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Effect of Long-term Storage on Saccharides and Fructooligosaccharides (FOS) of Onion Bulb Allium Cepa L. Var. Tenshin

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Abstract : The effect of long-term storage at 15°C for 24 weeks on the content of saccharides and fructooligosaccharides (FOS) in onion bulbs var. Tenshin was investigated. Initial glucose, fructose and sucrose contents averaged 19.5%, 19.2% and 19.6% respectively of total carbohydrates. Tri-saccharides averaged 12.4%, while tetra-saccharides averaged 11% of total carbohydrates. High-polymerized fructo-oligosaccharides averaged 8.9%, 3.8%, 1.9%, 1.3% and 2.3% for DP 5, 6, 7, 8 and up to 12 respectively. After 24 weeks, glucose and fructose contents were relatively the same (20.5 and 21% respectively), while sucrose content decreased significantly and averaged 14.6%. FOS content decreased significantly by 2.5, 58.7 and 72.6% after 8, 16 and 24 weeks respectively. Eight weeks storage did not affect total FOS content, while 24 weeks storage duration influenced significantly the FOS than saccharides contents of onion bulbs.

Keywords: saccharides, fructooligosaccharides, long-term storage, onion

Introduction

About 80% of onion dry matter is non-structural carbohydrates (Darbyshire and Henry, 1981). The predominant of these non-structural carbohydrates are glucose, fructose, sucrose and low-molecular-weight fructans, while starch and raffinose are absent (Benkeblia *et al.*, 2002; Darbyshire and Henry, 1981). The most important biochemical changes occurring during long-term storage of bulbs, as other vegetables, are the quantitative variation in the carbohydrate constituents, which have an important impact on the storability of onion bulbs (Rutherford and Whittle, 1984). Variations in mono and disaccharides level in onion bulbs during storage were previously reported (Benkeblia *et al.*, 2002, Rutherford and Whittle, 1982). However, variation of fructo-oligosaccharides during long term storage were not extensively investigated, despite the large data available on the storability and quality attributes of onions stored at different temperatures.

Fructooligosaccharides (FOS), a polyfructosylsucrose of varying molecular size, are the main reserve carbohydrate of onions, as well as other vegetative organs and plants including alliaceous organs (bulbs) (Hendry, 1993). Fructans may have function other than carbon storage: they have been implicated in protecting plants against water deficit by drought or low temperature, in inducing a resistance to drought or cold stress (Hendry and Wallace, 1993, Vijn and Smeekens, 1999) or as osmoregulator (Livingstone and Henson, 1998), but the molecular mechanism behind these putative roles still remains unclear. Fructans accumulated during bulbing, are catabolized during the regrowth and the sprout development of the bulbs (Darbyshire, 1978),

The aim of this fundamental investigation was to assess the effect of long-term storage on the saccharides and FOS content of onion bulbs regardless of the commercial qualities that are well documented. Bulbs were stored at 15 ± 0.1°C and 45 ± 1 % relative humidity (RH) for 24 weeks. The concentration of glucose, fructose, sucrose and other FOS were then determined.

Materials and Methods

Onion Bulbs : Onion bulbs (*Allium cepa* var. Tenshin, long day onion cultivated in Hokkaido region) freshly harvested and dried in the field for two weeks were obtained from the local farm of Rakuno Gakuen University. They were sorted for uniformity and absence of defects, packed in commercial plastic (PVC) trays each of 15 kg.

Storage Conditions : Four trays, each of 12 kg, of onions bulbs were stored in dark and placed in a refrigerated chamber (Eyelatron, model FLI 301 NH, Rikakikai, Tokyo) at 15 ± 0.1°C and 45 ± 1 % RH.

Saccharides and Fructooligosaccharides Extraction : Saccharides (glucose, fructose and sucrose) and FOS were extracted as described by Shiomi (1992). Tissues (10 g) were homogenized in 80 ml of aqueous ethanol (70 %) containing small amount of calcium carbonate. The homogenate was then boiled under reflux in a water bath for ten minutes. Then homogenate was filtered and the residue extracted three times with aqueous ethanol and one time with water in the same conditions. This extraction (aqueous ethanol + heating) allows extracting total soluble carbohydrates. 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 evaporator. The concentrated sugars were collected in one ml of water and passed through a 0.45 μm filter and analyzed by high performance anion exchange chromatography (HPAEC, Dionex, Sunnyvale, CA, USA). All processes were run in triplicate.

