

Fructan:fructan 1-fructosyltransferase and inulin hydrolase activities relating to inulin and soluble sugars in Jerusalem artichoke (*Helianthus tuberosus* Linn.) tubers during storage

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Revised: 17 November 2016 / Accepted: 25 January 2017 / Published online: 14 February 2017
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Abstract Influences of harvest time and storage conditions on activities of fructan:fructan 1-fructosyltransferase (1-FFT) and inulin hydrolase (InH) in relation to inulin and soluble sugars of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers were investigated. Maturity affected 1-FFT-activity, inulin contents, and inulin profiles of the tubers harvested between 30 and 70 days after flowering (DAF). Decreases in 1-FFT activity, high molecular weight inulin, and inulin content were observed in late-harvested tubers. The tubers harvested at 50 DAF had the highest inulin content ($734.9 \pm 20.5 \text{ g kg}^{-1} \text{ DW}$) with a high degree of polymerization (28% of DP >30). During storage of the tubers, increases in InH activity (reached its peak at 15 days of storage) and gradual decreases in 1-FFT activity took place. These changes were associated with inulin depolymerization, causing decreases in inulin content and increases in soluble sugars. As well, decreasing storage temperatures would retain high inulin content and keep low soluble sugars; and freezing at $-18 \text{ }^\circ\text{C}$ would best retard 1-FFT, InH, and inulin changes.

Keywords Fructan · Inulin content · 1-FFT activity · InH activity · Soluble sugars

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Introduction

Jerusalem artichoke (*Helianthus tuberosus* Linn.) is one of the primary sources for inulin from higher plants. Inulin is a polydisperse fructan which has 2–60 or higher degree of polymerization (DP) with fructosyl units linked by β (2 → 1) linkages and an end glucose residue (AACC 2009; Coussemont 1999). Inulin is used as a raw material for fiber fortification of processed foods, as a prebiotic, a sweetener, substrate for bio-ethanol and in animal feed. In Thailand, this plant is becoming a commercial crop for extraction of inulin as an ingredient in processed foods and health foods. Jerusalem artichoke and chicory have inulin content of $>150 \text{ g kg}^{-1}$ fresh weight basis (FW) and $>750 \text{ g kg}^{-1}$ dry weight basis (DW). The DP of inulin varies according to species, cultivar, production conditions, physiological age, and other factors (De Leenheer 1996). As a health-related supplement, inulin is a prebiotic to stimulate the growth of probiotic microorganisms in the intestinal tract and to suppress putrefactive pathogens. As well, inulin is purported to reduce glucose, cholesterol, phospholipids, and triglycerides in blood (Campbell et al. 1997).

Inulin is synthesized in the plant in two steps according to the model of Edelman and Jefford (1968). The initial step is the conversion of two molecules of sucrose into 1-kestose and glucose by sucrose:sucrose 1-fructosyltransferase (1-SST). In the next step, 1-kestose is converted to higher DP inulin by fructan:fructan 1-fructosyltransferase (1-FFT). Thus, 1-kestose is considered to be an important intermediate for the production of inulin. On the other hand, inulin depolymerization takes place by the action of fructan 1-exohydrolase (1-FEH), releasing free fructose, which in turn is used for sucrose synthesis (Itaya et al. 2002). Therefore, 1-SST, 1-FFT, and 1-FEH are key enzymes in inulin metabolism. Inulin hydrolase (InH) is

localized in the cell's vacuole of Jerusalem artichoke tubers and is developmentally regulated. It is low in growing tubers but increases during dormancy and sprouting. During sucrose depletion, InH activity would increase to breakdown fructans, resulting in fructose, sucrose, kestose, and nystose accumulation (Gupta and Kaur 2000).

Inulin content of Jerusalem artichoke tubers depends on climate, growing conditions, and harvest maturity (Saengthongpinit and Sajjaanantakul 2005). After harvest, the tubers have a short storage life because of rapid onset of rotting. Low temperature storage normally helps reduce inulin losses. For most fleshy products, freezing is not used as it can cause cell damages depending on cultivars, seasons, preconditioning, rate of freezing and others factors (Kays and Nottingham 2007).

Changes in inulin profiles, inulin content, 1-FFT activity, and InH activity in Jerusalem artichoke tubers cultivated in the northeast of Thailand during harvest and low temperature storage have not been fully investigated for maximizing commercial value of the crop. This study aimed to identify suitable harvest time and low temperature storage of Jerusalem artichoke tubers for the highest inulin content, and more broadly to conditions to obtain high DP of inulin to increase the value of the produce and its products.

Materials and methods

Plant materials and harvest time

Jerusalem artichoke (*Helianthus tuberosus*; Accession no. CN52867, Name of accession: PGR-2367, Origin: USSR, Genetic resources: the Plant Gene Resource of Canada) used in this work was obtained from the Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. It had high yield and high inulin content (Pimsaen et al. 2010). Jerusalem artichoke tubers were grown at the Khon Kaen University Farm from July to November, 2010. Tubers were harvested at different maturities; 30, 40, 50, 60 and 70 days after flowering (DAF). All tubers were washed to remove soil and soaked in sodium hypochlorite solution (280 ppm available chlorine) for 30 min to reduce microbial load and then rewashed with distilled water. Tubers of 200 g were sampled on the harvest date for fresh tuber analyses. The tubers were peeled, sliced, and freeze dried before milling with an IKA M 20 Universal mill. Each ground sample was passed through a 150 μm sieve. Prepared samples were vacuum-packed in high density polyethylene (HDPE) bags, then kept in an aluminum-laminated polyethylene bag and stored at $-20\text{ }^{\circ}\text{C}$ for further analyses of inulin profiles, inulin content, soluble sugars, and 1-FFT activity.

