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Postharvest Biology and Technology 37 (2005) 93–100
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Influence of harvest time and storage temperature on characteristics of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers

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Received 24 August 2004; accepted 1 March 2005

Abstract

Jerusalem artichoke (*Helianthus tuberosus* L.) tubers were harvested 16, 18 and 20 weeks after planting at Kanchanaburi Research Station, Kasetsart University, Thailand. Tuber maturity contributed to changes in inulin characteristics. A decrease in the more polymerised fractions (degree of polymerisation, DP > 10) with an increase in fructose and sucrose composition was observed for late-harvested (20 weeks) tubers. The inulin DP distribution profile from tubers, stored at 2 and 5 °C, significantly changed with increased storage time and temperature. Sucrose and DP 3–10 fractions increased while DP > 10 decreased, particularly after 4–6 weeks of storage. Changes in inulin composition were reflected by formation of a second fructan series, as revealed by HPAEC-PAD chromatograms. These peaks corresponded to inulo-*n*-ose fructan where inulo-*tri*-ose (3′) and inulo-*tetra*-ose (4′) were predominantly found after 2 weeks of tuber storage at 2 and 5 °C. Inulo-*n*-ose (5′) up to DP 17′ increased as a percentage with longer storage time. Tubers in frozen storage of tubers at −18 °C maintained their DP distribution profiles.

Keywords: Jerusalem artichoke; Inulin; Harvest time; Storage temperature; Inulo-*n*-ose; HPAEC-PAD

1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is a native plant of North America and is one of the primary sources for inulin in higher plants. Inulin is a polydisperse fructan which has a degree of polymerisation (DP) 2–60 or higher. The fructosyl units are linked by β (2 → 1) linkages with the end glucose residue (Hoebregs, 1997; Coussement, 1999). Jerusalem artichoke tubers are difficult to store outside the soil because of the rapid onset of rotting. Therefore, the crop must be harvested according to the daily capacity of processing facilities (Frese, 1993). Early-harvested tubers contain a greater amount of highly polymerised sugar fractions, which offer more industrial value than late-harvested tubers or those after storage

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doi:10.1016/j.postharvbio.2005.03.004

(Schorr-Galindo and Guiraud, 1997). Degradation of inulin to sucrose and fructo-oligosaccharides is highest after cold shock. Storage of Jerusalem artichoke tubers at low temperature (4 °C) for 34 days also increases the fructo-oligosaccharide content (Kang et al., 1993).

Pinpong (1997) found that the optimum harvesting stage of Jerusalem artichokes in Thailand was between 18–20 weeks after planting. Weight of fresh tubers increased rapidly over 12–18 weeks. After 20 weeks, loss of weight and firmness, and reduction in specific gravity and carbohydrates of tubers occurred rapidly. The structure of inulin depends upon many factors, such as the plant source from which it is extracted, the climate and growing conditions, the harvesting maturity and the storage time after harvest (De Leenheer and Hoebregs, 1994; Coussement, 1999). The objectives of this study were to assess the influence of harvest time and storage temperature on carbohydrate composition and changes in inulin characteristic profiles in Jerusalem artichoke tubers grown in Thailand. Such information is not available for Jerusalem artichokes grown in the tropical climate of south-east Asia.

2. Materials and methods

2.1. Jerusalem artichoke tubers

Jerusalem artichokes (*H. tuberosus* L.) were planted on November 22, 2001 at Kanchanaburi Research Station, Research and Development Institute for Agricultural Systems Under Adverse Conditions (IASAC), Kasetsart University, Thailand. The average temperature for the growing season was 28 °C. Initial flowering began 12 weeks after planting, and the artichokes were harvested for different maturities at 16, 18, and 20 weeks. All tubers were washed and soaked in 0.038 M sodium hypochlorite for 30 min to eliminate soil and reduce micro-organisms. Some samples were selected for fresh tuber analysis on the harvested date. The remaining tubers were packed in sealed polyethylene bags (0.075 mm thickness) and kept in duplicates at 5, 2, and −18 °C. These tubers were analyzed for inulin composition at 2 weeks intervals.