Saccharides and fructooligosaccharides analysis : The saccharides and 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 acetate-Na in 150 mM NaOH): 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, 0.06 and −0.6 V respectively, and the output range was 1 μC.
Glucose, fructose and sucrose standard were purchased from Nacalai Tesque Inc. (Kyoto, Japan), 1-kestose and nystose were previously prepared in the laboratory as described by Takeda *et al.* (1994). All standards are high-grade purity. Other FOS were identified according to previous works (Shiomi *et al.*, 1976, Shiomi *et al.*, 1979, Shiomi, 1981).

Data Analysis : All determinations were carried out in triplicate and the experiment was repeated twice. Results were expressed on a fresh weight basis, and data were averaged and compared by Student-t-test using Statistica 5.0 software.

Results

The distribution of the saccharides and FOS contents of onion bulb is shown in Fig. 1. Glucose, fructose and sucrose constitute a major proportion of non-structural carbohydrates, while tri and tetra saccharides contents are lower. It is observed that concentration of high-polymerized fructans decreased with their degree of polymerization. It is noted that initial contents of glucose, fructose and sucrose constitute major part of the dry matter and non-structural carbohydrates averaging 19.5%, 19.2% and 19.6% of total carbohydrates respectively. Tri-saccharides averaged 12.4%, while tetra-saccharides averaged 11% of total carbohydrates. High-polymerized FOS averaged 8.9%, 3.8%, 1.9%, 1.3% and 2.3% for DP 5, 6, 7, 8 and up to 12 respectively.
Fig. 2 illustrates the variation contents of glucose, fructose and sucrose in bulb tissues stored for 24 weeks at 15 °C. Glucose content was relatively the same after 24 weeks, while fructose content increased slightly after ten weeks and remained little high during the last 14 weeks. On the other hand, sucrose content peaked to 15.23 mg g^{-1} FW after six weeks, and then decreased to 7.57 mg g^{-1} FW four weeks later. During the 14 last weeks, sucrose content remained in steady state varying from 7.76 and 8.76 mg g^{-1} FW. The sharp decrease of sucrose is a consequence of the high catabolism due to the onset of sprouting of onions. This high catabolism also is responsible of the exhausting of high polymer carbohydrates, which are hydrolyzed producing sucrose and fructose. During storage, [fructose]/[glucose] and [glucose+fructose]/[sucrose] ratios were showed by Fig. 3. It was noted that [F]/[G] ratio increased from 1 to 1.26 after eight weeks coinciding with the sharp decrease of sucrose (Fig. 3A). This level remained high during the six last weeks ranging from 1.24 and 1.34, and then ratio decreased to 1.02 during the six last weeks. On the other hand, [G+F]/[S] ratio decreased slightly from 2 to 1.6 during the 6 first weeks, and then increased abruptly to 3.7 four weeks later (Fig. 3B). After a steady state of four weeks, [G+F]/[S] ratio decreased progressively to 2.8 during the 10 last weeks. The high level of [F]/[G] and [G+F]/[S] ratios observed after eight weeks, are due to the relative high metabolism of the sprout tissues which need a continuous availability of an important quantity of energy and substrate for their development and elongation
As illustrated in Fig. 4, FOS contents decreased significantly after 24 weeks storage (Fig. 3A and B). After 24 weeks, FOS decreased by 58%, 76%, 82%, 74%, 81%, 82% and 83% for DP 3, DP 4, DP 5, DP 6, DP 7, DP 8 and DP ~12 respectively. This decease of FOS is due to the metabolism of bulb tissues initiated by the break of dormancy and sprout elongation. This physiological state creates a high demand of substrate (saccharides) which are supplied by FOS, thus these polymers exhaust during the development of the sprout. However, as shown by Fig. 5, storage time affected total FOS content only after two months. During the 8 first weeks, total FOS content remained the same, but after four months this content decreased by 58.7% after 16 weeks and by 72.6% after 24 weeks.

