Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 33–37

Tetra- and penta-fructooligosaccharide (FOS) isomers assessment in onion bulb tissues: effect of temperature and storage time

Noureddine Benkeblia, Natsuko Takahashi, Keiji Ueno, Shuichi Onodera and Norio Shiomi*

Department of Food and Nutrition Sciences, Graduate School of Dairy Science Research, Rakuno Gakuen University, 582 Bunkyodai Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

Received 12 October 2004; accepted 17 November 2004
Available online 25 December 2004

Abstract—Tetra- and penta-fructooligosaccharide (FOS) isomers and their hydrolysis parameters—% hydrolysis, hydrolysis rate constant (k_{obs}) and half-lifes ($t_{1/2}$) in onion bulb tissues kept for 6 months at 10, 15, and 20 °C, were investigated. The hydrolysis of the tetra- and pentaFOS isomers ranged between 67% and 86% and isomers of b type **4b** and **5b** were hydrolyzed preferentially. The k_{obs} showed large variation from 51×10^{-3} week^{-1} (lowest value) to 82×10^{-3} week^{-1} (highest value), while the half-life $t_{1/2}$ of the isomers ranged between 8.5 and 16.6 weeks. DP 4 content showed close correlation at 15 and 20 °C but different at 10 °C. At 10 °C, DP 4 isomers decreased sharply during the first 2 months but remained stable during the last 4 months. At 15 and 20 °C, DP 4 increased slightly within the first month, and then decreased during the second month. During the last 4 months, the **4a** isomer remained stable, while **4b** and **4c** increased slightly. PentaFOS isomers varied in a similar fashion to tetraFOS isomers. At 10 °C, DP 5 isomers decreased sharply during the first 2 months and remained stable during the last 4 months except for a slight increase observed during month 3 for **5b** and **5c** + **5d**. At 15 and 20 °C, they showed a similar pattern to those of the tetra-isomers. It was concluded that pentaFOS isomers are preferentially hydrolyzed with respect to tetraFOS isomers, because they have a relatively high content of fructosyl ends chain. Results also allow us to distinguish that the DP *x*b (type b) isomers are preferentially hydrolyzed.

1. Introduction

About 80% of bulb dry matter consists of non-structural carbohydrates.[1] The predominant of these non-structural carbohydrates are glucose, fructose, sucrose, and low-molecular-weight fructans, while starch and raffinose are absent.[1,2] Fructans, also known as fructooligosaccharides (FOS), polyfructosylsucroses of varying molecular size, are the main carbohydrate reserve of onion, as well as of other vegetative organs and plants including alliaceous organs (bulbs). Fructans accumulate during bulbing, then are catabolized during the regrowth and the sprout development of the bulbs.[3]

Tetra-fructooligosaccharides isomers (DP 4), that is, nystose **4a** [1,1-kestotetraose], **4b** [1,6G-kestotetraose] and **4c** [1 and 6G-kestotetraose]; and penta-fructooligosaccharide isomers (DP 5), that is, **5a** [1,1,1-kestopentaose], **5b** [1,1,6G-kestopentaose], **5c** [1,1- and 6G-kestopentaose] and **5d** [1 and 1,6G-kestopentaose] constitute a part of the different FOS found in onion bulb tissues and their contents vary between 9% and 11%, and 7% and 9% of total carbohydrates for DP 4 and DP 5, respectively.[2,4,5] During the past decade, FOS have received considerable interest as food ingredients. They are used as non-digestible dietary fiber and texturing properties in many foodstuff.[6–8]

Some studies have been focused on the FOS properties with respect to their polydispersities, but 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 important to determine the in vivo kinetics of

* Corresponding author. Tel.: +81 11 388 4754; fax: +81 11 387 5848; e-mail: n-shiomi@rakuno.ac.jp

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.11.036

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] while no one investigated the kinetic of hydrolysis of these isomers during storage.

The present work is devoted to the study of the in vivo hydrolysis of three tetra- and four pentaFOS isomers during storage under three temperature regimes.

