Fructooligosaccharides changes during maturation in inflorescences and seeds of onion (*Allium cepa* L. ‘W202’)

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Shiomi, N., Benkeblia, N., Onodera, S. and Kawazoe, N. 2006. **Fructooligosaccharides changes during maturation in inflorescences and seeds of onion (*Allium cepa* L. ‘W202’).** *Can. J. Plant Sci.* *86:* 269–278. The distribution of fructooligosaccharides (FOS) and their related enzyme activities during the development of the inflorescences and the maturation of seeds in onion (*Allium cepa* L.) were investigated. Concentrations of glucose, fructose and sucrose in three inflorescence parts – complete floral parts (FP), pedicels (PD) and involucres (IV) – increased steadily during the first 8 wk of sampling and then decreased over for the next 6 wk. However the final glucose, fructose and sucrose contents were not significantly higher than those observed at the beginning of sampling. Surprisingly, FOS concentration in the inflorescence parts for the saccharides studied showed similar patterns with concentrations increasing during the first 6 wk and decreasing during the last 6 wk of the study. On the other hand, FL and PD parts contained higher amounts of FOS than IV parts. The activities of sucrose:sucrose 1-fructosyltransferase (SST), fructan:fructan 6–fructofuranosyltransferase (6G-FFT) and fructan:fructan 1–fructosyltransferase (1-FFT) showed similar patterns in seeds, decreasing slowly during the first weeks and remaining stable thereafter. The ratio 6G-FFT to 1-FFT decreased after 9 wk from 2.40 to 1.44 with an average of 2.1. In contrast, activities of invertase and β-fructofuranosidase increased progressively, although β-fructofuranosidase activity was twice as high as invertase activity during the last 2 wk. The ratio of synthesizing to hydrolysing activities decreased progressively to 0.2 and was below the value 1 after 9 wk. Thus, it seems that seed maturation was reached during the last week of August, which is 2 to 3 wk earlier than seed harvest normally occurs in Hokkaido.

**Key words:** Fructooligosaccharides, flower organs, seeds, onion


**Mots clés:** Fructooligosaccharides, inflorescences, grains, oignon.

Onion (*Allium cepa* L.), probably one of the first cultivated crops, requires two growing seasons to complete the cycle from seed to seed, although onions grown commercially for seed are produced as an annual crop. Seeds are produced in two systems: seed-to-seed and bulb-to-seed, and in either system seed maturity is reached (McGregor 1976).

Onion seed production requires low-humidity and temperate ambient conditions during spring and summer seasons. Flowering and seed formation occur in the late spring or early summer in response to vernalisation during the winter. The onion inflorescence is an umbel that produces 50 to 2000 flowers (florets). Flowering can be as long as two weeks and is not uniform since the umbel actually consists of aggregation of smaller 5- to 10-flowered inflorescences called “cymes” (McGregor 1976).

**Abbreviations:** 1-FFT, fructan:fructan 1–fructosyltransferase; FOS, fructooligosaccharides; FP, floral parts; 6G-FFT, fructan:fructan 6–fructofuranosyltransferase; HPAEC, high performance anion exchange chromatography; IV, involucres; PD, pulsed amperometric detector; PD, pedicels; SST, sucrose:sucrose 1-fructosyltransferase
have looked at onion or other edible investigators. While studies on fructans in tulip have been metabolism of fructans during flowering has rarely been of the latter can be found in the flowering of Hemerocallis (Bieleski 1993) and inflorescence development in Phippsia algida (Solhaug and Aeres 1994). The metabolism of fructans during flowering has rarely been investigated. While studies on fructans in tulip have been done (Lambrechts et al. 1994; Vergauwen et al. 2000), none have looked at onion or other edible Alliaceae flowers.

Seed formation and development constitute a critical phase in the life cycle of plants, and, thus, it is essential to understand the timing and location of metabolite accumulation during seed development (Jakobsen et al. 1994; Griffith 2000; Spurr et al. 2002). Soluble carbohydrates, and particularly sugars, are present in large and variable amount in plant seeds (Kuo et al. 1988), and have been implicated to protect both membrane (Crowe et al. 1984) and the cytoplasm (Leopold 1990). Sucrose and other non-reducing sugars have also been associated with the onset of desiccation tolerance during the seed development and with seed storability (Steadman et al. 1996; Obendorf et al. 1998). Moreover, timing of harvest of vegetable seeds is recognized as an important factor in the production of quality seeds (Brocklehurst 1985). Thus, selection of optimum harvest time coinciding with physiological maturity is of great interest, and accumulation of carbohydrates reserve could be a good indicator to evaluate full maturity and harvest time.