Discussion

Variations of glucose, fructose and sucrose in stored onion were subjected of many investigations. Benkeblia and Varoquaux (2003) and Benkeblia *et al.* (2004a, 2004b) reported close results on other cultivars of onion bulbs stored at 4, 10 and 20 °C, although a peaks of glucose and fructose were noted after six and ten weeks. However, similar pattern of sucrose was observed but the peak was noted after four weeks.
Salama et al. (1990) reported a decrease in total sugars and glucose in onions stored five months at 0, 15 and

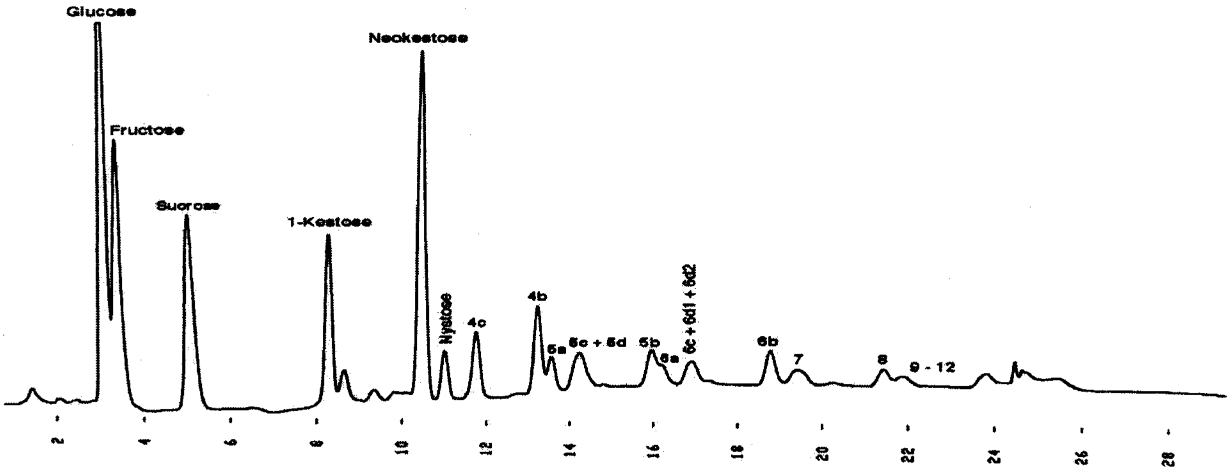

Fig. 1: HPAEC-PAD chromatogram of saccharidies and fructooligosaccharidies of onion bulb Alium cipaL. Var Tenshin I-Kestose 1^F-β -D-Fructofuranosyl sucrose; neokestose; 6^G-β -D-Fructofuranosyl sucrose; nystose(4a):1^F(1-β -D-Fructofuranosyl)$_2$ sucrose; 4b:6^G(1-β -D-Fructofuranosyl)^2 sucrose;4c; 1^F,6^G-di-β -D-Fructofuranosyl sucrose;5a:1^F(1-β -D-Fructofuranosyl)$_3$ sucrose; 5b: 6^G (1-β -D-Fructofuranosyl)$_3$ sucrose; 5c: 1^F(1-β -D-Fructofuranosyl)$_2$-6^G-β -D-fructofuranosyl sucrose;5d:(1-β -D-fructofuranosyl)$_2$ sucrose;6a: 1^F(1-β -D-fructofuranosyl)$_4$ sucrose; 6b: 6^G(1-β -D-fructofuranosyl)$_4$ sucrose; 6c: 1^F(1-β -D-fructofuranosyl)$_3$-6^G-β -D-fructofuranosyl sucrose;6d^1: 1^F-β -D-fructofuranosyl-6^G (1-β -D-fructofuranosyl)$_3$ sucrose; 6d$_2$$1^F$(1-β -D-fructofuranosyl)$_2$ 6^G(1-β -D-fructofuranosyl)$_2$ sucrose;DP7:1^F(1-β -D-fructofuranosyl)$_m$-6^G(1-β -D-fructofuranosyl)$_n$ sucrose ($m+n=5$); DP8:1^F(1-β -D-fructofuranosyl)$_m$-6^G(1-β -D-frutofuranosyl)$_n$ sucrose ($m+n=6$); DP9-12: 1^F(1-β -D-fructofuranosyl)$_m$-6^G(1-β -D-fructofuranosyl)$_n$ sucrose ($m+n>7$)