2. Results and discussion

2.1. Hydrolysis rate

The hydrolysis of the tetra- and pentaFOS isomers is shown in Table 1. The hydrolysis of the DP 4 isomers ranged from 67% to 81%, while it ranged from 70% to 86% for DP 5 isomers. Comparatively, it is interesting to note that isomers of type b (**4b** and **5b**) were hydrolyzed preferentially particularly at 15 and 20 °C.

Table 1. Percentage of hydrolysis (%) of the tetra- and penta-fructooligosaccharide isomers in onion bulb tissues after 24 weeks

	Hydrolysis (%)		
Temperature (°C)	10	15	20
Nystose **4a**	71	63	75
4b	71	72	81
4c	67	70	79
5a	82	80	77
5b	75	86	81
5c + **5d**	70	70	78

Previous investigations showed that the percentage of hydrolysis of one tetraFOS isomer (nystose) vary considerably (from 20% to 90%) in six varieties of onion bulbs stored at 0 °C for 6 months.[12] L'Homme et al.[10] also reported that hydrolysis of nystose in mineral-buffered aqueous solutions followed straight lines, and increased with high temperatures and low pH values.

2.2. Hydrolysis rate constant and half-life times

The hydrolysis rate constant (k_{obs}) and the half-lifes ($t_{1/2}$) are shown in Tables 2 and 3, respectively. The k_{obs} shown large variation from 51×10^{-3} week^{-1} (lowest value) to 82×10^{-3} week^{-1} (highest value). However, we have noted that **4b** and **5b** isomers showed the highest hydrolysis rates among the DP 4 and the DP 5 isomers, respectively. The half-lifes of the isomers are shown in Table 3, and we noted that $t_{1/2}$ varied from 8.5 to 16.6 weeks, with similar observation where **4b** and **5b** isomers showed shortest time among the DP 4 and DP 5 isomers, respectively. This indicates that probably the affinity of the hydrolyzing enzymes for the DP *x*b isomers is higher and these last are preferably hydrolyzed.

Table 2. Hydrolysis rate constant (k_{obs}) of the tetra- and penta-fructooligosaccharide isomers in onion bulb tissues after 24 weeks

	k_{obs} (week^{-1})		
Temperature (°C)	10	15	20
Nystose **4a**	51×10^{-3}	42×10^{-3}	57×10^{-3}
4b	61×10^{-3}	54×10^{-3}	68×10^{-3}
4c	46×10^{-3}	42×10^{-3}	58×10^{-3}
5a	71×10^{-3}	53×10^{-3}	61×10^{-3}
5b	58×10^{-3}	82×10^{-3}	69×10^{-3}
5c + **5d**	51×10^{-3}	50×10^{-3}	62×10^{-3}

Table 3. Half-lifes ($t_{1/2}$) of the tetra- and penta-fructooligosaccharide isomers in onion bulb tissues after 24 weeks

	$t_{1/2}$ (weeks)		
Temperature (°C)	10	15	20
Nystose **4a**	13.5	16.6	12.2
4b	11.3	13	10.2
4c	15	16.5	12
5a	10	13	11.4
5b	12	8.5	10
5c + **5d**	13.7	14	11

To the best of our knowledge, no investigation has been carried out in these terms in either onion bulb or other crops tissues. Nevertheless, in vitro study showed that k_{obs} was temperature and pH dependent, while higher polymerized FOS showed shorter half-lifes whatever the temperature and pH of incubation.[10]

2.3. Tetra- and pentaFOS isomers variation

2.3.1. TetraFOS variation. The variation of the tetraFOS isomers content is shown in Figure 1. Within the first month, DP 4 content showed close correlation at 15 and 20 °C but was different at 10 °C. At 10 °C, DP **4a**, **4b**, and **4c** decreased sharply during the first 2 months to 0.20, 0.46, and 0.40 mg g^{-1} FW, respectively. Afterwards, they remained stable over 4 months ranging between 0.28 and 0.40, 0.59 and 0.86, and, 0.54 and 0.72 mg g^{-1} FW, respectively; although a slight increase was noted during month 3 especially for **4b**. At 15 and 20 °C, DP 4 increased slightly within the first month, and then decreased during the second month to 0.18 and 0.16, 0.33 and 0.30, and, 0.24 and 0.23 mg g^{-1} FW for **4a**, **4b**, and **4c**, respectively. However, **4a** remained stable during the last 4 months at 15 and 20 °C, while **4b** and **4c** increased slightly, ranging between 0.82 and 1.07, and 0.69 and 0.81 mg g^{-1} FW at 15 °C; and, 0.61 and 0.91, and, 0.48 and 0.71 mg g^{-1} FW at 20 °C, respectively.