Storage temperature

Jerusalem artichokes were again grown at the Khon Kaen University Farm from September 2010 to January 2011 for the storage study. At 50 DAF, the tubers were harvested, disinfected in sodium hypochlorite solution (280 ppm available chlorine) for 30 min, drained, washed, packed and sealed in polyethylene bags (200 g each), and then kept at different temperatures: -18 , 0, 5, 10, 15, and $25\text{ }^{\circ}\text{C}$. The tubers were stored for 0, 5, 10, 15, 20, 25, and 30 days. Fresh tubers were sampled from each pack for the analyses before the rest was freeze-dried, ground, packed, and stored in the same manner as above. The samples were analyzed for 1-FFT activity, InH activity, inulin, fructose, glucose, sucrose, kestose, and nystose.

Enzyme extraction

Enzyme extraction was conducted according to the method of Henson and Livingston (1996). Thawed Jerusalem artichoke tubers (5 g) were homogenized in 30 mL of ice-cold 10 mM sodium phosphate buffer containing 2 mM dithiothreitol (pH 6.5) using an ACE homogenizer (model AM4), at full speed. Homogenates were squeezed through 3 layers of cheesecloth and centrifuged at $10,000g$ for 20 min. This extraction was conducted in triplicate. Supernatants were then collected and brought to 100 mL with extraction buffer. Aliquots of 12 mL were concentrated to 1 mL via centrifugation at $3000g$ using an Amicon Ultra15-10,000 NMWL filter (Amicon Bioseparation, Millipore, Bedford, MA, USA). Concentrates were diluted to 10 mL with extraction buffer and re-concentrated twice in the same manner. The extracts were used for enzyme assays.

1-FFT activity assay

1-FFT activity was assayed according to the method of Ishiguro et al. (2010). One unit of 1-FFT activity is defined as the amount of the enzyme which catalyzes the fructosyl transfer from 1-kestose to another 1-kestose to synthesize 1 nmol of nystose in 1 min under the following conditions. A mixture of enzyme (100 μL), 0.05 g kg^{-1} 1-kestose solution (200 μL), and McIlvaine buffer (pH 5.5, 200 μL) was incubated at $30\text{ }^{\circ}\text{C}$ for 3 h. The reaction was stopped in boiling water for 10 min. The activity of 1-FFT was calculated from the amount of nystose formed in the filtered supernatant and analyzed by a high-performance anion exchange chromatography unit with a pulsed-ampereometric detector (HPAEC-PAD), Dionex BioLC (Sunnyvale, CA, USA) with a CarboPac PA200 column (2 mm \times 250 mm) and a guard column. The injection volume was 25 μL using an autosampler (Dionex AS50) and 150 mM sodium hydroxide was used as the eluent at a

flow rate of 0.25 mL min^{-1} . Nystose content was calculated based on the peak areas from the chromatograms, as integrated by the Chromeleon software version 6.2 from Dionex. An appropriate dilution of nystose standard solution was used for calibration.

InH activity assay

InH activity was determined by the method of Ishiguro et al. (2010). One unit of InH activity is defined as the amount of enzyme which catalyzes the hydrolysis of 1 nmol of inulin in 1 min. A mixture of enzyme (100 μL), 0.05 g kg^{-1} inulin solution (200 μL), and McIlvaine buffer (pH 5.5, 100 μL) was incubated at $30 \text{ }^\circ\text{C}$ for 5 h. The reaction was stopped in boiling water for 10 min. InH activity was calculated from the amount of fructose released as analyzed by HPAEC-PAD in the same manner, as described above. The amount of free fructose was calculated from peak areas of the chromatograms, calibrated with an appropriate dilution of fructose standard solution.

Determination of inulin contents

Inulin was extracted using hot deionized water according to the method of Raessler et al. (2008) with modification. Each ground sample (500 mg) was weighed in a centrifuge tube. Deionized water (50 mL) was added, and then the tube was shaken in a water bath at $80 \text{ }^\circ\text{C}$ for 30 min (Raessler et al. 2008). The sample was cooled to room temperature and transferred in to centrifuge tubes before centrifuging at $10,000\text{g}$ for 20 min. After centrifugation, the supernatant (10 mL) was transferred to another centrifugation tube. To this solution, $340 \mu\text{L}$ of 300 g L^{-1} HCl solution was added before the tube was again placed in a shaking water bath at $85 \text{ }^\circ\text{C}$ for 60 min to obtain complete hydrolysis of inulin. After cooling to room temperature, the supernatant was transferred to a 50 mL volumetric flask and filled to volume with deionized water. The aliquot was filtered and transferred to sample vials. Products from inulin hydrolysis, i.e. fructose and glucose were analyzed by HPAEC-PAD, as described in 1-FFT activity assay. Peak areas of the chromatograms were used for sugar calculation. An appropriate dilution of a standard solution containing glucose and fructose was used for calibration with subtractive correction for fructose from sucrose. The inulin final content was obtained from $0.91 \times \text{fructose}$.