2.2. Inulin extraction

One-hundred and fifty grams of tuber were taken randomly from each pack and chopped into small pieces with a Waring blender. Inulin was extracted by hot deionized water in a modification of the method of Van Waes et al. (1998). Eighty-five grams of deionized water at 85 °C was added to 11.5 g crushed tubers, and the slurry was shaken at 130 rpm at 85 °C for 1 h in a water bath. After cooling to room temperature, the total weight was adjusted to 100 g with deionized water, and the slurry was then centrifuged for 20 min at 12,000 × *g*. The supernatant was stored at −20 °C until being completely thawed in a water bath at 40 °C before inulin analysis.

2.3. Inulin analysis by HPAEC-PAD

The degree of polymerisation (DP) profile of inulin extracts was analyzed by high-performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD), using a Dionex BioLC (Sunnyvale, CA, USA), equipped with an ED 50-pulsed electrochemical detector with gold, working electrode and silver chloride as a reference electrode. The inulin extracts were diluted to appropriate concentration with deionized water, and filtered through a 0.45 μm Satorius cellulose acetate membrane before injection. The injection volume was 25 μl by autosampler (AS50). A CarboPac PA1 column (2 mm × 250 mm) with guard column was used with two gradient eluents at a flow rate of 0.25 ml min^{-1}. Eluent A was 150 mM sodium hydroxide, while eluent B was 150 mM sodium hydroxide/500 mM sodium acetate. The concentration of sodium hydroxide was kept constant to ensure an optimal pH for the analysis (Van Waes et al., 1998). The gradient condition was programmed to obtain a separation of high-DP inulin, as described below. The system was equilibrated with eluent A for 10 min before analysis. The elution gradient was 0–15 min with 100% eluent A, 15–45 min with linear gradient from 0 to 60% eluent B, 45–90 min with linear gradient from 60 to 90% eluent B, 90–110 min with linear gradient from 90 to 100% eluent B, and 110–120 min with linear gradient from 100 to 0% eluent B. The potential and time periods for the pulsed amperometric detector were: E1, +0.1 V for 400 ms; E2, −2.0 V for 20 ms; E3, +0.6 V for 10 ms; and E4, −0.1 V for 60 ms. Detection potential was 0.1 V. The other potentials were used to clean the electrode. High and low potential were used to eliminate the gold oxide at the surface of the working electrode to avoid electrode fouling (Cataldi et al.,

2000). Relative percentage DP composition of sugars and inulin were calculated based on the peak area from the chromatogram, as integrated by the Chromeleon™, software version 6.2 from Dionex. Quantification of each DP inulin was limited due to the lack of appropriate standard inulin at specific DP.

2.4. Total soluble solids and dry matter

Total soluble solid of tubers was measured by hand refractometer. Dry matter of the tuber was determined by drying in a hot-air oven at 105 °C for 24 h (AOAC, 1990). Each sampling was performed in duplicate.

2.5. Statistical analysis

All statistical analyses were performed, using the SPSS for Windows software version 10. Data were analyzed by multivariate analysis of variances (MANOVA) one-way fixed factor. Duncan's multiple range test was calculated for multiple mean comparisons at a significance level of $*P < 0.05$.

3. Results and discussion

3.1. Effect of harvest time

After flower initiation, Jerusalem artichoke tubers start translocating photosynthetic assimilates from stems to tubers (Meijer et al., 1993; Zubr and Pedersen, 1993). The tubers harvested at a late maturity of 20 weeks had a spongy texture at the base of the stem. The dry matter of tubers increased with maturity from 16 to 20 weeks (Table 1). Carbohydrate content of Jerusalem artichoke tubers is related to dry matter and reaches a maximum at the end of growth (Ben Chekroun et al., 1994). Total soluble solids were about the same in each maturity stage. However, the composition of the sugars was different. Fructose content increased rapidly up to nine-fold in the 20-week tubers compared to those at 16 weeks. Sucrose content increased only slightly in late-maturity tubers. Glucose was more abundant at early maturity and decreased at late maturity. Edelman and Jefford (1968) indicated that fructan synthesis was controlled by sucrose–sucrose fructosyltransferase (SST) and fructan–fructan fructosyltransferase (FFT). SST is the first step of fructan synthesis in growing tubers, using sucrose as the primary source of fructosyl donor and releasing free glucose. Glucose usually appeared in growing tubers and decreased to a very low level in the mature tubers. The increase in free fructose could indicate increased activity of inulinase as the tuber grows older (Limami and Fiala, 1993).

