Inulin content of Jerusalem artichoke (Helianthus tuberosus L.) tubers stored at 5 °C in a refrigerator for different durations

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Abstract
Jerusalem artichoke is a perennial crop, but it is grown as an annual crop for its inulin containing tubers. Various factors affect inulin content, in which storage duration is one of the significant causes. Therefore, the effect of different storage durations on inulin content of Jerusalem artichoke was investigated in this study. Four Jerusalem artichoke varieties were assigned as factor A, and six durations of storage at 5 °C were assigned as factor B. The 24 treatment combinations of a 4×6 factorial were arranged in a completely randomized design with four replications. After harvest, the tubers were cleaned and stored at 5 °C for five months. Inulin content was analyzed consecutively at 0, 1, 2, 3, 4 and 5 months after storage. Times of storage and varieties were significantly different (P<0.01) for inulin content. Stored tubers had inulin contents ranging from 35.9 to 43.7 % compared to 47.8 % of freshly-harvested tubers. The lowest inulin content was found after 2 months of storage. JA 89 had the highest inulin content (47.80%), whereas CNS2867 had the lowest inulin content (35.85%). Regression analysis showed small and insignificant effect of storage time on inulin content, and the results are discussed. The results will be useful for consumers and industries for their concern on storage loss of inulin content after storage.

Keywords: functional food, Helianthus tuberosus L. storage duration, storage loss, tuber preservation.

Introduction
Jerusalem artichoke (Helianthus tuberosus L.) is a perennial tuber crop, originating in central-eastern North America (Bock et al. 2014). The crop has been also known as a promising material for ethanol production in biofuel industry and functional food industry (Yang et al. 2015). Jerusalem artichoke tuber is used as a raw material for animal feed and functional food products. The tuber contains inulin, a fructose polymer, and its degraded product called oligofructose is the major compound for functional food industry. Inulin can stimulate health-promoting gut microflora, prevent obesity, heart disease and diabetes, and may reduce the risk of cancer (Orafti 2005). Various factors including variety, growing environment, planting date, harvest time and storage condition affect inulin content (Danilčenko et al. 2008; Puangbut et al. 2012; Puttha et al. 2012; Saengthongpinit and Sajjaanantakul 2005). Previous studies reported that storage of tubers stimulated the depolymerization of inulin (Cabezas et al. 2002). However, harvested tubers sometimes cannot be further processed or consumed instantly after harvest. Therefore, various methods have been used for tuber storage. Jerusalem artichoke tubers can be successfully stored for long period under cold temperatures. Long duration of storage could depolymerize inulin. Storing the tubers at 5 °C for two weeks reduced inulin DP>10 (Saengthongpinit and Sajjaanantakul, 2005). To the best of our knowledge, the study was completed with only one Jerusalem artichoke variety and the storage was limited to 10 weeks. Therefore, the results so far have not been conclusive. The information on the effect of long-term storage on inulin content of different genotypes of Jerusalem artichoke is still lacking. Therefore, this study investigated the effect of storage duration on inulin content of four Jerusalem artichoke genotypes.

Results and discussion
Storage times and varieties of Jerusalem artichoke were significant different (P≤0.05) for inulin content, but the interaction between Jerusalem artichoke variety and storage time was not significant (Table 1.). Freshly-harvest tubers (0 month) had the highest inulin content, which was significantly higher than the tubers stored for 1, 2, 3 and 5 months (Table 2.). However, it was not significantly higher than the tubers stored for 4 months. This might be caused by the inherent heterogeneity of the samples because the experimental error was acceptably low, and the larger sample size is recommended for determination of inulin content in Jerusalem artichoke. JA 89 had the highest inulin content, and it was significantly higher than the other varieties. The lack of significant interaction between storage time and variety indicated that the varieties performed similar pattern for inulin content across storage times. Storage of Jerusalem artichoke tubers at 5 °C in a refrigerator for one to five months reduced inulin content although the reduction as identified by regression analysis was not significant (Fig 1.). The results showed a slow
Table 1. Analysis of variance for inulin content of four Jerusalem artichoke varieties after storage at 5 °C in a refrigerator at different durations from 0 to five months.

| Source of variation | df | Sum square | Mean square | F-ratio | Probability |
|---------------------|----|------------|-------------|---------|-------------|
| Month (M)           | 5  | 1659.31    | 331.862     | 9.07    | 0.000       |
| Variety (V)         | 3  | 588.84     | 196.278     | 5.36    | 0.0022      |
| M×V                 | 15 | 838.71     | 55.914      | 1.53    | 0.1184      |
| Error               | 72 | 2635.8     | 36.608      |         |             |
| Total               | 95 | 5722.65    |             |         |             |

CV 14.92%.

