

## Hydrolysis Kinetic Parameters of DP 6, 7, 8, and 9–12 Fructooligosaccharides (FOS) of Onion Bulb Tissues. Effect of Temperature and Storage Time

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The objective of this study was to report on the variation of DP 6 isomers (**6b**, **6c** + **6d**<sub>1</sub> + **6d**<sub>2</sub>), **7a**, **8**, and **9–12** fructooligosaccharides (FOS) and their hydrolysis parameters [percent hydrolysis, consumption rate, hydrolysis rate constant ( $k_{\text{obsd}}$ ), and half-life time ( $t_{1/2}$ )] in onion bulb tissues stored for 6 months at 10, 15, or 20 °C. The hydrolysis of DP 6 isomers, **7a**, and **8** ranged from 74 to 85%, whereas that of DP **9–12** averaged 86%. The consumption rate of **6b**, **6c** + **6d**<sub>1</sub> + **6d**<sub>2</sub>, **7a**, **8**, and **–12** averaged 25, 58, 38, 26, and 48  $\mu\text{g/g}$  of fresh weight per week, respectively. The  $k_{\text{obsd}}$  showed large variation from  $53 \times 10^{-3} \text{ week}^{-1}$  (lowest value) to  $92 \times 10^{-3} \text{ week}^{-1}$  (highest value), whereas the half-life  $t_{1/2}$  of the different FOS ranged between 7.5 and 13.1 weeks. DP **6b** isomers increased during the first month, and then the content decreased sharply during the second month at 20 °C but remained stable during the last 4 months, whereas at 10 and 15 °C, **6b** decreased progressively from the first to the six month. In contrast, DP **6c** + **6d**<sub>1</sub> + **6d**<sub>2</sub> decreased abruptly within the first 3 months at 10, 15, and 20 °C; however, during the last 3 months they remained stable, ranging between 0.32 and 0.39. Variation of DP **7a**, **8**, and **9–12** FOS was close to that of DP 6 FOS isomers. DP **7a** increased slightly during the first month, and afterward **7a** started decreasing progressively during the last 5 months. DP **8** FOS showed a similar pattern independent of temperature regime: they increased slightly within the first month, but from the second month, DP **8** began decreasing progressively and continued decreasing to the sixth month. DP **9–12** FOS also varied similarly to DP **6c** + **6d**<sub>1</sub> + **6d**<sub>2</sub>. They decreased sharply within the first 2 months, and during the last 4 months, they continued to decrease slowly. Surprisingly, these variations occurred independently of temperature regimes and were affected only by storage duration. It was concluded that highly polymerized FOS are preferably hydrolyzed rather than low DP FOS isomers because they have a relatively high content of fructosyl end chains.

**KEYWORDS:** Fructooligosaccharides; hydrolysis; half-life time;  $k_{\text{obsd}}$ ; temperature; *Allium cepa*; storage

### INTRODUCTION

About 80% of onion bulb (*Allium cepa*) dry matter consists of nonstructural carbohydrates (1). The predominant of these nonstructural carbohydrates are glucose, fructose, sucrose, and low molecular weight fructans, whereas starch and raffinose are absent (1, 2). Fructans, also known as fructooligosaccharides (FOS), which are oligo- and polyfructosylsucroses of various molecular sizes, are the main carbohydrate reserve of onion, as well as of other vegetative organs and plants including alliaceous organs (bulbs). Fructans accumulate during bulbing and are then catabolized during the regrowth and sprout development of the bulbs (3).