The harvesting time of Jerusalem artichokes also affected the quality of inulin (Table 1). The amount of DP 3–10 was not significantly different within the 16 to 20-weeks maturity period, while the DP 11–20 component decreased in the 20-week tubers. This suggests that inulin composition changed with maturity.

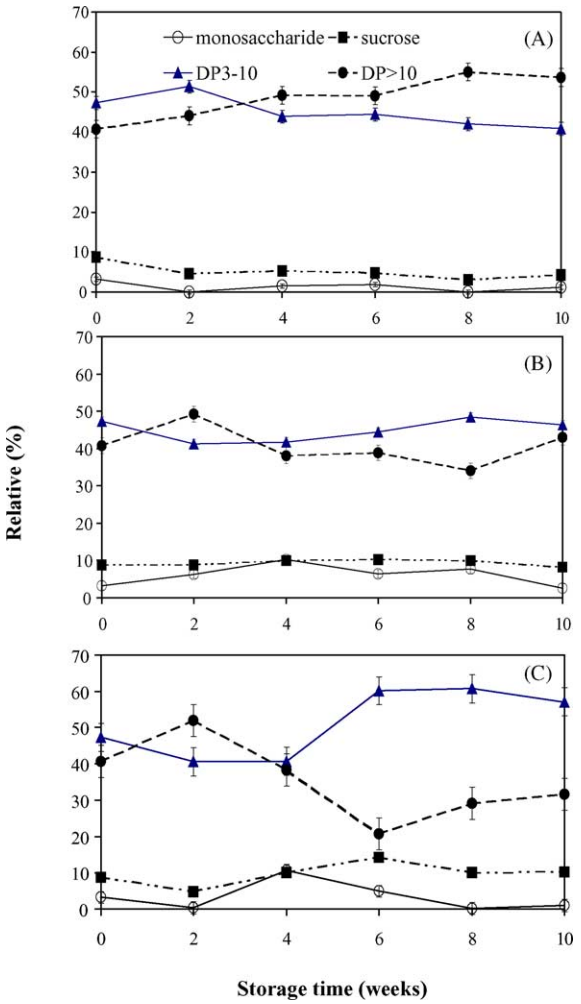

Fig. 1. Relative percentage (mean ± S.D.) of sugars and inulin profiles from 20-week maturity Jerusalem artichoke tubers stored at −18 °C (A), 2 °C (B), and 5 °C (C) for 10 weeks.

Table 1
Dry matter, total soluble solids and relative percentage composition of sugars and inulin of Jerusalem artichoke tubers with different maturity[a]

Composition	Maturity (weeks)		
	16	18	20
Dry matter (%)	19.63[b] ± 0.33	24.77[a] ± 1.38	23.55[a] ± 0.06
Total soluble solid (%)	23.25[a] ± 1.77	22.50[a] ± 0	23.50[a] ± 0.71
Relative %			
Glucose	0.96[a] ± 0.03	0.80[b] ± 0.01	0.26[c] ± 0.06
Fructose	0.34[c] ± 0.04	0.74[b] ± 0.08	3.00[a] ± 1.10
Sucrose	7.51[b] ± 0.35	7.50[b] ± 0.04	8.76[a] ± 0.27
Inulin DP 3–10	47.01[a] ± 0.75	47.15[a] ± 0.04	47.28[a] ± 0.42
DP 11–20	29.19[a] ± 0.28	29.56[a] ± 0.24	26.71[b] ± 0.13
DP 21–30	10.24[a] ± 0.37	9.99[a] ± 0	9.52[a] ± 0.15
DP > 30	4.79[a] ± 0.46	4.30[a] ± 0.18	4.48[a] ± 0.08

Means with different letters in a row are significantly different at $*P < 0.05$ according to Duncan's multiple range test.
[a] All data are the mean S.D. of duplicates.