In an attempt to improve our understanding of FOS accumulation in plant organs and their potential role in seed physiology and quality, the distribution of FOS and their related enzyme activities [sucrose:sucrose 1-fructosyltransferase (SST), fructan:fructan 6-O-fructosyltransferase (6G-FFT) and fructan:fructan 1-F-fructosyltransferase (1-FFT)] were investigated during the development of the inflorescences, and the maturation of seeds in onion (Allium cepa L.).

**MATERIALS AND METHODS**

**Plant Material**
Onion bulbs *Allium cepa* L. var. ‘W202’ (summer cultivar obtained from Wisconsin University) were grown on the local farm of the university, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan. From June to September, the average daylength is 15, 15, 14 and 13 h, the average temperature is 12, 16, 20 and 18°C, and the average rainfall is 122, 160, 198 and 173 mm for June, July, August and September, respectively (Sapporo Office Information). The onions were planted on May 17, and samples were assessed for flowering and seeds maturation 14 times from Jun. 26 to Sep. 20. Due to the asynchronous pattern within and between umbels, sampling was carried out on umbels showing a close phenological stage as described by Vanden Broeck et al. (2003). Sample harvest commenced 2 wk after flowers completely emerged and were first observed blooming. Subsequent harvests were done at 1-wk intervals until the capsules dehisced and the seeds were released. The umbel heads were separated into three parts: complete floral parts (FL), pedicels (PD) and involucres (IV, top of inflorescence stalk). In parallel, immature and mature seeds were separated during the experiment and enzyme activities were assessed. After separation, tissue samples were stored at –40°C until use.

**Fructooligosaccharide Extraction**
Fructooligosaccharides were extracted by the method of Shiomi (1992). Tissues (10 g) were homogenized in 80 mL of aqueous ethanol (70%) using a small amount of calcium carbonate. The homogenate was boiled under reflux in a water bath for 10 min. Homogenate was then filtered and the residue extracted three times with aqueous ethanol and once with water under the same conditions. The filtrates were combined and filled up to 500 mL with distilled water. An aliquot of the filtrate (10 mL) was concentrated under vacuum at 35°C to dryness using a Büchi rotavapor (Büchi Laboratoriums-Technik, Flawil, Switzerland). The concentrated sugars were collected in 1 mL of water and passed through a 0.45-µm filter and analysed by high performance anion exchange chromatography (HPAEC, Dionex, Sunnyvale, CA). 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) 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; and 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 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 are expressed in g 100 g−1 fresh matter (g 100 g−1 FM).

Glucose, fructose and sucrose standards were purchased from Nacalai Tesque Inc. (Kyoto, Japan). 1-Kestose [3a, 1β-D-fructofuranosylsucrose, 1-kestotriose] and nystose [4a, 1β-(1β-D-fructofuranosyl) sucrose, 1,1-kestotetraose] were previously prepared in the laboratory as described by Takeda et al. (1994). Neokestose [3b, 6G-β-D-fructofuranosylsucrose, 6G-kestotriose], 4b [6G (1-β-D-fructofuranosyl)2 sucrose, 1,1-kestotetraose], 4c [1β, 6G-di-β-D-fructofuranosyl sucrose, 1 and 6G-kestotetraose], 5a [1β (1-β-D-fructofuranosyl)2 sucrose, 1, 1, 1-kestotetraose], 5b [6G (1-β-D-fructofuranosyl)3 sucrose, 1, 1, 1, 6G-kestotetraose], 5c [1β (1-β-D-fructofuranosyl)$_2$-6G-β-D-fructofuranosyl sucrose, 1, 1 and 6G-kestotetraose], 5d [1β(1-β-D-fructofuranosyl)-6G (1-β-D-fructofuranosyl)$_2$, sucrose, 1 and 1, 6G-kestotetraose], and DP 6 isomers ($6b, 6c, 6d_1, 6d_2$) and DP up to 12 were obtained from asparagus roots as described in previous papers (Shiomi 1992; Shiomi 1992a).
et al. 1976, 1979; Shiomi 1981). The standards 6a \([1^F\ (1-\beta-D\-fructofuranosyl)\_2\ sucrose, 1, 1, 1, 1-kestohexaose] and 7a \([1^F\ (1-\beta-D\-fructofuranosyl)\_3\ sucrose, 1, 1, 1, 1-kestohexaose]\) were prepared from Jerusalem artichoke tubers in our laboratory. Because fructan structure and nomenclature is very complex, Lewis (1993) and, Waterhouse and Chatterton (1993) nomenclatures were used in this study. All isolated and synthesized standards are of high grade purity (≥ 99.8%).