**6a** [ $1^{\text{F}}$  (1- $\beta$ -D-fructofuranosyl)<sub>4</sub> sucrose; 1,1,1,1-kesto-hexaose], **6b** [ $6^{\text{G}}$  (1- $\beta$ -D-fructofuranosyl)<sub>4</sub> sucrose; 1,1,1,6G-kesto-hexaose], **6c** [ $1^{\text{F}}$  (1- $\beta$ -D-fructofuranosyl)<sub>3</sub>- $6^{\text{G}}$ - $\beta$ -D-fructofuranosyl sucrose; 1,1,1 and 6G-kesto-hexaose], **6d**<sub>1</sub> [ $1^{\text{F}}$ - $\beta$ -D-fructofuranosyl- $6^{\text{G}}$  (1- $\beta$ -D-fructofuranosyl)<sub>3</sub> sucrose; 1 and 1,1,6G-kesto-hexaose], **6d**<sub>2</sub> [ $1^{\text{F}}$  (1- $\beta$ -D-fructofuranosyl)<sub>2</sub>- $6^{\text{G}}$  (1- $\beta$ -D-fructofuranosyl)<sub>2</sub> sucrose; 1,1 and 1,6G-kesto-hexaose], **7a** [ $1^{\text{F}}$  (1- $\beta$ -D-fructofuranosyl)<sub>5</sub> sucrose; 1,1,1,1,1-kestoheptaose], **8** [ $1^{\text{F}}$  (1- $\beta$ -D-fructofuranosyl)<sub>*m*</sub>- $6^{\text{G}}$  (1- $\beta$ -D-fructofuranosyl)<sub>*n*</sub> sucrose ( $m + n = 6$ )] and FOS of DP  $\sim 12$  constitute a part of the different FOS found in onion bulb tissues, and their contents vary between 4 and 5%, between 1.5 and 2.5%, between 1.5 and 2%, and between 3 and 4% of total carbohydrates for DP **6**, **7**, **8**, and **9–12**, respectively (2, 3–5). During the past decade, FOS have received considerable interest as food ingredients.

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They are used as nondigestible dietary fiber and texturing properties in many foodstuffs (6–8).

Some studies have been focused on the FOS properties with respect to their polydispersities, and a few take into account their degradation parameters *in vitro* (9, 10). Actually, it has been stated that the chemical breakdown of FOS (hydrolysis) is easy, and conditions during which partial or total hydrolysis of the FOS occurs are frequently met during long-term storage. The main effects are shortening of the FOS chains and production of free sugars, that is, glucose, fructose, and sucrose. Thus, it seems to be important to determine the *in vivo* kinetics of the hydrolysis of these FOS during long-term storage because the estimation of the amount of the exhausted sugars and the losses of these nutritive compounds in tissues are of great importance for technologists and nutritionists. Little has been done on the variation of the different FOS during storage (4, 11, 12), and no studies have investigated the kinetics of hydrolysis of these isomers during storage except that of Benkeblia et al. (13), who reported kinetic parameters of tetra- and penta-fructooligosaccharide isomers in onion bulbs during storage.

The present work is devoted to the study of the *in vivo* hydrolysis and kinetic parameters of DP 6 isomers, 7a, 8, and 9–12 FOS of onion bulbs during storage under three temperature regimes.

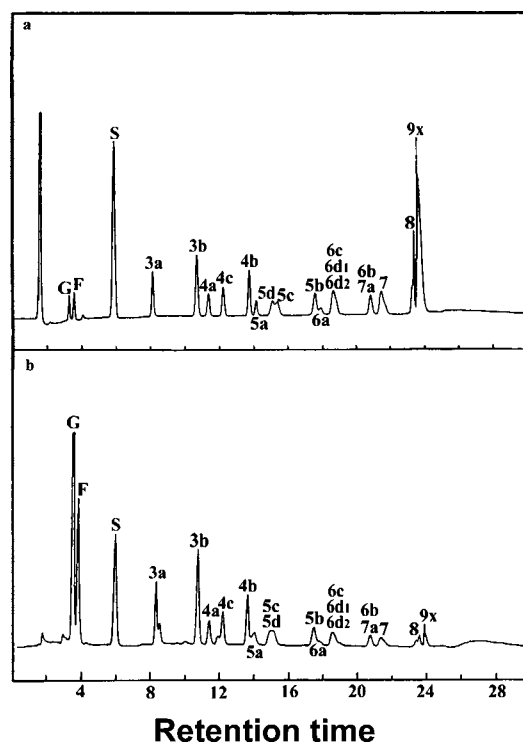
## MATERIALS AND METHODS

**Materials.** Onion bulbs, *Allium cepa* cv. Tenshin (summer cultivar), that had been freshly harvested and dried (cured) in the field for 2 weeks were obtained from the local farm of the university, Ebetsu, Hokkaido, Japan. During the drying period (end of August), the average temperature was 20–22 °C and the average humidity was 65–70%. The final moisture of the bulbs averaged 89.7%. The bulbs were sorted for uniformity and absence of defects and then packed in commercial plastic (PVC) trays of 10 kg each. Five trays were kept in storage as described below. FOS contents were determined immediately after harvesting and after every month during storage duration.