2.3.2. PentaFOS variation. As shown in Figure 2, variation of pentaFOS isomers was very close to those of tetraFOS isomers. At 10 °C, penta-isomers decreased sharply during the first 2 months and remained stable during the last 4 months except for a slight increase observed during month 3 for **5b** and **5c** + **5d**. At 15 and 20 °C, penta-isomers showed a similar pattern to those of tetra-isomers: they increased slightly within the first

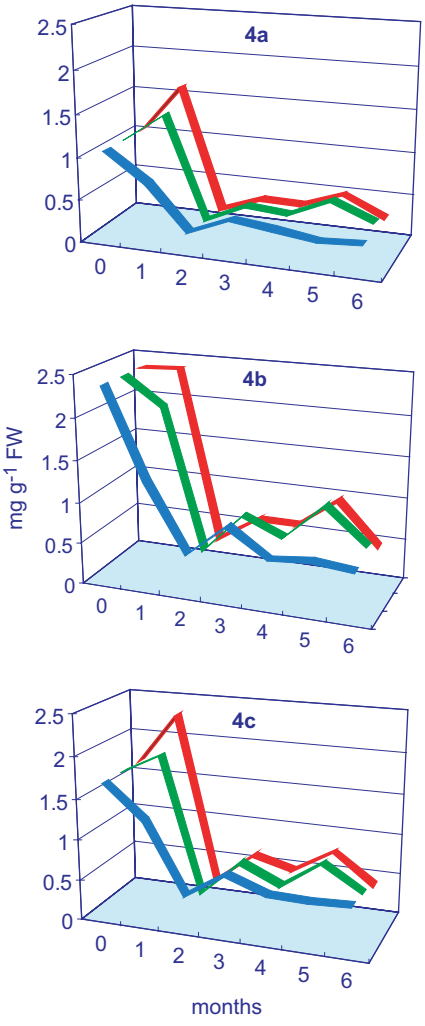

Figure 1. Variation of tetra-fructooligosaccharide isomers content in onion bulb tissues at 10 (■), 15 (■), and 20 °C (■).

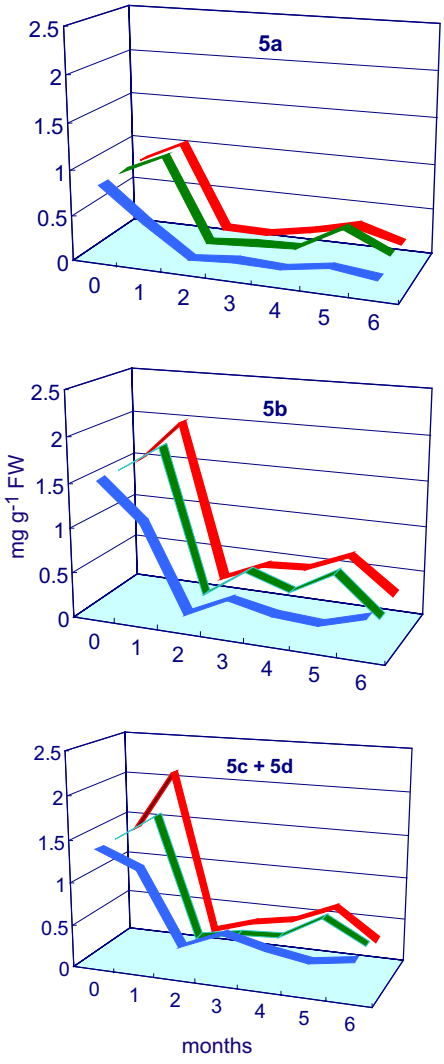

Figure 2. Variation of penta-fructooligosaccharide isomers content in onion bulb tissues at 10 (■), 15 (■), and 20 °C (■).