**Enzyme Extraction**

All operations of enzyme extraction were carried out on ice. Seeds (1 g) were homogenized in 20 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 × g at 0°C for 30 min. The supernatant was then collected and dialysed for 48 h against the same phosphate buffer using cellulose tube. The dialysate was then concentrated, to a final volume of 5 mL, by centrifugation (3000 × g) using an Amicon Centriprep YM 10 filter (Amicon Bioseparation, Millipore, Bedford, MA). This enzyme extract was used for enzyme assays.

**Enzyme Assays**

**SST Activity**

One katal of SST activity was defined as the amount of enzyme that catalysed the fructosyl transfer from sucrose to 1-kestose to produce 1 mole of 1-kestose (3a) in 1 s 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 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 analysed by HPAEC-PAD as described above.

**1-FFT Activity**

One katal of 1-FFT activity was defined as the amount of the enzyme that catalysed the fructosyl transfer from 1-kestose to another 1-kestose to synthesis 1 mole of nystose (4a) in 1 s 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 a boiling water bath for 3 min, and the mixture was analysed by HPAEC-PAD as described above.

**6G-FFT Activity**

One katal of 6G-FFT activity was defined as the amount of the enzyme that catalysed the fructosyl transfer from 1-kestose to another 1-kestose to synthesis 1 mole of \(1^F\-6\beta\-D\-fructosyl sucrose (4c)\) in 1 s. The activity of 6G-FFT was tested using the mixture for 1-FFT assay, and calculated from the amount of 4c formed.

**Invertase Activity**

One katal of invertase activity was defined as the amount of enzyme that hydrolysed 1 mole of sucrose in 1 s. The experimental conditions were those described for SST measurements. Invertase activity was calculated from the amount of fructose released.

**β-Fructofuranosidase Activity**

One katal of β-fructofuranosidase activity was defined as the amount of enzyme that hydrolysed 1 mole of 1-kestose in 1 s. The experimental conditions were those described for 1-FFT measurements. β-Fructofuranosidase activity was calculated from the amount of fructose released.

**Statistical Analysis**

All determinations were carried out in triplicate (three plants were sampled per assay date) and expressed on a fresh weight (FW) basis, and the experiment was repeated twice and data averaged. The data were analysed statistically by comparison of means by Student t-test and determination of least significant difference (LSD at \(P = 0.05\)) using GraphPad Instat 3.06 (GraphPad Software Inc, San Diego, CA).

**RESULTS AND DISCUSSION**

**FOS Variations**

During flowering and seed maturation, variations of glucose, fructose and sucrose contents in FP, PD and IV showed similar patterns (Fig. 1). Glucose content increased progressively from 4.35, 4.35 and 6.73 to 9.18, 16.69 and 12.52 mg g\(^{-1}\) FW, and then decreased also progressively to 2.59, 8.01 and 7.55 mg g\(^{-1}\) FW in FL, PD and IV parts, respectively. During August, it was noted that higher levels of glucose accumulated in PD, ranging from 12.92 to 16.69 mg g\(^{-1}\) FW, while in FP and IV, glucose content ranged from 8.19 to 9.16, and 10.33 to 12.52 mg g\(^{-1}\) FW, respectively. Moreover, significant differences in glucose content were seen between FL and IV parts during July, August and September.

Fructose content varied similarly and increased from 2.33, 2.33 and 5.83 to 11.41, 18.02 and 13.22 mg g\(^{-1}\) FW, then decreased to 2.49, 7.59 and 7.46 mg g\(^{-1}\) FW in FL, PD and IV parts, respectively. During August and September, fructose content in PD was the highest, ranging from 40 to 47% of total fructose of umbels, while in FP and IV, it ranged from 20 to 30%, and from 27 to 44%, respectively. Statistically, significant differences in fructose content was found between FL and IV parts during the end of August and September.

The pattern for sucrose content was similar to glucose and fructose except there was little difference from the end of June to the beginning of September. Sucrose content increased from 4.84, 4.84 and 4.38 to 9.35, 12.07 and 11.65 mg g\(^{-1}\) FW, then decreased to 4.09, 4.55 and 9.44 mg g\(^{-1}\) FW in FL, PD and IV parts, respectively. From July to August, sucrose concentrations ranged from 22 to 36%, from 25 to 36%, and from 32 to 52% of total sucrose in FP, PD and IV, respectively, and there were significant differences found during the last 3 wk among FL and PD, and IV parts.