**Storage Conditions.** Onion bulbs were stored in the dark at three temperatures. For the lower temperature regimes, onions were placed in a refrigerated chamber (Eyelatron, model FLI 301 NH, Rikakikai Co. Ltd., Tokyo, Japan) at 10 ± 0.1 °C and 65 ± 1% relative humidity (RH) and at 15 ± 0.1 °C and 55 ± 1% RH. For the higher temperature regime, 20 °C, onions were placed in a ventilated room with an average RH of 50%.

**Fructooligosaccharides Standards.** 6b [6<sup>G</sup> (1-β-D-fructofuranosyl)<sub>4</sub> sucrose; 1,1,1,6G-kestoheptaose], 6c [1<sup>F</sup> (1-β-D-fructofuranosyl)<sub>3</sub>-6<sup>G</sup>-β-D-fructofuranosyl sucrose; 1,1,1 and 6G-kestoheptaose], 6d<sub>1</sub> [1<sup>F</sup>-β-D-fructofuranosyl-6<sup>G</sup> (1-β-D-fructofuranosyl)<sub>3</sub> sucrose; 1 and 1,1,6G-kestoheptaose], 6d<sub>2</sub> [1<sup>F</sup> (1-β-D-fructofuranosyl)<sub>2</sub>-6<sup>G</sup> (1-β-D-fructofuranosyl)<sub>2</sub> sucrose; 1,1 and 1,6G-kestoheptaose], and FOS of DP 9–12 were obtained from asparagus roots as described in previous papers (14–16). The standards 6a [1<sup>F</sup> (1-β-D-fructofuranosyl)<sub>4</sub> sucrose; 1,1,1,1-kestoheptaose], 7a [1<sup>F</sup> (1-β-D-fructofuranosyl)<sub>5</sub> sucrose; 1,1,1,1,1-kestoheptaose], 8 [1<sup>F</sup> (1-β-D-fructofuranosyl)<sub>m</sub>-6<sup>G</sup> (1-β-D-fructofuranosyl)<sub>n</sub> sucrose (m + n = 6)], and 9–12 [1<sup>F</sup> (1-β-D-fructofuranosyl)<sub>m</sub>-6<sup>G</sup> (1-β-D-fructofuranosyl)<sub>n</sub> sucrose (m + n ≥ 7)] were prepared from Jerusalem artichoke tubers in our laboratory. Fructan nomenclature is not simple due to the complex structures, the nomenclatures for FOS proposed by Lewis (17) and Waterhouse and Chatterton (18) were used. All isolated and synthesized standards are of high-grade purity (>99.8%).

**FOS Extraction.** FOS were extracted according to the method of Shiomi (19). Bulbs of average size were cut longitudinally into fourths, and the large middle part of one fourth was sampled. Tissues (10 g) were homogenized in 80 mL of aqueous ethanol (70%) using a small amount of calcium carbonate (0.5 g/L). The homogenate was boiled under reflux in a water bath for 10 min. Homogenate was filtered and



**Figure 1.** HPAEC chromatogram of (a) standards mixture and (b) onion extract.

the residue 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 1 mL of water, filtered through a 0.45 μm filter, and analyzed by high-performance anion exchange chromatography (HPAEC Dionex, Sunnyvale, CA). All processes were run in triplicate (three samples from three different bulbs).

**FOS Analysis.** FOS were separated on an HPLC carbohydrate column PA1, Carbo Pack (Sunnyvale, CA) with a Dionex Bio LC series HPLC (Sunnyvale, CA) and pulsed amperometric detector (PAD), and the separation profile of the standard mixture and sample is shown in Figure 1. The gradient was established by mixing eluent A (150 mM NaOH) with eluent B (500 mM sodium 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, 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 as follows: 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. The applied PAD potentials for  $E_1$  (500 ms),  $E_2$  (100 ms), and  $E_3$  (50 ms) were 0.01, 0.60, and –0.60 V, respectively, and the output range was 1 μC. Samples (25 μL) were injected using an autosampler (Intelligent autosampler, model AS-4000, Hitachi Ltd., Tokyo, Japan). FOS are estimated by comparison with standard peaks and expressed in milligrams per gram of fresh weight (mg/g of FW).