Determination of inulin profiles

Fresh tubers of 150 g were sampled from each pack and homogenized with a homogenizer (AM4 model, ACE, Nihon Seiki Kaisha Ltd., Tokyo). Inulin was extracted with hot deionized water (Van Waes et al. 1998). Eighty-five grams of deionized water were added to a flask containing

11.5 g homogenized sample, and then the flask was shaken at 130 rpm for 1 h in a hot ($85 \text{ }^\circ\text{C}$) water bath. After cooling to room temperature, the total weight was adjusted to 100 g with deionized water before the slurry was centrifuged for 20 min at $10,000\text{g}$ and then the supernatant was collected and stored at $-20 \text{ }^\circ\text{C}$ until use. Prior to inulin profile analysis, the sample was thawed in a $40 \text{ }^\circ\text{C}$ water bath. Inulin profiles were obtained from HPAEC-PAD, as described above. Two gradient eluents were 150 mM sodium hydroxide (eluent A) and 150 mM sodium hydroxide/500 mM sodium acetate (eluent B) running at a flow rate of 0.25 mL min^{-1} . The system was equilibrated with eluent A for 10 min before analysis. The elution gradient was programmed at 0–15 min with 100% eluent A, 15–45 min with 0–60% eluent B, 45–90 min with 60–90% eluent B, 90–110 min with 90–100% eluent B, and 110–120 min with 100–0% eluent B. The relative percentage of inulin DPs was calculated from peak areas of the chromatograms, as shown in Fig. 1.

Determination of fructose, glucose, sucrose, kestose, and nystose

Each ground Jerusalem artichoke sample (500 mg) was weighed into a flask. Deionized water (50 mL) was added, and the preparation steps until centrifugation were the same as described in the inulin determination. Supernatants were collected in a 200 mL volumetric flask and filled to volume with deionized water. Aliquots were filtered and transferred to sample vials. Fructose, glucose, sucrose, kestose and nystose were analyzed by HPAEC-PAD, as described above. Sugar contents were calculated from the peak areas of the chromatograms. An appropriate dilution of a standard solution containing glucose, fructose, sucrose, 1-kestose and nystose was used for calibration.

Statistical analysis

Statistical analyses were performed using SPSS version 16 for Windows. Data were analyzed using one-way fixed factor analysis of variance (ANOVA) with Duncan's multiple range test for mean comparison at the significance level of $P < 0.05$.

Results and discussion

Influence of harvest time

Activity of 1-FFT

Activity of fructan:fructan 1- fructosyltransferase (1-FFT) in harvested tubers continuously decreased from 30 to 70

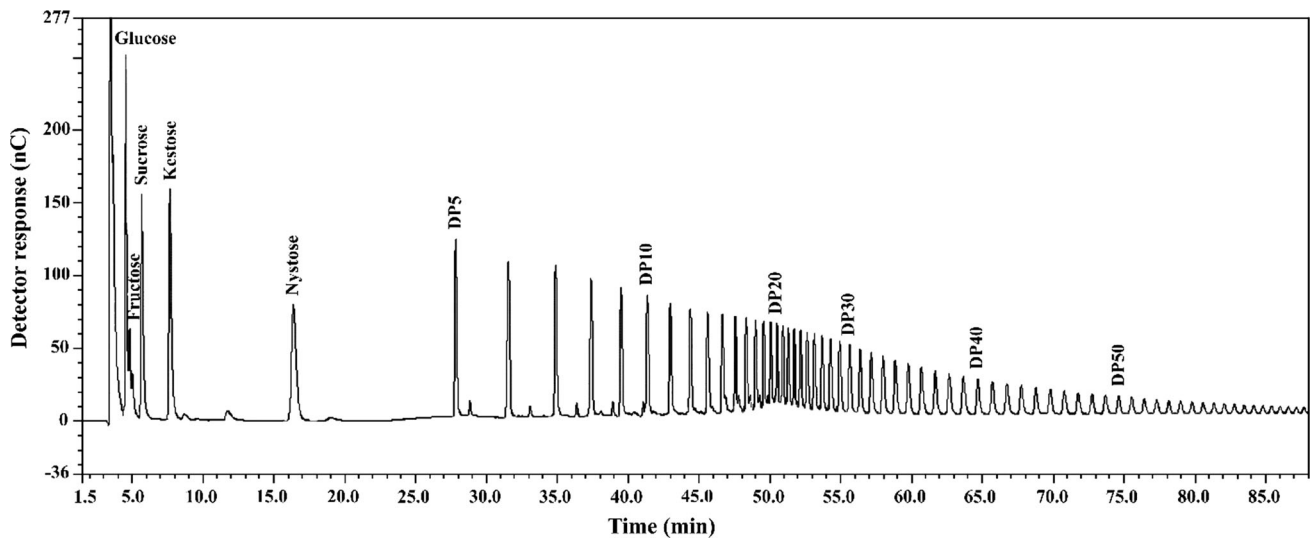


Fig. 1 HPAEC-PAD chromatograms of inulin in Jerusalem artichoke tubers

Table 1 Activity of fructan:fructan 1-fructosyltransferase (1-FFT) and Inulin content in Jerusalem artichoke tubers at different harvest time