Furthermore, it is interesting to note that final glucose and fructose contents were higher in PD and IV parts than in FL.
parts, while final sucrose content of IV parts was higher than those of FL and PD parts.

Trisaccharides showed a similar pattern, although higher contents were observed at a little earlier stage than for mono- and disaccharides (Fig. 2). The content of 1-kestose was higher from the beginning, particularly in PD, and decreased progressively over time. 1-Kestose content ranged from 0.77 to 3.99 mg g\(^{-1}\) FW in PD, while ranging from 0.27 to 2.61 mg g\(^{-1}\) FW, and from 0.63 to 3.25 mg g\(^{-1}\) FW in FP and IV, respectively. Neokestose content increased from 0.33, 1.16 and 0.34 to 2.23, 2.96 and 2.47 mg g\(^{-1}\) FW, then decreased progressively to 0.16, 0.86 and 0.63 mg g\(^{-1}\) FW in FL, PD and IV parts, respectively. Statistically, significant differences were found during August between FL and PD, and between FL and IV parts. Trisaccharides were higher at the beginning and increased finally to value less than those observed at the beginning of flowering. The PD also showed a high content of trisaccharides, varying from 40 to 52% of total trisaccharides, and statistically significant differences were seen from June to the end of August between PD and IV, FL and PD, and FL and IV.

Tetrasaccharides showed significant variation, with lower amounts during the beginning and the end of flowering period (Fig. 3). Nystose (4a) increased from 0.16, 0.74 and 0.10 to
0.99, 2.45 and 1.35 mg g\(^{-1}\) FW, then decreased to 0.19, 0.43 and 0.31 mg g\(^{-1}\) FW in FL, PD and IV parts, respectively. On the other hand, 4b increased from 0.25, 0.85 and 0.18 to 1.50, 2.02 and 1.92 mg g\(^{-1}\) FW in FL, PD and IV parts, respectively.

Fig. 3. Accumulation of nystose (4a), 4b, 4c and tetrasaccharides (total DP 4) in flowers (●), pedicels (□) and involucres (×) of onion inflorescences during seed maturation. Vertical bars above values represent SD (only + SD was shown so as to retain clarity in the figure). LSD was calculated at \(P \leq 0.05\).

Fig. 4. Accumulation of 5a, 4b, 4c and pentasaccharides (total DP 5) in flowers (●), pedicels (□) and involucres (×) of onion inflorescences during seed maturation. Vertical bars above values represent SD (only + SD was shown so as to retain clarity in the figure). LSD was calculated at \(P \leq 0.05\).
and then decreased to 0.10, 0.48 and 0.45 mg g$^{-1}$ FW, respectively. The tetrasaccharide 4c increased from 0.16, 1.10 and 0.15 to 1.75, 1.95 and 1.03 mg g$^{-1}$ FW, then decreased to 0.28, 0.26 and 0.47 mg g$^{-1}$ FW in FL, PD and IV parts, respectively. Total tetrasaccharides of the three umbels parts varied from 3.69 to 13.76 mg g$^{-1}$ FW and then decreased to 2.97 mg. It was noted that major individual or total tetrasaccharides were contained in PD during the beginning of flowering, while FP contained the majority during the last period of flowering. Moreover, the high peaks of tetra-saccharides were nearly always found during August.

Pentasaccharides exhibited a similar patterns to tri- and tetrasaccharides (Fig. 4). The pentasaccharide 5a increased from 0.10 and 0.10 to 0.68 and 0.40 mg g$^{-1}$ FW during June, July and August in FL and PD parts, respectively, and then decrease sharply to 0.18 and 0.10 mg g$^{-1}$ FW, respectively, during September. In IV parts, 5a increased to 0.75 mg g$^{-1}$ FW during June and July and decreased to during August and September to a final content of 0.19 mg g$^{-1}$ FW. The
pentasaccharide $5b$ increased to $1.11, 1.22$ and $1.19$ mg g$^{-1}$ FW in FL, PD and IV parts, respectively. During June and July, a balance of $5b$ content was noted between FP, PD and IV, while during the last 6 wk FP content was significantly greater than PD and IV combined. The pentasaccharides $5c + 5d$ increased sharply during July and August from $0.11, 0.48$ and $0.10$ to $2.41, 1.98$ and $2.70$ mg g$^{-1}$ FW, then decreased to $0.16, 0.46$ and $0.86$ mg during September in FL, PD and IV parts, respectively. No significant difference in content of $5c + 5d$ was observed during the first 5 wk, while their content was higher in FP during the 5 following weeks. Similarly, total pentasaccharides increased from $1.32$ to $11.06$ mg g$^{-1}$ FW and decreased to $3.07$ mg during the last 6 wk, and the major part was observed in FP with a significant difference between this part, and PD and IV.