**Estimation of Degradation Parameters.** *Percentage of Hydrolysis.* The percentage of hydrolysis of the isomers was estimated after 6 months by the equation

$$\text{hydrolysis (\%)} = \left| \frac{C_f}{C_i} - 1 \right| \times 100$$

where  $C_i$  is concentration at month 0 and  $C_f$  the concentration at month 6.

*Consumption Rate.* The consumption rate was estimated by fitting linear regression lines to the FOS content versus storage time (in weeks)

**Table 1.** Percentage of Hydrolysis of DP 6 Isomers, **7a**, **8**, and **9–12** Fructooligosaccharides in Onion Bulb Tissues Stored for 6 Months at Different Temperatures

	hydrolysis <sup>a</sup> (%) at		
	10 °C	15 °C	20 °C
<b>6b</b>	73a	72a	72a
<b>6c + 6d<sub>1</sub> + 6d<sub>2</sub></b>	82b	79b	81b
<b>7a</b>	81b	83c	82b
<b>8</b>	85b	84c	83b
<b>9–12</b>	87bc	83c	89c

<sup>a</sup> Values with different letters within the same column are significantly different at  $P = 0.05$ .

as shown by the following equation:

$$\text{consumption rate } (\mu\text{g/g per week}) = \frac{\Delta[\text{FOS}]}{\text{time (weeks)}}$$

**Hydrolysis Rate Constant and Half-Life Time.** The hydrolysis rate constant ( $k_{\text{obsd}}$ ), which showed a pseudo-first-order kinetics according to the models of Logan (20), is estimated by the equation

$$C_t = C_i e^{(-k_{\text{obsd}}t)}$$

where  $C_i$  is the concentration at month 0,  $C_t$  the concentration after 6 months, and  $t$  time in weeks.

The half-life time ( $t_{1/2}$ ) is estimated as follows:

$$t_{1/2} = \frac{\ln 0.5}{k_{\text{obsd}}}$$

**Statistical Analysis.** The experiment was repeated in two successive seasons (2003 and 2004). 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).

## RESULTS AND DISCUSSION

**Hydrolysis and Consumption Rates.** The hydrolysis of the DP 6 isomers, **7a**, **8**, and **9–12** FOS is shown in **Table 1**. The hydrolysis of the DP 6 isomers, **7a**, and **8** ranged from 72 to 82%, from 81 to 83%, and from 83 to 85%, respectively, whereas it ranged from 83 to 89% for DP **9–12** FOS. It is interesting to note that the high DP FOS were hydrolyzed preferably, particularly **9–12** FOS, which were almost completely exhausted after 6 months. This is due mainly to the sprout development, which has great need of energy and higher DP FOS constitute the main source of this energy, providing lower FOS and saccharides during the sprouting process. We also noted that FOS hydrolysis during storage followed sprouting of the bulbs (data not shown).

We observed significant differences in the consumption rates of the different FOS during 6 months of storage (**Table 2**). Consumption rates of DP **6b** isomers, **7a**, and **8** ranged from 24.6 to 39.2  $\mu\text{g/g}$  of FW per week, and the consumption rate of **6c + 6d<sub>1</sub> + 6d<sub>2</sub>** together ranged between 57.5 and 59.6  $\mu\text{g/g}$  of FW per week. Notably, the consumption rates of **6c + 6d<sub>1</sub> + 6d<sub>2</sub>** and DP **9–12** were statistically higher (48–50  $\mu\text{g/g}$  of FW per week), showing that hydrolysis and consumption of higher DP are more rapid and confirming their role of reserve and energy regulators.

Despite their important role in carbohydrate reserve, no investigation has been carried out on high DP kinetics in onion or other fructan-containing crops. However, an investigation carried out on low DP FOS, that is, DP **3** and **4**, showed that

**Table 2.** Consumption Rate of DP 6 Isomers, **7a**, **8**, and **9–12** Fructooligosaccharides in Onion Bulb Tissues Stored for 6 Months at Different Temperatures

	consumption rate <sup>a</sup> ( $\mu\text{g/g}$ per week) at		
	10 °C	15 °C	20 °C
<b>6b</b>	25.0a	24.6a	24.6a
<b>6c + 6d<sub>1</sub> + 6d<sub>2</sub></b>	59.6b	57.5b	58.8b
<b>7a</b>	39.2c	37.9c	38.8c
<b>8</b>	26.7a	25.8a	26.3a
<b>9–12</b>	50.1d	47.9d	47.1d

<sup>a</sup> Values with different letters within the same column are significantly different at  $P = 0.05$ .