The decrease in DP 11–20 with increase in free fructose and glucose might be caused by the depolymerisation of fructan by fructan exohydrolase (FEH) (Edelman and Jefford, 1968). It has been shown that FEH exhibits a high affinity for fructan with a DP up to 30 (Bonnett and Simpson, 1993). Ben Chekroun et al. (1994), using HPLC and TLC techniques, found that only the late-maturity tubers had maximum contents of polyfructan. The drying period of Jerusalem artichoke leaves and stems is accompanied by a small increase in reducing sugar, which is due to depolymerisation of high-molecular weight carbohydrate molecules (Schorr-Galindo and Guirand, 1997). Tubers at 16 and 18 weeks contained high-DP fructan, compared to 20 weeks, where inulin depolymerisation may occur. Jerusalem artichoke tubers at 16–18 weeks were preferable for the production of inulin. When considering dry matter content, we found that 18 weeks after planting seemed to be the optimum maturity.

3.2. Effect of storage temperature

Tubers, stored at 2 and 5 °C for 10 weeks were still firm and crisp, and exhibited no sign of spoilage or sprouting. The effect of storage temperature on the distribution profile of inulin DP (Fig. 1) was more pronounced with longer storage time. A gradual increase in sucrose and DP 3–10, and a decrease in DP > 10 fractions were seen in inulin composition extracted from 2 and 5 °C stored tubers, particularly at 5 °C after 4 weeks of storage (Fig. 1(C)). Composition of inulin extracted from −18 ° stored tubers remained stable throughout the storage time (Fig. 1(A)). Most enzymatic and chemical reactions were drastically reduced or stopped at

Table 2
Relative percentage of sugars and inulin composition of 20-week maturity Jerusalem artichoke tubers stored at different temperatures for 10 weeks[a]

Composition (relative %)	Storage temperature (°C)			
	Fresh	−18	2	5
Monosaccharide	3.26[a]	1.26[b]	2.51[ab]	1.05[b]
Sucrose	8.76[b]	4.33[c]	8.22[b]	10.23[a]
Inulin DP 3–10	47.28[b]	40.82[c]	46.33[b]	57.06[a]
DP 11–20	26.71[b]	31.67[a]	27.48[b]	23.64[c]
DP 21–30	9.52[c]	15.29[a]	11.93[b]	6.77[d]
DP > 30	4.48[b]	6.65[a]	3.54[b]	1.27[c]

Means with different letters in a row are significantly different at $*P < 0.05$ according to Duncan's multiple range test.
[a] All data are the mean of duplicates.