30°C, but fructose increased particularly at 0° C. Similar results on fructose were reported by Rutherford and Whittle (1982) at 0°C. Hurst *et al.* (1985) noted a decrease in total sugars of onion kept during six months at 1 and 4° C, but no variation was noted at 21° C. Benkeblia *et al.* (2002) also did not observe any significant difference in total saccharides (glucose, fructose and sucrose) stored for six months at 4, 10 and 20 °C. The variation in saccharides -especially sucrose- reported by these numerous investigations suggest that metabolism of sucrose is not clearly understood. This component plays a central role in growth and development in plant, and demand for products of sucrose degradation are important factors.

The ratios of [F]/[G] and [G+F]/[S] were not extensively reported by literature. Benkeblia *et al* (2004a) reported that [F]/[G] ratio in onion var. Tenshin increased after six weeks and then decreased and remained stable during 18 weeks at 10 and 20 °C, while [G+F]/[S] ratio showed similar pattern. The same authors noted that [F]/[G] ratio did not vary during six months storage at 4, 10 and 20 C of onion bulbs var. Rouge Amposta (Benkeblia and Varoquaux, 2003). These results suggest that catabolism of saccharides especially sucrose is not completely elucidated (Koch and Zeng, 2002). This component plays a central role in growth and development in plant, and demand for products of sucrose degradation are important factors particularly during break of dormancy of the bulb. Changes in FOS levels in tissues remain still unknown. Rutherford and Whittle (1982) reported that total fructans decreased after six months storage and their degradation is higher at low temperature. Suzuki and Cutcliffe (1989) reported similar results with a slight increase of DP 5 - 8 fructopolymers. Benkeblia *et al.* (2002) noted a slight increase of DP 5 - 8, of onion bulbs var. Rouge Amposta after six months at 10 and 20°C. On the other hand, a decrease of FOS was observed in var. Jaune d' Espagne stored for six months at the same temperatures (Benkeblia *et al.* 2004b). These conflicting results could probably due to the difficulty to separate the fructans, and the presence of a multiple isomers of the DP 5 and DP 6 FOS depending on: (1) type of onions, (2) maturity at harvesting, and (3) varieties. Furthermore, little has been done on the enzymology and regulation of FOS degradation and the molecular mechanism behind their metabolism and their putative roles remain still unclear