**Table 3.** Hydrolysis Rate Constant ( $k_{\text{obsd}}$ ) of DP 6 Isomers, **7a**, **8**, and **9–12** Fructooligosaccharides in Onion Bulb Tissues Stored for 6 Months at Different Temperatures

	$k_{\text{obsd}}$ <sup>a</sup> (1/week) at		
	10 °C	15 °C	20 °C
<b>6b</b>	$55 \times 10^{-3}$ a	$53 \times 10^{-3}$ a	$53 \times 10^{-3}$ a
<b>6c + 6d<sub>1</sub> + 6d<sub>2</sub></b>	$71 \times 10^{-3}$ b	$65 \times 10^{-3}$ b	$68 \times 10^{-3}$ b
<b>7a</b>	$74 \times 10^{-3}$ b	$68 \times 10^{-3}$ b	$72 \times 10^{-3}$ b
<b>8</b>	$80 \times 10^{-3}$ c	$73 \times 10^{-3}$ bc	$76 \times 10^{-3}$ bc
<b>9–12</b>	$85 \times 10^{-3}$ d	$75 \times 10^{-3}$ c	$92 \times 10^{-3}$ d

<sup>a</sup> Values with different letters within the same column are significantly different at  $P = 0.05$ .

**Table 4.** Half-Lives ( $t_{1/2}$ ) of DP 6 Isomers, **7a**, **8**, and **9–12** Fructooligosaccharides in Onion Bulb Tissues Stored for 6 Months at Different Temperatures

	$t_{1/2}$ <sup>a</sup> (weeks) at		
	10 °C	15 °C	20 °C
<b>6b</b>	12.6a	13.1a	13.1a
<b>6c + 6d<sub>1</sub> + 6d<sub>2</sub></b>	9.8b	10.7b	10.2b
<b>7a</b>	9.3b	10.2c	9.6c
<b>8</b>	8.7c	9.5d	9.1b
<b>9–12</b>	8.2d	9.3e	7.5d

<sup>a</sup> Values with different letters within the same column are significantly different at  $P = 0.05$ .

percentage of hydrolysis of one tetra-FOS isomer (nystose) varies considerably (from 20 to 90%) in six varieties of onion bulbs stored at 0 °C for 6 months (12). However, these authors reported a large variation of FOS contents of the cultivars studied, and after 6 months of storage, the FOS contents of three cultivars varied positively, whereas they varied negatively in three other cultivars. In our present (and previous) results, we did not observe this, because FOS were assessed directly using a HPAEC-PAD method and purified standards, rather than enzymatic analysis of total fructan. L'Homme et al. (10) also reported that hydrolysis of nystose in mineral-buffered aqueous solutions was linear and increased with high temperatures and low pH values. Furthermore, FOS hydrolysis in vitro might be different from that occurring in bulb tissues, because onion bulbs go through different physiological stages during storage, which are characterized by different metabolic reactions concerning FOS (11, 21).

**Hydrolysis Rate Constant and Half-Life Times.** The hydrolysis rate constant ( $k_{\text{obsd}}$ ) and the half-life time ( $t_{1/2}$ ) are shown in **Tables 3** and **4**, respectively. The  $k_{\text{obsd}}$  showed increasing values from  $53 \times 10^{-3}$  to  $84 \times 10^{-3}$  week<sup>-1</sup>. These values increased significantly with the increase of degree of polymerization of FOS, and DP **9–12** showed a hydrolysis