Harvest time (DAF)	1-FFT activity ($\text{Ug}^{-1} \text{DW}$)	Inulin ($\text{g kg}^{-1} \text{DW}$)
30	79.9 ± 5.6^a	640.9 ± 15.0^c
40	57.0 ± 3.1^b	715.6 ± 13.4^{ab}
50	43.3 ± 0.8^c	734.9 ± 20.5^a
60	35.9 ± 0.6^d	710.0 ± 29.9^{ab}
70	28.0 ± 0.6^e	681.3 ± 17.8^b

Values are the mean \pm standard deviation

Different letters indicate significant difference at $P < 0.05$

DAF in a progressively pseudo-linear regression scheme, from 79.9 to 28.0 $\text{Ug}^{-1} \text{DM}$ (65.0% decrease), as shown in Table 1. From the activity of 1-FFT and inulin content, results suggested that 1-FFT was active before 30 DAF and its remaining activity continued catalyzing the formation of inulin as demonstrated by rising inulin levels until 60 DAF. It was reported that fructan synthesis was controlled by 1-SST and 1-FFT (Edelman and Jefford 1968). The DP of fructans produced by the plant depended mainly on the enzymatic activity of 1-FFT which provided a wide range of DP, from small amounts of short-chain oligomers to large amounts of high-DP fructans (Van Laere and Van Den Ende 2002). In addition, the action of 1-SST and 1-FFT resulted in the formation of fructans with different chain lengths. In Jerusalem artichoke tubers, both 1-SST and 1-FFT were observed to be active during fructan accumulation (Pollock 1986). Starting from sucrose, 1-SST produced 1-ketose which would then be elongated by 1-FFT, resulting in the formation of inulin (Vijn and Smeekens 1999).

Inulin content

Inulin content trended to a peak at 50 DAF with an average of $735 \text{ g kg}^{-1} \text{ DW}$ and gradually decreased in tubers harvested at 60 and 70 DAF (by 7.3% from 50 to 70 DAF) (Table 1). There were no significant differences among inulin contents in tubers harvested during 40–60 DAF, though 50 DAF seemed to be a good harvest time considering that long chain polymerized fructans were also highest at this time point.

Inulin profile

The harvest time for Jerusalem artichoke tubers affected DP of inulin, as shown in Fig. 2a. The inulin profile was significantly different during the period of 30–70 DAF. DP of inulin increased both in proportion and in chain length during 30–50 DAF. The DP >30 relatively reached by 28% at 50 DAF and then decreased with sharp increases in DP <10 at 60 and 70 DAF. The decrease in higher DP

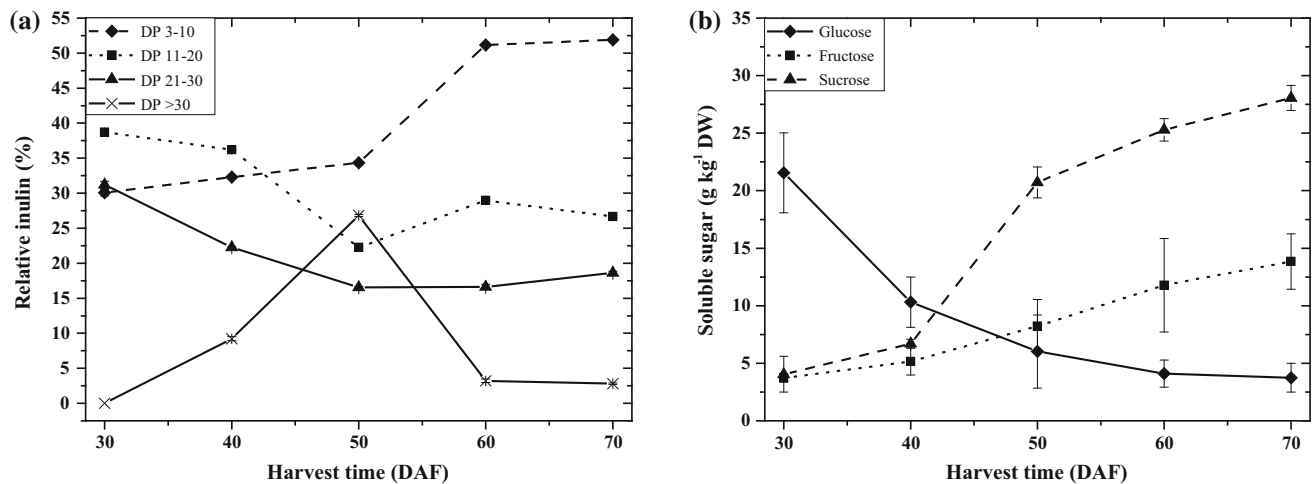


Fig. 2 Inulin profile (a), glucose, fructose, and sucrose (b) in Jerusalem artichoke tubers at different harvest time

inulin at 60–70 DAF was likely caused by the depolymerization of fructans by fructan 1-exohydrolase (1-FEH) (Edelman and Jefford 1968). Similar changes were reported in Jerusalem artichoke tubers harvested at 56 DAF or 20 weeks maturity (Saengthongpinit and Sajjaanantakul 2005). It was reported that the drying period of Jerusalem artichoke leaves and stems in the field was accompanied by a slight increase in reducing sugars that resulted from depolymerization of high molecular weight carbohydrates (Schorr-Galindo and Guiraud 1997). Results from the present study suggest that tubers harvested at 50 DAF would be preferable for the production of long chain inulin, because the tubers contained the highest DP of fructans among 30–70 DAF harvested tubers.