month, and then they decreased during the second month to 0.13 and 0.12, 0.14, and 0.19, and, 0.28 and 0.21 mg g^{-1} FW for **5a**, **5b** and **5c** + **5d**, respectively. During the last 4 months, penta-isomers increased slightly especially during month 5, but they remained however lower than the values of the beginning of storage, ranging from 0.23 to 0.46 at 15 °C and 0.19 to 0.32 mg at 20 °C, 0.21 to 0.63 at 15 °C, and 0.29 to 0.67 mg at 20 °C, and, 0.38 to 0.68 at 15 °C and 0.31 to 0.65 mg at 20 °C for **5a**, **5b**, and **5c** + **5d**, respectively (Fig. 3).

A few studies have investigated the variation of the different FOS during the keeping under different temperatures of onion bulb tissues,[4,11,12] however none take into account the distribution and the variation of the different isomers particularly the numerous isomers considered by this investigation. Jaime et al. reported that nystose **4a** and one pentaFOS (which isomer of GF_4 was not identified) decreased significantly in onion bulb tissues of five cultivars after 6 months storage at 0 °C. However, the results of our other studies,[4,11] although they did not show the different isomer variations, are in agreement with the present results and they showed that keeping onion bulbs under 10 and 20 °C affected significantly the total FOS among them tetra- and pentaFOS.

3. Conclusions

In summary, in this study and with regard to the different hydrolysis parameters, it is suggested that higher oligomers (pentaFOS isomers) are more hydrolyzed than the lower oligomers (tetraFOS isomers) because they have a relatively high content of fructosyl end chains. On the other hand, results allow us to distinguish that the isomers DP *x*b (type b) are preferentially hydrolyzed. Furthermore, it appeared that the hydrolysis of the different isomers took place mainly during the first 2 months, while the increases observed during the last 3 months are due to the production of new tetra- and pentaFOS isomers provided by higher DP-FOS. The results obtained here could allow estimation of the potential degradation of the tetra- and pentaFOS isomers met in crops during storage. However, further investigations are needed to estimate these parameters either for other FOS isomers especially highly polymerized FOS found