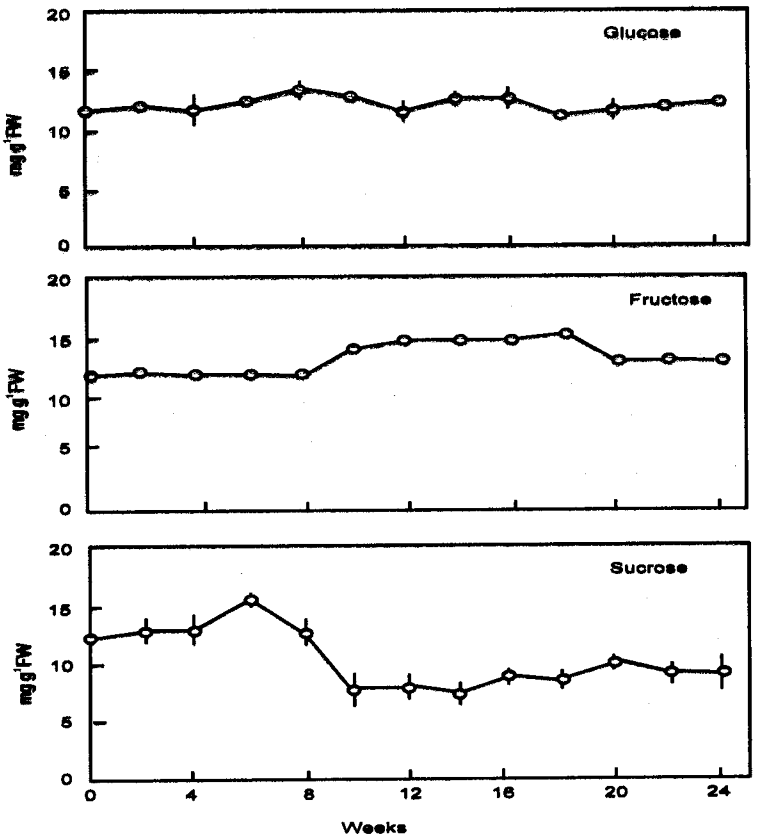

Fig. 2: Variation of glucose, fructose and sucrose contents in onion bulb tissues during storage at 15°C(vertical bars represent SD)

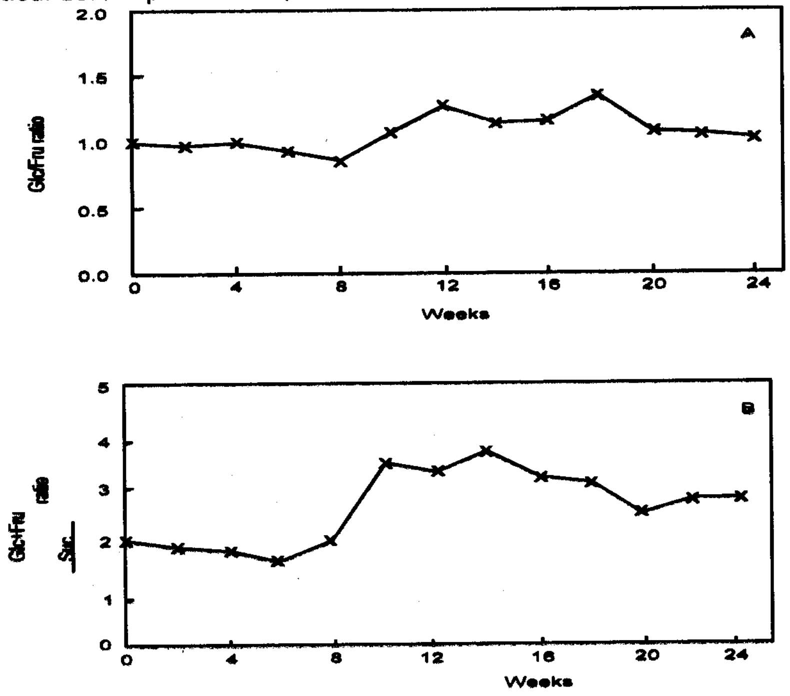

Fig. 3: Effect of storage time on [fructose],[glucose] ratio (A) and [glucose + fructose]/[sucrose] ratio (B) of onion bulbs kept 24 weeks at 15°C.