Soluble sugar content

Soluble sugar contents were significantly different over harvest times (Fig. 2b). After 50 DAF, free glucose decreased while free fructose and sucrose increased. These results were similarly found in more mature (from 16 to 20 weeks) Jerusalem artichoke tubers measured as relative percentage of sugars (Saengthongpinit and Sajjaanantakul 2005). Decreases in free glucose could be explained by its utilization in the respiration process and through metabolic activities of the tubers (Jaime et al. 2000). 1-SST catalyzes the first step of fructan synthesis in the growing tuber, using sucrose as the primary source of the fructosyl donor and releasing free glucose. Thus, glucose usually appears in growing tubers and decreases to a low level in mature tubers.

Increasing in fructose and sucrose at 60 and 70 DAF was coincident with increasing in low DP inulin. This was the result of high molecular weight inulin hydrolysis as shown in Fig. 2a. Jerusalem artichoke tubers use free fructose and sucrose for metabolic activities as glucose substitutes.

Changes in soluble sugars were the result of inulin depolymerization catalyzed by 1-FEH and 1-FFT to release free fructose. The released fructose would then be used for the synthesis of sucrose supply to other plant tissues (Itaya et al. 2002).

Changes during low temperature storage

Activity of 1-FFT

Activity of 1-FFT in the tubers harvested at 50 DAF was significantly different over the whole range of temperatures, -18 , 0 , 5 , 10 , 15 , and 25 °C during 30 days storage (Fig. 3a). Storage at -18 °C provided the highest retention of 1-FFT activity compared with other higher storage temperatures. It should be noted that freezing storage at -18 °C could best maintain 1-FFT activity with gradual decreases in 1-FFT activity during the assigned period. This implied that 1-FFT was still active at this storage temperature. In addition, activity of this enzyme could be further reduced during thawing and storing at higher temperatures above freezing point. The enzyme could be denatured at the ice-liquid interface during freezing due to additional interfacial tension on the entrapped enzyme from ice recrystallization during thawing (Cao et al. 2002). During storage, 1-FFT activity increasingly decreased at high temperatures (Fig. 3a). The higher storage temperature applied led to greater 1-FFT activity loss. The loss of 1-FFT activity at all storage temperatures would be the results of gene 1-FFT transcription and expression affected by soluble sugars changes in the tubers. It was found that carbon deficit in the inulin producing plant, *Chrysolaena obovata* (Less.) Dematt., inhibited transcription of the gene 1-FFT and that sugar supplementation provided negative correlation between 1-FFT activity and gene expression (Trevisan et al. 2015). Supplemented sugars were glucose,

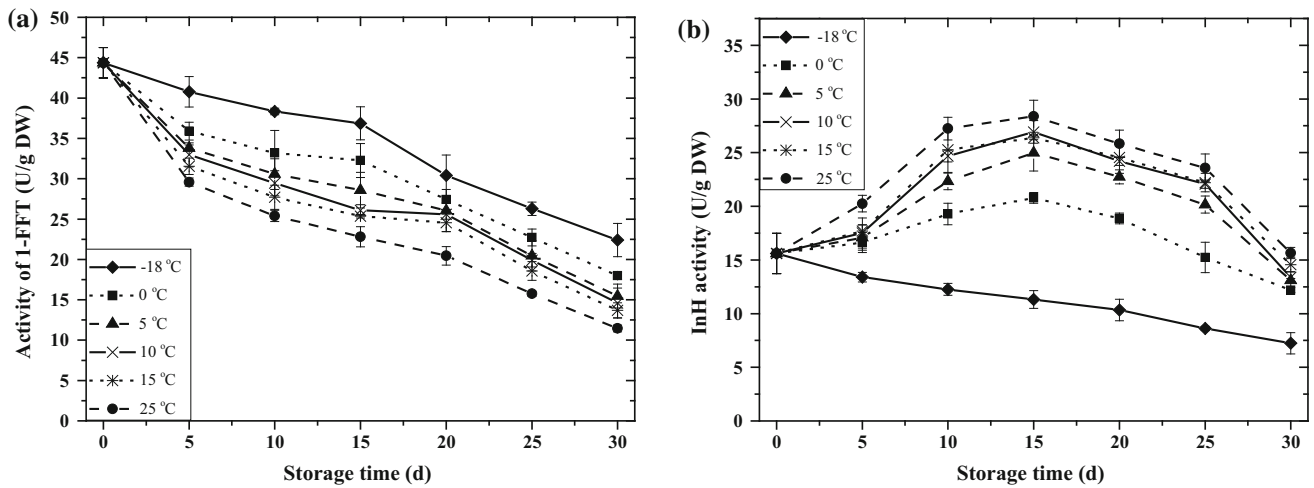


Fig. 3 Activities of 1-FFT (a) and InH (b) in Jerusalem artichoke tubers stored at different temperatures for 30 days