**Table 5.** Percentage of Hydrolysis, Consumption Rate, Hydrolysis Rate Constant ( $k_{\text{obsd}}$ ), and Half-Lives ( $t_{1/2}$ ) of DP 6 Isomers, **7a**, **8**, and **9–12** Fructooligosaccharides in Onion Bulb Tissues after 2 Months at Different Temperatures

	hydrolysis <sup>a</sup> (%) at			consumption rate <sup>a</sup> ( $\mu\text{g/g}$ per week) at			$k_{\text{obsd}}$ <sup>a</sup> (1/weeks) at			$t_{1/2}$ <sup>a</sup> (weeks) at		
	10 °C	15 °C	20 °C	10 °C	15 °C	20 °C	10 °C	15 °C	20 °C	10 °C	15 °C	20 °C
<b>6b</b>	24.4a	7.4a	62.2a	8.3a	1.7a	21.3 <sup>a</sup>	$12 \times 10^{-3}$ a	$7 \times 10^{-3}$ a	$41 \times 10^{-3}$ a	59.5a	96.6a	17.1 <sup>a</sup>
<b>6c + 6d<sub>1</sub> + 6d<sub>2</sub></b>	66.3b	31.4b	60.6ad	48.3b	22.9b	44.2b	$45 \times 10^{-3}$ b	$16 \times 10^{-3}$ b	$39 \times 10^{-3}$ a	15.3b	44.1b	17.9a
<b>7a</b>	55.8c	42.5c	42.5b	26.3c	20.1b	20.1a	$34 \times 10^{-3}$ c	$23 \times 10^{-3}$ c	$23 \times 10^{-3}$ b	20.4c	30.1c	30.1b
<b>8</b>	10.7d	22.7d	8.0c	3.3d	7.1c	2.5c	$7 \times 10^{-3}$ d	$11 \times 10^{-3}$ d	$9 \times 10^{-3}$ c	95.4d	64.7d	74.6c
<b>9–12</b>	55.1c	60.9e	57.32d	37.3	35.1d	39.2d	$33 \times 10^{-3}$ c	$39 \times 10^{-3}$ e	$35 \times 10^{-3}$ d	20.8c	17.7e	19.6a

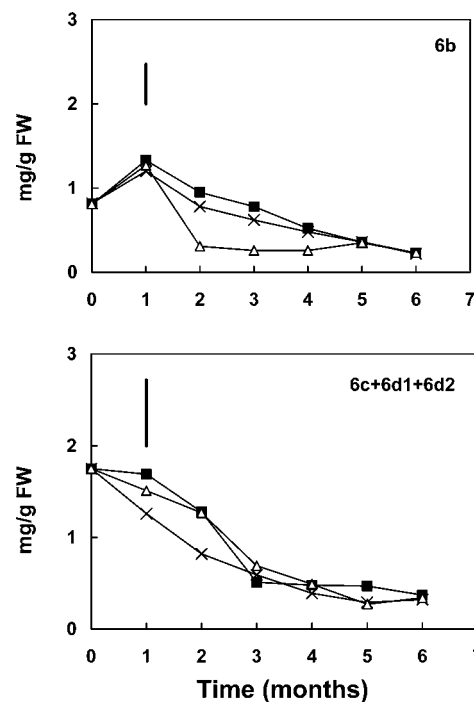
<sup>a</sup> Values with different letters within the same column are significantly different at  $P = 0.05$ .

constant rate 50% higher than that of DP 6 isomers. On the other hand, the half-life of the DP 6 isomers, **7a** and **8**, showed similar kinetics, with  $t_{1/2}$  of 8.3 and 13.1 weeks, whereas higher DP FOS (**9–12**) have shorter half-lives of only 2 months (**Table 4**). These results confirm that high DP FOS are primarily catabolized independent of temperature, balancing the content of other hydrolyzed low DP FOS.

Because of the importance of the first 2 months of storage life, during which dormancy release leads to onset of sprouting, the percentage of hydrolysis, consumption rate, hydrolysis rate constant ( $k_{\text{obsd}}$ ), and half-lives ( $t_{1/2}$ ) of DP 6 isomers, **7a**, **8**, and **9–12** FOS were characterized at 2 months of storage. Surprisingly, hydrolysis was >40% after only 2 months for almost all FOS (**Table 5**). Similarly, the consumption rate was also high and the half-life time somewhat short, particularly for DP 6 isomers, **7a**, and **9–12**. Considering DP **9–12** FOS because they exhaust continuously to lower DPs, we note that after 2 months, the percentage of hydrolysis is two-thirds of final hydrolysis, the consumption rate is close to that observed after 6 months, and the half-life times were only 1 time lower than those estimated after 6 months. This suggests that FOS exhaust more rapidly during the first 2 months of storage, due to dormancy release and sprout development.