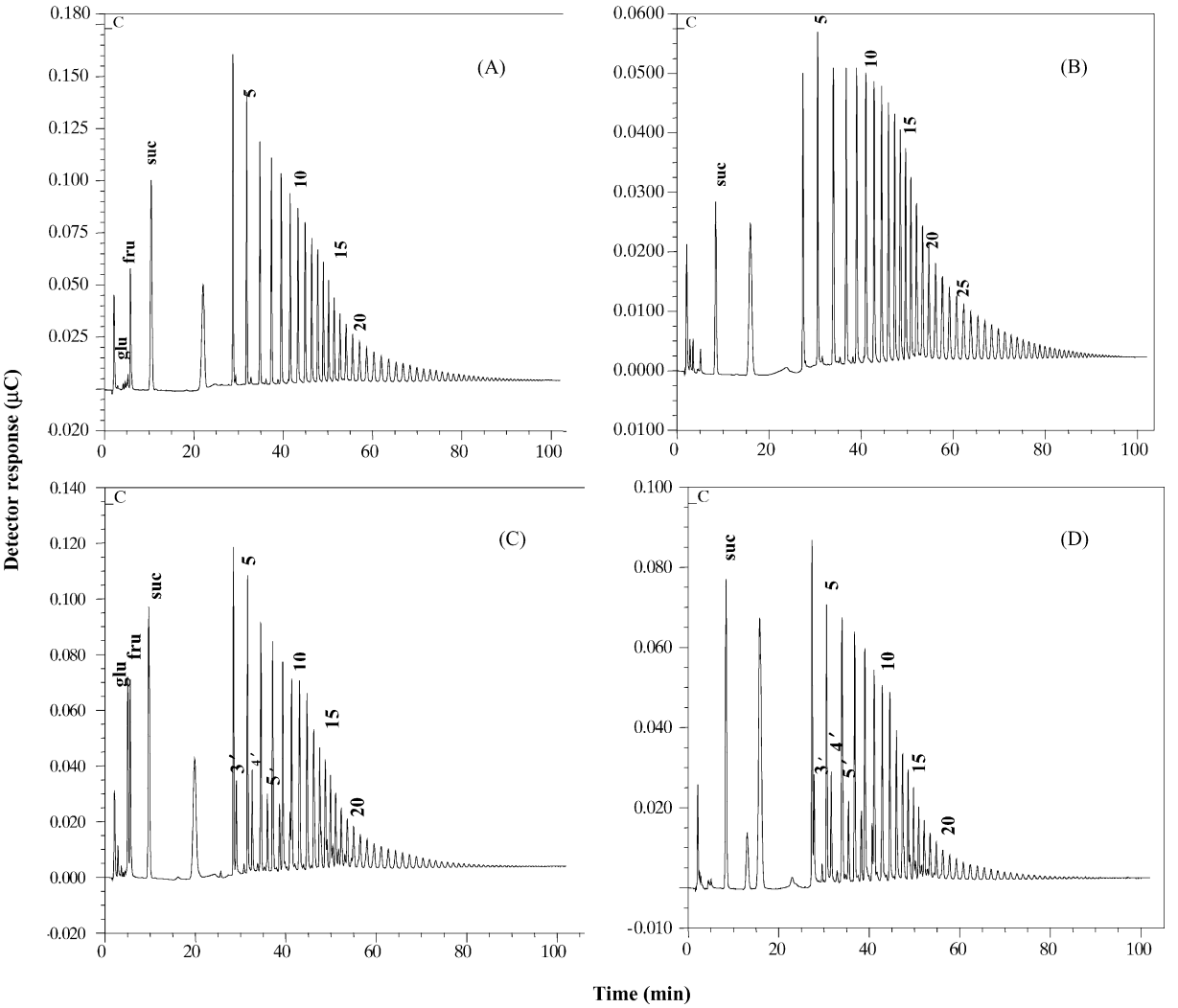

Fig. 2. HPAEC-PAD chromatograms of inulin and new fructan series from 20-week maturity Jerusalem artichoke tubers as fresh tuber (A), and stored at −18 °C (B), 2 °C (C), and 5 °C (D) for 8 weeks. The series of small peaks in (C) and (D) indicate new fructan series. Glu = glucose, Fru = fructose, Suc = sucrose. Number of peak represents DP fractions.

freezing temperatures, while Jerusalem artichoke tissue metabolism could continue at a slow rate, even at 2 °C storage temperature. Cold storage would therefore retard undesirable changes in the inulin characteristics for a certain period of time, e.g. 4 weeks at 5 °C in this study. Frozen storage would maintain Jerusalem artichoke tubers and their inulin quality for a much longer time.