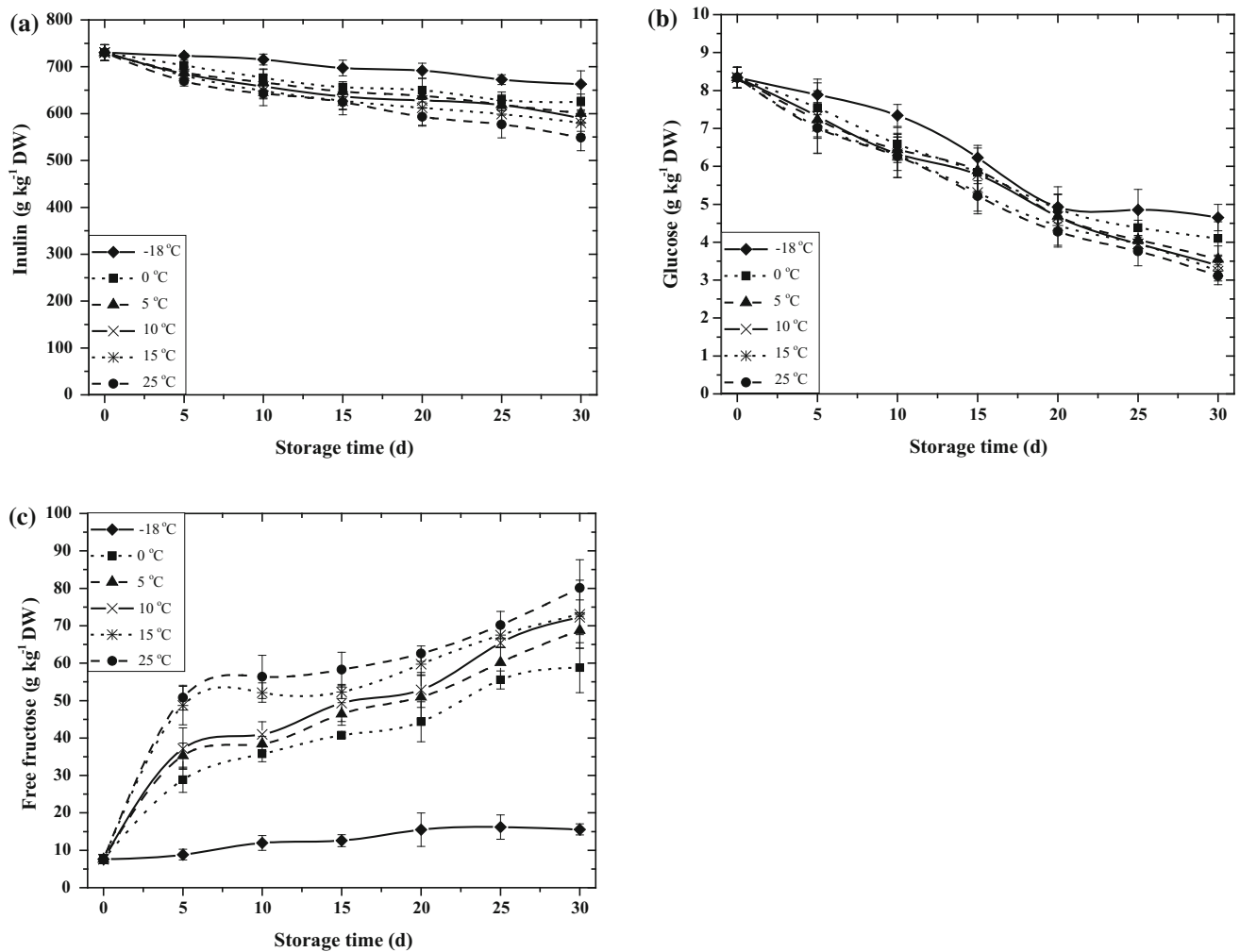


Fig. 4 Inulin content (a), glucose (b), and free fructose (c) in Jerusalem artichoke tubers harvested at 50 DAF and stored at different temperatures for 30 days

fructose, and sucrose, all of which decreased 1-FFT activity. Among these sugars, fructose markedly exhibited 1-FFT activity reduction. Evidently, soluble sugars rise (Figs. 4c, 5a) supported 1-FFT activity loss during storage.