Furthermore, it is pertinent to note that hydrolysis of DP **9–12** does not lead exclusively to the formation of lower DP FOS, assuming that these low DP FOS are also hydrolyzed to maintain a balance among the FOS. In fact, these biochemical changes do not respond exclusively to “chemical” balance, but they also are under the influence of other biological and physiological parameters, for example, osmotic pressure and intra- and intercellular fluxes. However, our results were close to that observed in vitro as discussed below.

To our knowledge, no previous investigation of these parameters has been conducted in either onion bulb or other crop tissues. Nevertheless, in vitro study showed that  $k_{\text{obsd}}$  was temperature- and pH-dependent, whereas higher polymerized FOS showed shorter half-lives whatever the temperature and pH of incubation (10). These studies demonstrated that FOS degradation in aqueous media of low DP (3–5) follows pseudo-first-order kinetics and takes place mainly at acidic pH rather than neutral or basic values, in agreement with our results. It also reported that the pH of dry onion bulb (DM of 13–14%) is somewhat acid, ranging between 5.8 and 6.2 (personal data), whereas intracellular pH values in higher plants usually fall below 5.5 and undoubtedly reflect the pH of the vacuoles (22) where hydrolysis and synthesis of fructans takes place. It was previously found that acid hydrolysis of five different FOS samples showed that the fructose production rate was much more important for the smaller oligomers (low DP) (9), whereas the inverse process was observed in vivo (onion tissues). These conflicting results make it difficult to compare in vitro hydrolysis

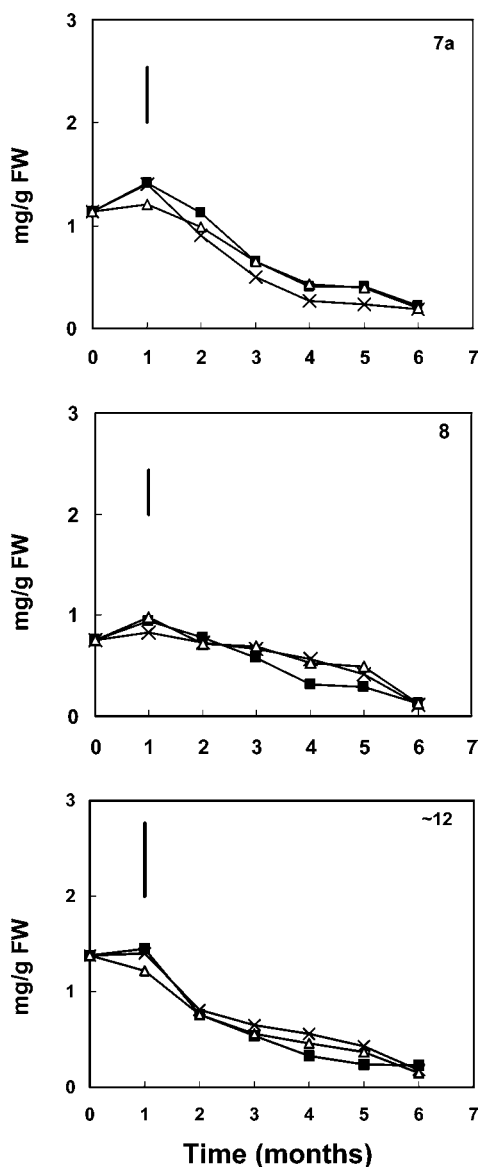


**Figure 2.** Variation of hexa-fructooligosaccharide isomers in onion bulbs during storage at 10 (×), 15 (Δ), and 20 °C (■). Vertical bar represents LSD at  $P = 0.05$ .

of FOS with in vivo results, even though chemical results might reveal effects such as pH and temperature. These conflicting results observed in vivo could probably reflect roles of fructans other than carbohydrate reserve in fructan-containing plants and the complexity of their metabolism in vivo (23, 24).