Increases in sucrose and DP 3–10 were found as a result of high storage temperature (Table 2). These changes corresponded with significant decreases in higher DP inulin (DP > 10) and monosaccharide at 5 °C as compared to 2 °C. Modler et al. (1993) also found that higher storage temperature encouraged breakdown of inulin and utilization of monosaccharide formed from the breakdown, presumably due to higher respiration and other metabolic activities. The lower proportion of monosaccharide, sucrose and DP 3–10 in frozen samples (−18 °C), compared to fresh tubers was probably due to drip loss during thawing, which is in

Table 3
Relative percentage of sugars and inulin composition of 20-week maturity Jerusalem artichoke tubers with different storage times at 5 °C[a]

Composition (relative %)	Storage time (weeks)					
	0	2	4	6	8	10
Glucose	0.26^{ab}	2.22^{ab}	3.56^{a}	0.5^{ab}	0^{b}	0.05^{b}
Fructose	3.00^{bc}	2.65^{bc}	7.16^{a}	4.43^{ab}	0.18^{c}	1.01^{bc}
Sucrose	8.76^{ab}	4.90^{b}	10.15^{ab}	14.29^{a}	10.03^{ab}	10.23^{ab}
Inulin DP 3–10	47.28^{c}	40.60^{d}	40.72^{d}	60.14^{a}	60.73^{a}	57.06^{b}
DP > 10	40.71^{b}	51.88^{a}	38.42^{b}	20.66^{d}	29.17^{cd}	31.68^{bc}
DP 11–20	26.71^{ab}	31.89^{a}	24.76^{ab}	16.43^{b}	22.24^{ab}	23.64^{ab}
DP 21–30	9.52^{b}	13.08^{a}	9.17^{b}	3.12^{d}	5.01^{cd}	6.77^{c}
DP > 30	4.48^{b}	6.91^{a}	4.49^{b}	1.11^{d}	1.92^{c}	1.27^{d}

Means with different letters in a row are significantly different at $*P < 0.05$ according to Duncan's multiple range test.
[a] All data are the mean of duplicates.

agreement with the preliminary results found on reductions in total soluble solids. Increase in the high-DP proportion of the −18 °C sample therefore reflected the losses of those low-molecular weight fractions.

3.3. Effect of storage time on inulin DP distribution

The effect of storage time on DP distribution changes can be seen in Fig. 1. Table 3 also demonstrates the effect of storage time at 5 °C upto 10 weeks. The reduction in DP > 10 and the increases in sucrose and DP 3–10 were significant after 4–6 weeks of storage. Therefore, long-term storage would inevitably affect inulin composition, i.e. degradation to shorter chains. In our case, 4-weeks storage time seemed to be the limit at 5 °C in order to maintain high-DP inulin (Fig. 1).

Further examination of the HPAEC-PAD chromatogram revealed that not only was the distribution profile of inulin changed but also new carbohydrates were formed (Fig. 2). Series of small peaks, especially from tubers stored at 20 and 5 °C were found, as illustrated in Fig. 2(C) and (D), while samples stored at −18 °C retained their original chromatographic pattern (Fig. 2(B)). A new second fructan series, DP 2′–DP 5′, was found in fresh Jerusalem artichoke in trace amounts by Ernst et al. (1996). These were characterized as inulo-*n*-ose that contained only β (2 → 1)-linked fructose molecules without an end glucose moiety (Ernst et al., 1996). These inulo-*n*-ose products, for example, 2′ for inulo-*bi*-ose and 3′ for inulo-*tri*-ose, might be derived from large inulin molecules by hydrolysis of terminal glucose or fructose molecules. These new fructans were probably formed during inulin

Table 4
Relative percentage of inulo-*n*-ose as compared to sugar and inulin composition of 16-week maturity Jerusalem artichoke tubers stored at 2 °C and 5 °C upto 12 weeks

	Inulo-*n*-ose (relative %)										
Storage conditions	3′	4′	5′	6′	7′	12′	13′	14′	15′	16′	17′
2 °C											
2 weeks	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4 weeks	1.56^{b}	1.30^{b}	1.01^{b}	0.86^{b}	0.48^{b}	0.15^{b}	0.19^{b}	0.22^{b}	0.22^{b}	ND	ND
12 weeks	4.54^{a}	4.04^{a}	3.31^{a}	2.64^{a}	2.06^{a}	1.04^{a}	0.91^{a}	0.64^{a}	0.46^{a}	0.31	ND
5 °C											
2 weeks	0.50^{c}	0.47^{c}	ND	ND	ND	ND	ND	ND	ND	ND	ND
4 weeks	0.94^{b}	0.97^{b}	0.75^{b}	0.65^{b}	0.35^{b}	ND	0.20^{b}	0.20^{b}	ND	ND	ND
12 weeks	2.91^{a}	2.73^{a}	2.32^{a}	1.94^{a}	1.58^{a}	0.29^{a}	0.90^{a}	0.75^{a}	0.62	0.48	ND