Activity of 1-FFT decreased progressively in a pseudo-linear regression manner as found in fresh tubers during growing. During 30 days storage, 1-FFT activity reduced from 44.4 to 22.4 and to 11.5 Ug^{-1} DM at -18 and 25 °C, respectively. Similar results that the activity of 1-FFT decreased progressively in a pseudo-linear regression scheme were reported in burdock roots stored at 0, 15, and 25 °C (Ishiguro et al. 2010). In Jerusalem artichoke tubers, 1-SST and 1-FFT were active during fructan accumulation (Pollock 1986), though 1-FFT involving both inulin synthesis and hydrolysis decreased during storage (Fukai et al. 1997). At appropriate conditions for synthesis, 1-FFT catalyzes inulin chain elongation. However, depolymerization would be catalyzed by 1-FFT to move fructosyls from the longer-chain inulin to obtain free sucrose (Luscher et al. 1993). In the current work, 1-FFT activity continuously

reduced from the first maturity stage being studied (30 DAF) to the end of postharvest storage, but the enzyme was still able to contribute to changes in inulin content.

Activity of InH

Activity of inulin hydrolase (InH) was significantly different at all temperatures during 30 days storage (Fig. 3b). At -18 °C, InH steadily lost its activity during storage which was similar to 1-FFT, though was unlike storage at 0, 5, 10, 15, and 25 °C where InH activity steadily increased over the first 15 days of storage with the maximum increase on day 15 regardless of storage temperature. After InH activity reached its peak, the activity at all storage temperatures on day 25 was higher or similar to day 0. On the other hand, activity of InH progressively increased as storage temperatures increased. Similar results were reported in burdock roots stored in modified atmosphere packaging at 2, 8 and 20 °C where activity of inulinase (InH) also increased sharply after 1 week storage

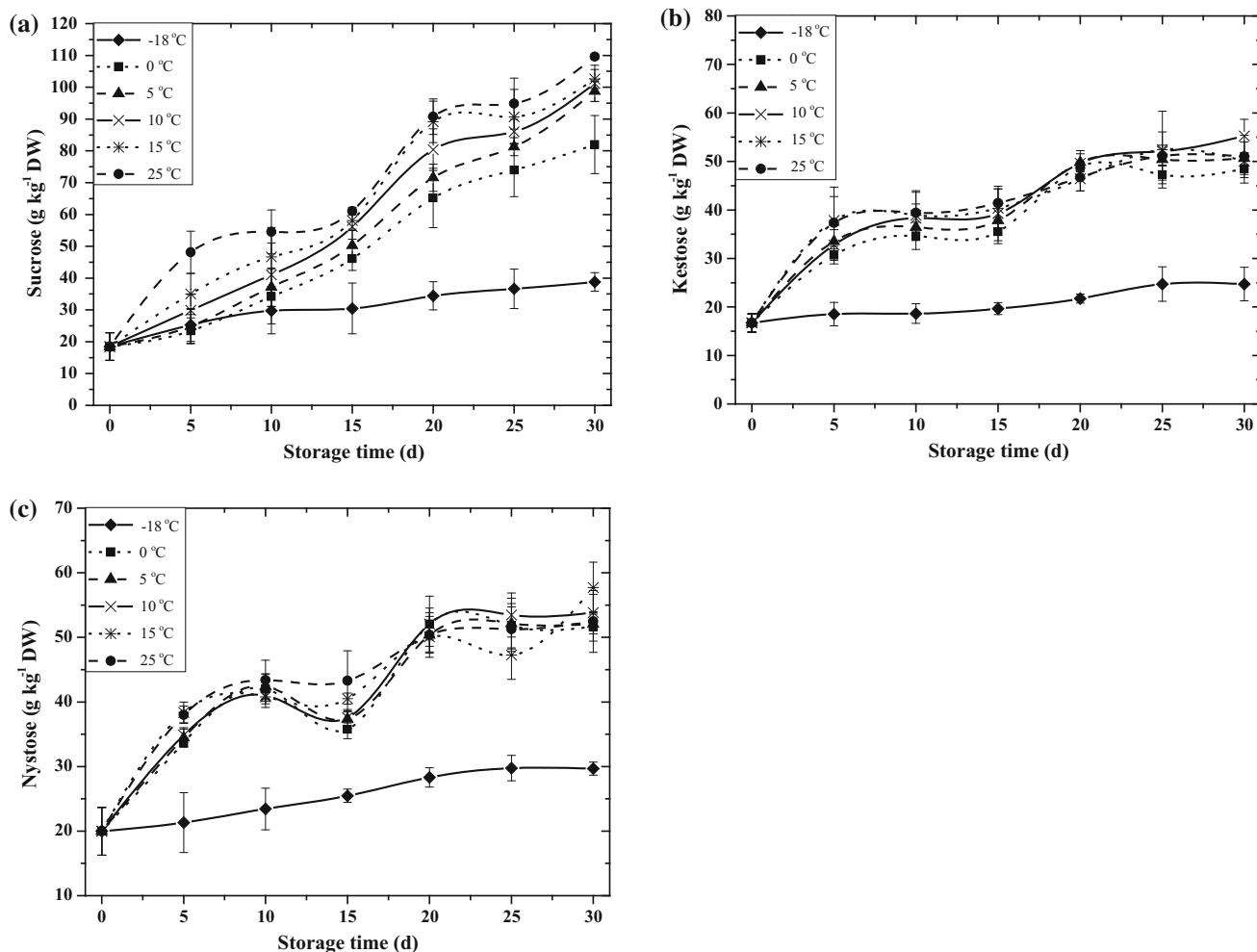


Fig. 5 Sucrose (a), kestose (b), and Nystose (c) in Jerusalem artichoke tubers stored at different temperatures for 30 days