**FOS Variation.** The variation of DP 6 FOS isomers content is shown in **Figure 2**. Within the first month, **6b** content increased to 1.20, 1.33, and 1.27 mg/g of FW at 10, 15, and 20 °C, respectively. Afterward, at 20 °C **6b** decreased sharply during the second month to 0.31 mg/g of FW and remained stable during the last 4 months, ranging between 0.23 and 0.35 mg/g of FW. At 10 and 15 °C, **6b** decreased progressively from month 1 to month 6 to 0.22 and 0.23 mg/g FW, respectively, and the content of **6b** was significantly different after 2 months at 20, 10, and 15 °C. On the other hand, DP **6c + 6d<sub>1</sub> + 6d<sub>2</sub>** decreased abruptly within the first 3 months from 1.75 to 0.59, 0.51, and 0.69 mg/g of FW at 10, 15, and 20 °C, respectively. In contrast, during the last 3 months DP **6c + 6d<sub>1</sub> + 6d<sub>2</sub>** remained stable, ranging between 0.32 and 0.39 mg/g of FW, between 0.37 and 0.48 mg/g of FW, and between 0.29 and 0.93 mg/g of FW at 10, 15, and 20 °C, respectively. However, no significant difference was observed among temperatures during the 6 months of storage.

As shown in **Figure 3**, variation of DP **7a**, **8**, and **9–12** FOS was close to that of DP 6 FOS isomers. DP **7a** increased slightly



**Figure 3.** Variation of DP 7a, 8, and 9–12 fructooligosaccharides in onion bulbs during storage at 10 (x), 15 (Δ), and 20 °C (■). Vertical bar represents LSD at  $P = 0.05$ .

during the first month from 1.13 to 1.40, 1.14, and 1.20 mg/g of FW at 10, 15, and 20 °C. After that, 7a started decreasing progressively during the last 5 months, and final contents were 0.19, 0.22, and 0.20 mg/g of FW at 10, 15, and 20 °C, respectively. Similarly, no significant difference was noted among temperatures during the 6 months of storage.

DP 8 FOS showed a similar pattern independent of temperature regime: they increased slightly within the first month from 0.75 to 0.83, 0.94, and 0.98 mg/g of FW at 10, 15, and 20 °C, respectively. From the second month, DP 8 began decreasing progressively and continued to decrease up to month 6. The final contents of DP 8 were 0.11, 0.13, and 0.12 mg/g of FW at 10, 15, and 20 °C, respectively, and during the 6 months of storage no significant difference of DP 8 contents was observed among temperature regimes.

DP 9–12 FOS also showed a similar pattern, particularly to that of DP 6c + 6d<sub>1</sub> + 6d<sub>2</sub>. They decreased sharply within the first 2 months from 1.88 to 0.81, 0.76, and 0.76 mg/g of FW at 10, 15, and 20 °C, respectively. During the last 4 months, they continued to decrease slowly, reaching a final content of 0.18, 0.24, and 0.25 mg/g of FW at 10, 15, and 20 °C, respectively.

Few studies have investigated the variation of the different FOS in onion bulb tissues during different storage temperatures (4, 11, 12). To date, only one study has reported the variation of lower DP FOS and their kinetic parameters in onion bulbs during storage under different temperature regimes (13). However, despite the technological importance of FOS in storage and quality attributes of onion bulbs, no investigations have taken into account the distribution and the variation of the different high DP FOS, especially the numerous isomers considered by this investigation. Jaime et al. (12) reported that low DP FOS (DP 4 and one unidentified isomer penta-FOS named GF<sub>4</sub>) decreased significantly in onion bulb tissues of five cultivars after 6 months of storage at 0 °C. However, the results of our other studies (4, 11) showed that storage duration of onion bulbs at 10 and 20 °C significantly affected the total FOS independent of temperature. Moreover, FOS hydrolysis in onion bulbs has not yet been adequately explained in terms of biochemical and enzymological pathways. Undoubtedly, the postharvest catabolism of FOS in onion tissues, such as in other roots and tubers, is unique in terms of both reaction products and the enzymes that catalyze their degradation, but unfortunately this significance is not yet clear.

**Conclusion.** This study suggests that higher DP FOS are hydrolyzed preferentially to lower ones. These compounds play a major role as reserve carbohydrate, providing the end substrates of the catabolic activity and sprout development during regrowth (sprouting). Our results show that DP 9–12 FOS are preferentially and primarily hydrolyzed, and this could maintain a steady state of low DP FOS in the tissues. Furthermore, it was observed that the hydrolysis of the different high DP FOS occurred mainly during the first 2 months, whereas the increases observed during the first 2 months at 15 and 20 °C are due to the production of new DP 6 FOS arising from the hydrolysis of higher DP FOS at higher temperatures. The results obtained here could allow estimation of the potential degradation of the high DP FOS observed during storage.

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