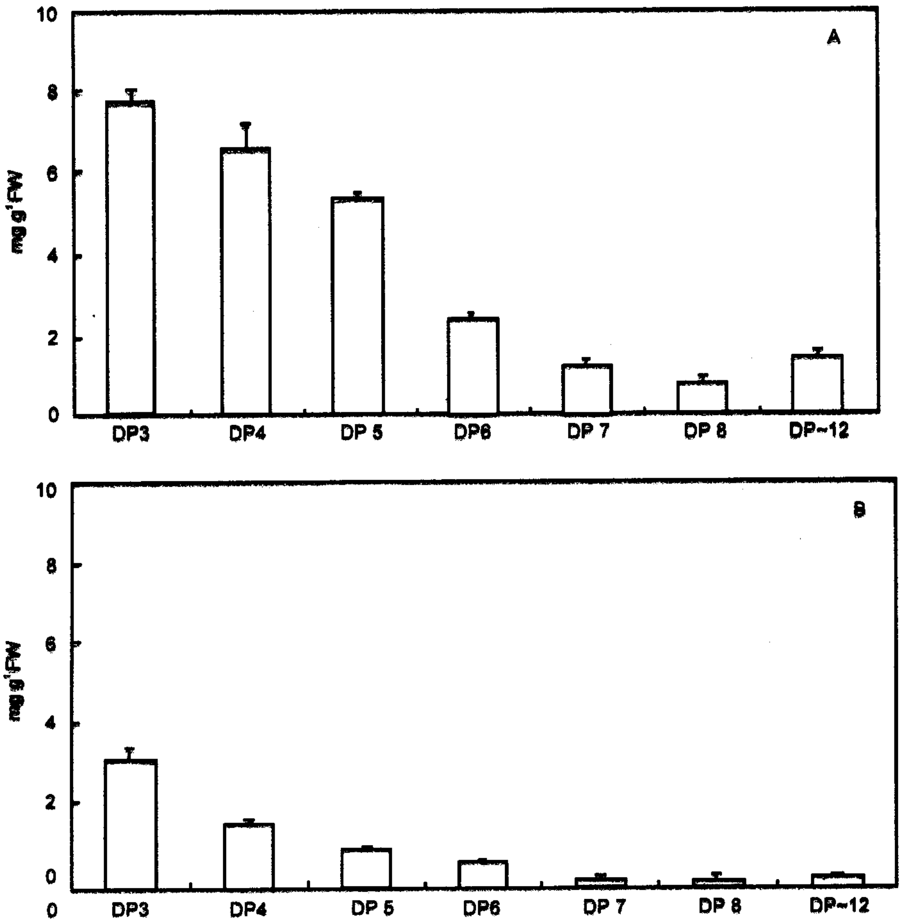

Fig. 4: Effect of storage time on fructooligosaccharides of onion bulb kept 24 weeks at 15°C A:fresh onion bulbs, B after 24 weeks (vertical bars represent SD)

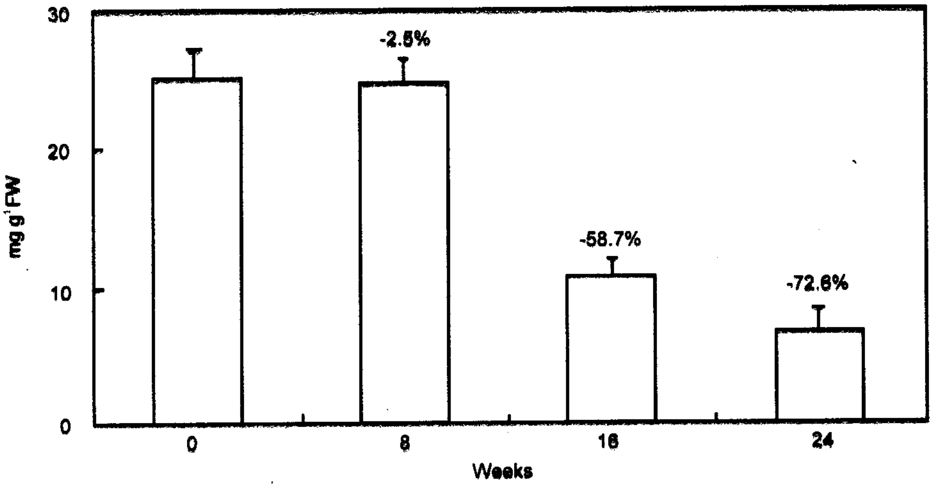

Fig. 5: Effect of storage time on total fructooligosaccharides content in onion bulbs (vertical bars represent SD)

Conclusion

Results showed that storage time influenced significantly the fructooligosaccharides content of onion bulb. The physiological state (break of dormancy and sprout growth) may create a greater demand for mobilization and utilization of carbohydrates particularly glucose and fructose and their direct dimer sucrose. Nevertheless, the metabolism of carbohydrates of onion bulb remains complex and further investigations are needed to determine their catabolism pathway and enzymes involved.

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