 8 refer to inner (youngest) → outer (oldest) leaf-bases of the bulb)
Discussion

Up to now, few studies have described the distribution of carbohydrates and FOS in onion bulb tissues, while none has considered the accumulation and distribution of FOS across leaf-bases and during bulb formation, except for a study by Shiomi et al. (1997).

First, Bacon (1959) examined the distribution of smaller fructans (up to DP 5) in mature onions and reported that tetra- and penta-saccharides are absent from the outer leaf-bases and increase to maximum concentration in the inner leaf-bases. Later de Miniac (1970) reported a similar distribution of the tri-, tetra- and penta-saccharides, except that DP 3 and DP 4 were present in the outer leaf-bases. Darbyshire and Henry (1978) investigated the non-structural carbohydrates content of individual leaf-bases of mature harvested onion bulbs and noted that DP 3 to 9 fructans were present in all leaf-bases and increased in concentration towards the younger leaf-bases. Thus, with the increasing DP, the concentration of each fructan declined and this relationship was consistent for all leaf-bases. These authors also noted that the glucose and sucrose levels tended to remain constant, while the fructose level was high in the inner leaf-bases. All these results are in agreement with those reported here, except for fructose, which tended to vary across the leaf-bases, as shown in Figure 3.

Recently, Ng et al. (1998) performed a comparative examination of the cell wall chemistry of different component tissues of five varieties of onion bulbs. They dissected the onion bulb into four different tissue regions [top+bottom, brown dry outer skins, outer fleshy leaves and inner fleshy leaves, respectively] and noted that the inner leaf-bases contained more soluble polysaccharides than the outer leaf-bases. Similar results were reported by Jaime et al. (2001), who used two methods for their assays to optimize the extraction procedure.

Unfortunately, none of these studies assayed the distribution and accumulation of the different FOS across leaf-bases during growth, only in mature harvested onions. Shiomi et al. (1997) studied the accumulation of different FOS and their metabolizing enzymes in three varieties of onion bulbs during growth. First, they identified two trisaccharides, three tetra-saccharides and four penta-saccharides, together with a mixture of hexa- and hepta-saccharides. Secondly, they assessed different FOS in whole onion bulb tissues of these three cultivars and noted that they increased from July 28 to August 26, then decreased, but to a higher level than that observed on June 26. They also noted that glucose increased in two cultivars, while sucrose and fructose increased in one. However, the general pattern of these variations was in agreement with the present study. The results showed that FOS increased from June to August, while sucrose and especially fructose remained low. This pattern was due to the mobilization of fructose and sucrose, which are the main substrates for the enzymes involved in FOS synthesis. The increase in FOS was
due to their translocation from the aerial leaves to the bulb, indicating the beginning of maturity. The decrease observed during September was probably caused simultaneously by (i) the reduction of FOS biosynthesis and their exportation from the green leaves to the bulb tissues as the bulb reached maturity, (ii) the increase in the water content of the bulb tissues, resulting from higher water uptake during this period of drought stress. This physiological stress is well known to be the main factor inducing bulb dormancy. On the other hand, as illustrated in Figure 6, the balance observed between saccharides and FOS in the carbohydrate content during bulb formation is in agreement with the claim that fructans have osmoregulator function, providing osmotic adjustment during bulbing.

Conclusions

There is evidence that the contents of different FOS in onion bulb tissues during growth vary differently in the leaf-bases depending mainly on the growth stage and maturity. The high levels observed in August gave a clear demonstration that the accumulation of fructans occurs during this hot period, suggesting the end of bulbing and the possible harvesting stage. The results also showed that some FOS isomers accumulated more than others across the leaves, probably because their synthesis pathway was more favoured or they were degraded to a lesser extent during growth. This accumulation also seemed to be osmotically regulated, maintaining a balance between mono- and di-saccharides and FOS. Moreover, the carbohydrate content could be used as an effective tool for the prediction of the onset of bulb development and for the better prediction of maturity, which is considered one of the main factors of good storability in onion bulbs. Total carbohydrates could also be of value for estimating optimal maturity and harvest dates, to avoid dry matter losses during the last days of growth. Finally, further studies are recommended to assess FOS accumulation under different environmental conditions and in different cultivars, because these are key factors in bulbing and FOS accumulation.

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Corresponding author: N. Shiomi
Phone/Fax: +81 11 388 4754
*E-mail: n-shiomi@rakuno.ac.jp*