Hexasaccharide $6b$ increased from $0.11, 0.11$ and $0.14$ to $0.87, 0.63$ and $0.47$ mg g$^{-1}$ FW in FL, PD and IV parts, respectively, and remained steady for 8 wk in PD and IV parts, but decreased sharply to $0.1$ mg on FL parts. Other hexasaccharides ($6c + 6d_1 + 6d_2$) increased progressively from $0.10, 0.11$ and $0.10$ to $2.28, 1.48$ and $1.89$ mg g$^{-1}$ FW in FL, PD and IV parts, respectively. Afterwards, $6c + 6d_1 + 6d_2$ decreased progressively to $0.14$ mg in FL parts, while they decreased sharply to $0.21$ and $0.24$ mg in PD and IV parts, respectively, and remained steady from the end to August to September (Fig. 5). It was noted that during the first 6 wk, contents of both $6c + 6d_1 + 6d_2$ and total hexasaccharides were very similar in FP, PD and IV, while during the following 5 wk, the majority (from 66 to 85%) was found in FP, which was significantly higher than the other two floral parts during this period.

The higher-order FOS, DP 7, DP 8, and DP 9x increased progressively during the first 10 wk and total contents of the three umbels parts were from $0.69, 0.49$ and $2.19$ to $4.47, 4.28$ and $11.38$ mg g$^{-1}$ FW, respectively (Fig. 6). Total FOS contents of umbels then decreased to $1.20, 1.01$ and $5.21$ mg g$^{-1}$ FW for DP 7, DP 8 and DP 9x, respectively. DP 7, DP 8 and DP 9x accumulated similarly in FL, PD and IV during June and July, but they were significantly higher in FP during August and the beginning of September.

Surprisingly, mono-, di- and fructooligosaccharides content of the flower organ varied in a close pattern, and each compound increased during June and July before decreasing during the last 5 wk (Fig. 7). Statistically significant differences were observed between July and August and June and September, while no significant difference was noted between July and August, or June and the beginning of September. It is also interesting to note that as seeds matured, a balance was observed among the different saccharides and FOS. Glucose, fructose, sucrose, DP 3, DP 4, DP 5, DP 6, DP 7, DP 8 and DP 9x averaged $24, 22, 24, 8.6, 4, 3, 2, 2$ and $5\%$ of total carbohydrate contents, respectively. We also noted that total mono- and disaccharides ranged from 60 to 80% of total carbohydrate content.

**Enzyme Activities in Seeds**

The activity of SST in seeds was lower and decreased slowly from $1.63$ to $0.50$ nkat g$^{-1}$ FW during the first 4 wk, and afterwards remained stable, ranging between $0.28$ and $0.48$ nkat g$^{-1}$ FW (Fig. 8A). 6G-FFT and 1-FFT showed similar patterns and decreased from $4.37$ and $1.82$ to $2.85$ and $1.35$ nkat g$^{-1}$ FW, respectively, during July (Fig. 8A). Both activ-
Fig. 8. Variation of activities of (A) synthesizing enzymes (SST, 6G-FFT and 1-FFT) and 6G-FFT to 1-FFT ratio (inset), (B) degrading enzymes (invertase, β-fructofuranosidase), and (C) synthesis- to hydrolysis-activity in onion seeds during their development and maturation. Vertical bars above values represent SD. LSD was calculated at $P \leq 0.05$. 
CONCLUSION

It appears that fructooligosaccharides are involved in both the development and flowering of onion inflorescences, although the details remain unclear. In addition, these chemicals are also strongly implicated in the maturation of the seeds; however, this role is also unknown. Our results showed that seed maturation was reached during the last week of August. Thus, from both agronomical and technical points of view, they could be harvested earlier than normal practices when FOS content is higher. This early harvest would be beneficial in improving seed quality by increasing tolerance to desiccation, and storage life. Given other parallels, it seems likely that similar and close phenomena are operating in the flowering of edible and ornamental bulbs. However, further investigation is needed to study in depth the relationship between the flowering stages, including seed formation, and the variations of the FOS content of the different parts of the inflorescence along these stages. It would also be of interest in future work to determine whether these FOS may substitute for the role of sucrose in providing desiccation tolerance and prolonged seed storability.

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