regardless of temperature (Ishimaru et al. 2004). The activity of InH at the end of 30 days storage changed from 15.6 to 7.2 and to 15.4 Ug^{-1} DW at -18 and 25 °C, respectively. With increased InH activity up to 15 days and relatively high activity at the end of storage, high increases in soluble sugars (fructose, sucrose, kestose, and nystose) were correspondingly observed in the tubers after maximum InH activity. Therefore, InH played an important role in storage changes of sugars in the tubers. This indicated that, storage for first 5 days could maintain quality of the tubers with minimal changes. To avoid high soluble sugars in the tubers, storage should be at 10 °C or below and for no longer than 15 days unless lower DP inulin is desirable for specific uses.

Inulin content

At 50 DAF, inulin content in the Jerusalem artichoke tubers averaged 734.2 g kg^{-1} DW (Fig. 4a). Inulin content varied significantly with different storage temperatures over the 30 days. Jerusalem artichoke tubers stored at -18 °C had the highest content of inulin, though inulin content decreased slightly (9.7% reduction) after 5 days of storage. During storage at 0, 5, 10, and 15, inulin contents only slightly decreased after 5 days, though showed significant loss when stored at 25 °C and linearly reduced to the end of the storage period. Final inulin contents at 30 days were reduced by 14.9, 18.2, 19.6, 20.9, and 25.2% for storage at incremental temperatures between 0 and 25 °C, respectively. This could be due to depolymerization of fructans catalyzed by 1-FEH. Depolymerization of inulin was found to be nearly complete within the first 6 weeks of cold storage and changes in DP of inulin were more pronounced at higher storage temperatures (Rutherford and Weston 2001; Modler et al. 1993). The abiotic stress of low temperature seemed to affect Jerusalem artichoke tubers during the growth period in the same manner. The amount of inulin in overwintered Jerusalem artichoke tubers was found to decrease, because of inulin conversion to sucrose together with forming lower DP inulin (Clausen et al. 2012).

Hydrolysis of inulin is necessary to obtain soluble sugars for metabolic activities of the tubers. Storage at low temperature would slow down respiration rate and delay changes of inulin contents. For long term storage of Jerusalem artichoke tubers, -18 °C would clearly be the best choice.

Soluble sugar content

During 30 days of storage, changes of fructose, sucrose, kestose, and nystose were significantly different at all

storage temperatures (Figs. 4b, c, 5a–c). Glucose content gradually decreased during storage whereas fructose, sucrose, kestose, and nystose contents noticeably increased, especially after storage for 15 days except for the -18 °C storage temperature. Increases in fructose and sucrose in the tubers stored at 5 °C for 30 days were similar to those found in mature Jerusalem artichoke tuber stored at the same temperature for 4 weeks, expressed as relative percentage (Saengthongpinit and Sajjaanantakul 2005). Glucose decreased during storage as a result of respiration and metabolic activities of the tubers (Jaime et al. 2001). Increased levels of free fructose, sucrose, kestose, and nystose in the stored tubers could have been caused by inulin hydrolysis as catalyzed by 1-FEH and 1-FFT to produce free fructose and sucrose as glucose substitutes for metabolic activities (Itaya et al. 2002). At low temperature storage, Jerusalem artichoke tubers would be essentially in dormancy and 1-SST activity was found idle, yet 1-FEH was active enough to catalyze the removal of fructosyl residues from the stored fructans, providing sucrose in vacuoles (Gupta and Kaur 2000). However, it is well recognized that enzyme activity is retarded at -18 °C since the majority of water becomes ice which limits movement of the enzyme and its substrates. Slight increase in soluble sugars of the tubers stored at -18 °C would, therefore, be due to the activity of hydrolytic enzymes prior to freezing and during thawing. During storage of the tubers, InH would work in concert with 1-FEH and 1-FFT, resulting in increased soluble sugars. On the basis of fructose released from inulin (Fig. 4c), storage below 10 °C for 10 days would be suitable for controlling the combined effects of these key enzymes.

Conclusion

Jerusalem artichoke tubers harvested at 50 DAF had the highest concentration of inulin and the highest proportion of long chain inulin polymers. Therefore, 50 DAF would be the optimum harvest time for commercial production of the tubers and for industrial uses of inulin. Inulin contents in the tubers were strongly influenced by storage temperatures. During storage of Jerusalem artichoke tubers, inulin depolymerization caused decreases in inulin content associated with increases in fructose, sucrose, kestose, and nystose. These changes were in association with increased InH and decreased 1-FFT activities, indicating high hydrolysis and low synthesis of inulin. Decreasing storage temperatures would slow down the loss of inulin and the rise in soluble sugars, except glucose. Particularly, freezing storage at -18 °C would best minimize changes of inulin contents, soluble sugars, and the activity of 1-FFT and InH.

Acknowledgements This research was supported by Suranaree University of Technology, Suratthani Rajabhat University, Khon Kaen University, Postharvest Technology Innovation Center, the Office of Higher Education, Thailand, and Whistler Center for Carbohydrate Research, Purdue University, USA.

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