ND = not detected.; means with different letters in a column for each storage temperature and each inulo-*n*-ose are significantly different at $*P < 0.05$ according to Duncan's multiple range test.

mobilization in plant tissue (Ernst et al., 1996). Our findings indicate that the second fructan series occurred in Jerusalem artichoke tubers during cold storage, but not under frozen storage at −18 °C. A previous study (Ernst et al., 1996) by gel permeation suggested that DP 2 (sucrose) and 2′ (inulo-*bi*-ose), DP 3 and 3′, etc. were of the same molecular size. However, their retention times on the HPAEC-PAD chromatogram were quite different; DP 2′ eluted after DP 3 (1-kestose), 3′ eluted after 4 (nystose), and so on (Ernst et al., 1996). Vogel (1993) also reported that sucrose (DP 2) was eluted very much earlier than inulo-*bi*-ose (DP 2′).

Table 4 shows the amount of inulo-*n*-ose designated as DP 3′ up to DP 17′ in relative percentage from tubers kept at 2 and 5 °C up to 12 weeks. Inulo-*n*-ose increased with time of storage. The amount of inulo-*tri*-ose (3′) increased about four-fold from 4 to 12 weeks of storage. Inulo-*tri*-ose (3′) and inulo-*tetra*-ose (4′) were the predominant new fructans found throughout the 12-week study period. Inulo-*tri*-ose (3′) and inulo-*tetra*-ose (4′) were first found after 2 weeks of tuber storage at 5 °C. Higher DP of inulo-*n*-ose was observed as storage time increased. Ernst et al. (1996) found a second fructan DP 2′ upto DP 18′ in chicory stored at 2–4 °C for 3 weeks. However, DP 2′ was not found in this study. There were no second fructan series found in the frozen-stored tubers. Freezing temperatures could cease hydrolytic activity in the tubers, as seen in Fig. 2(B). If the second fructan series is to be minimized, inulin should be extracted before indigeneous hydrolysis occurs. Cold (non-freezing) storage of Jerusalem artichoke tubers could result in degradation of high-DP inulin to short-chain inulin, and the formation of these β (2 → 1) linked fructans without end glucose moiety.

4. Conclusions

Decrease in the DP 11–20 component and increase in fructose and sucrose were seen in the 20-week mature tubers compared with 16–18 weeks, which had higher DP fractions and lower contents of sugar. However, the dry matter, which estimate carbohydrate content or tubers yield, was lower than for late-maturity tubers. Therefore, 18 weeks seems to be the optimum harvest time for locally grown Jerusalem artichokes on the basis of the amount of high-DP inulin and dry matter content. Storage temperature and duration affected the quality and DP distribution profile of the inulin. Increased sucrose and DP 3–10, with decreased DP > 10 fractions were observed in the 2 and 5 °C cold storage tubers after 4–6-weeks storage, whereas inulin composition remained unchanged with frozen storage (−18 °C) for 3 months. A series of second fructan, inulo-*n*-ose appeared in cold storage tubers. Inulo-*n*-ose increased as storage time increased, while inulo-*tri*-ose (3′) and inulo-*tetra*-ose (4′) were predominant throughout the 12-week study period. There were no second fructan series found with frozen storage.

Acknowledgements

This research was financially supported by the Postgraduate Education and Research Development in Postharvest Technology Program, Kasetsart University and the Split-mode Program, National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. Partial support also came from the Graduate School of Kasetsart University. The authors wish to thank the Research and Development Institute for Agricultural Systems under Adverse Conditions (IASAC), Kasetsart University for the kind contribution of Jerusalem artichoke tubers.

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