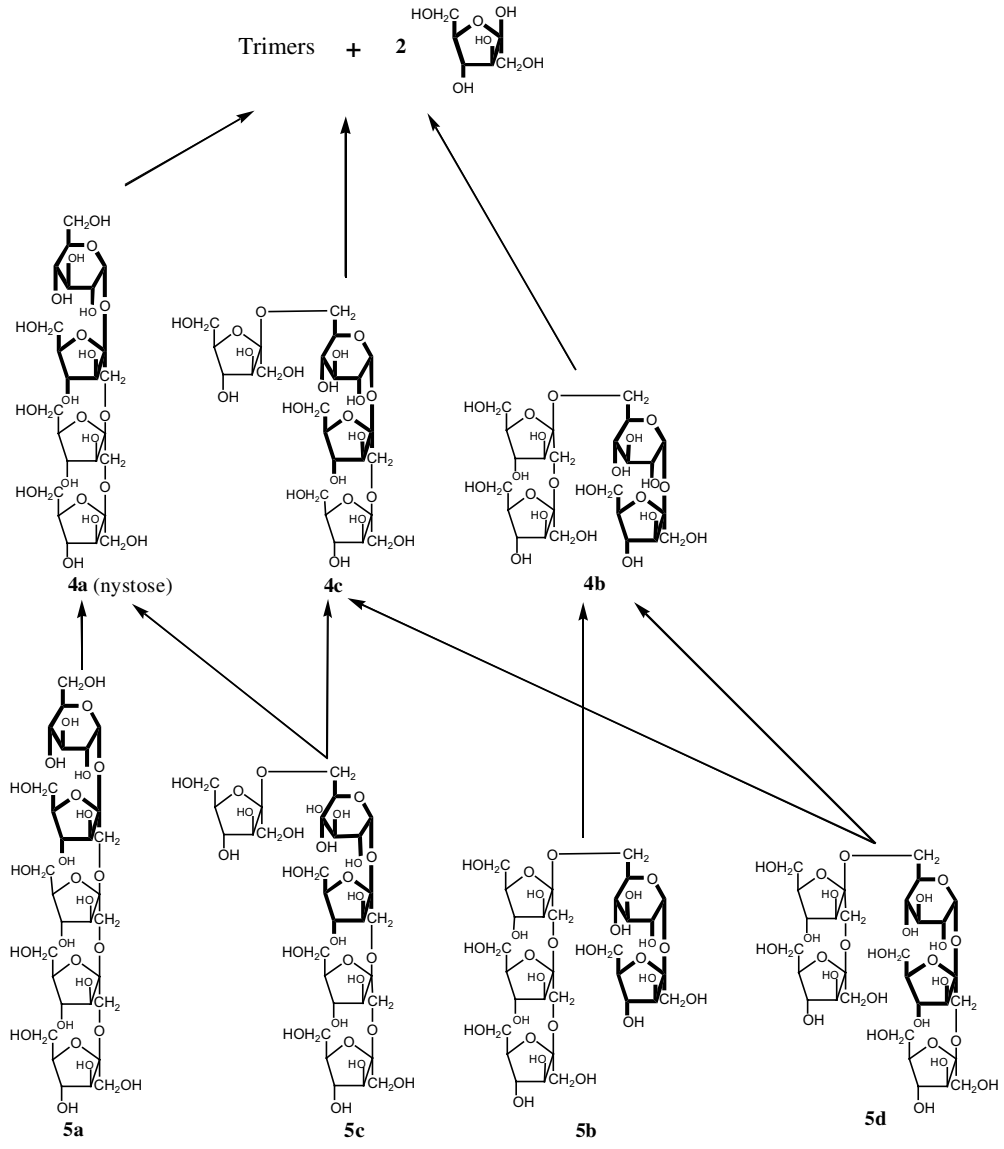

Figure 3. Hydrolysis pathway of the tetra- and penta-fructooligosaccharide isomers.

in onion, or in other crops because their compositions might be considerably different.

4. Experimental

4.1. Plant material

Onion bulbs *Allium cepa* cv. Tenshin (summer cultivar), which had been freshly harvested and dried in the field for 2 weeks were obtained from the local farm of the university, Ebetsu, Hokkaido, Japan. The bulbs were sorted for uniformity and absence of defects, packed in commercial plastic (PVC) trays of 12 kg each. Five trays were kept in store.

4.2. 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) at 10 ± 0.1 °C and $65 \pm 1\%$ relative humidity, and 15 ± 0.1 °C and $45 \pm 1\%$ RH. For the higher temperature regime, 20 °C, onions were placed in a ventilated room with an average RH of 50%.

4.3. Fructooligosaccharides standards

Nystose **4a** [1^F(1-β-D-fructofuranosyl)$_2$ sucrose, 1,1-kestotetraose] was previously prepared in the laboratory as described by Takeda et al.[13] **4b** [6^G(1-β-D-fructofuranosyl)$_2$ sucrose 1, 6G-kestotetraose], **4c** [1^F,6^G-di-β-D-fructofuranosyl sucrose, 1 and 6G-kestotetraose], **5a** [1^F(1-β-D-fructofuranosyl)$_3$ sucrose, 1,1,1-kestopentaose), **5b** [6^G(1-β-D-fructofuranosyl)$_3$ sucrose, 1,1,6G-kestopentaose], **5c** {1^F(1-β-D-fructofuranosyl)$_2$-6^G-β-D-fructofuranosyl sucrose, 1,1 and 6G-kestopentaose], **5d** [1^F-β-D-fructofuranosyl-6^G(1-β-D-fructofuranosyl)$_2$ sucrose, 1 and 1, 6G-kestopentaose] were obtained from

asparagus roots as described in previous papers.[14–16] Because the fructans nomenclature is not simple since the structures are very complex, 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%).

4.4. Estimation of degradation parameters

4.4.1. Percentage of hydrolysis. The percentage of hydrolysis of the isomers was estimated after 24 weeks by the following equation:

$$\text{Hydrolysis (\%)} = \left[\frac{C_f}{C_i} - 1\right] \times 100$$

where C_i is concentration at week 0 and C_f the concentration at week 24.

4.4.2. Hydrolysis rate constant and half-life time. The hydrolysis rate constant (k_{obs}), which showed a pseudo-first-order kinetics according to the models of Logan,[19] is estimated by the following equation:

$$C_f = C_i e^{(-k_{obs}t)}$$

where C_i is the concentration at week 0, C_f the concentration after 24 weeks and t time in weeks.

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

$$t_{1/2} = \ln\left[\frac{0.5}{k_{obs}}\right]$$

4.5. Fructooligosaccharides extraction

Fructooligosaccharides (FOS) were extracted by the method of Shiomi.[20] 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 during 10 min. Then, homogenate was filtered and the residue extracted three times with aqueous ethanol and one time with water in 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 labortechnik AG, Flawil Switzerland). The concentrated sugars were collected in 1 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.

4.6. Fructooligosaccharides analysis

FOS 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 min, 25 mM. System II: 0–1 min, 5 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. 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. FOS are expressed in mg per gram fresh weight (mg/g FW).

Acknowledgements

Financial support from the Japanese Society for the Promotion of Science is gratefully acknowledged.

References

1. Darbyshire, B.; Henry, R. J. *New Phytol.* **1981**, *87*, 249–256.
2. Benkeblia, N.; Varoquaux, P.; Shiomi, N.; Sakai, H. *Int. J. Food Sci. Technol.* **2002**, *37*, 169–176.
3. Darbyshire, B. J. *Hort. Sci.* **1978**, *53*, 195–201.
4. Benkeblia, N.; Onodera, S.; Shiomi, N. *Food Chem.* **2004**, *87*, 377–382.
5. Shiomi, N.; Onodera, S.; Sakai, H. *New Phytol.* **1997**, *136*, 105–113.
6. Spiegel, J. E.; Rose, R.; Karabell, P.; Frankos, V. H.; Schmitt, D. F. *Food Technol.* **1994**, *1*, 85–89.
7. Flamm, G.; Glinsmann, W.; Kritchevsky, D.; Prosky, L.; Roberfroid, M. *Crit. Rev. Food Sci. Nutr.* **2001**, *41*, 353–362.
8. Franck, A. *Br. J. Nutr.* **2002**, *87*(Suppl. 2), S287–S291.
9. Blecker, C.; Fougnies, C.; Van Herk, J. C.; Chevalier, J. P.; Paquot, M. J. *Agric. Food Chem.* **2002**, *50*, 1602–1607.
10. L'Homme, C.; Arbelot, M.; Puigserver, A.; Biagini, A. J. *Agric. Food Chem.* **2003**, *51*, 224–228.
11. Benkeblia, N.; Onodera, S.; Shiomi, N. *J. Sci. Food Agric.*, in press.
12. Jaime, L.; Martín-Cabrejas, M. A.; Mollá, E.; López-Andréu, F. J.; Esteban, R. M. J. *Agric. Food Chem.* **2001**, *49*, 982–988.
13. Takeda, H.; Sato, K.; Kinoshita, S.; Sasaki, H. *J. Ferment. Bioeng.* **1994**, *77*, 386–389.
14. Shiomi, N.; Yamada, J.; Izawa, M. *Agric. Biol. Chem.* **1976**, *40*, 567–575.
15. Shiomi, N.; Yamada, J.; Izawa, M. *Agric. Biol. Chem.* **1979**, *43*, 1375–1377.
16. Shiomi, N. *Phytochemistry* **1981**, *20*, 2581–2583.
17. Lewis, D. H. *New Phytol.* **1993**, *124*, 583–594.
18. Waterhouse, A. L.; Chatterton, N. J. In *Science and Technology of Fructans*; Suzuki, M., Chatterton, N. J., Eds.; CRC: Boca Raton, FL, 1993; pp 1–7.
19. Logan, S. R. *Fundamentals of Chemical Kinetics*; Longman Group: Harlow, 1996.
20. Shiomi, N. *New Phytol.* **1992**, *122*, 421–432.