Fig 1. Regression analysis for inulin contents of six storage durations (months) averaged across 4 Jerusalem artichoke varieties stored at 5 °C in a refrigerator.

Table 2. Means for inulin content of six storage durations from 0 to five months and four Jerusalem artichoke varieties across six storage durations.

| Month after storage | Inulin content (%) |
|---------------------|--------------------|
| 0                   | 47.80*             |
| 1                   | 39.47bc            |
| 2                   | 35.85c             |
| 3                   | 40.11bc            |
| 4                   | 43.69bc            |
| 5                   | 36.33bc            |

Means of storage durations and means of varieties with the same letter are not significantly different by LSD at 0.05 probability level.

reduction from the initiation to the end of the experiment. However, the inulin contents of the storages for 3 and 4 months were rather high and confounding. This might be possibly due to the inherent heterogeneity of the samples as discussed earlier.

Other than the reduction in inulin content, long term storage of Jerusalem artichoke tubers also caused the loss of aesthetic appearance, spoilage and germination of tuber buds. Therefore, Jerusalem artichoke tubers as fresh vegetable should not be stored too long. Based on the results, consuming fresh Jerusalem artichoke tubers seemed to be a compromising idea to gain highest inulin.

In the previous study on the storage of Jerusalem artichoke tubers at 5 °C in the variety CNS2867, which was also used in this study, the rapid reduction in inulin content occurred during storage for 0 to 5 days, and slow reduction occurred after 5 days of storage until 30 days of storage (Maicaurkaew et al., 2017). According to Leroy et al. (2010), storage time caused a decrease in inulin content and an average degree of polymerization, accompanied by an increase of free fructose and sucrose due to depolymerization of inulin. Inulin DP 3-10 was increased, while DP > 10 decreased with longer storage time at 5 °C (Saengthongpinit and Sajjaanantakul, 2005).

In this study, the highest inulin content was 47.8% at harvest date, and the lowest inulin content was found after 2 months of storage (35.9%). In previous studies, the range of inulin contents in fresh tubers of Jerusalem artichoke was from 7 to 30% (Kays and Nottingham, 2008; Saengthongpinit, 2005). In the most recent study, inulin contents of Jerusalem artichoke tubers ranged from 8.16 to 13.46% of fresh weight (Brkljača et al., 2014). Inulin contents in this study exceeded the range of inulin content in previous studies might be due to breeding attempt for high inulin content (Puangbut et al., 2017). However, the range of
inulin contents in fresh tubers is still wide even in the same variety. Studies so far have been conducted to store Jerusalem artichoke tubers under a wide range of temperatures. Saengthongpinit and Sajaanantakul (2005) reported that the compositions of inulin extract of frozen tubers (-18 °C) remained stable throughout the storage time. However, the proportions of monosaccharide, sucrose and DP 3–10 in frozen samples (-18 °C) were lower due to drip loss during thawing (Saengthongpinit and Sajaanantakul, 2005). Based on our preliminary experiment, the texture and taste of the tubers become unpleasant for consumers. Therefore, frozen storage of Jerusalem artichoke tubers is not recommended.

The quality of Jerusalem artichoke tubers under different storage conditions has been studied. Daničenko et al. (2008) found that tuber weight, soluble solid content, total sugar content and sucrose content reduced during four months of storage at 2 °C in chamber. However, the reduction in sugar content was found after storage. The temperature, 5 °C was used in this study to simulate actual storage method of consumers or growers.

Jerusalem artichoke varieties were significantly different for inulin content, ranging from 37.63 to 44.48% across all storage durations, and the highest inulin content was found in JA 89 (Table 2). JA 89 was significantly higher than HEL 65, CN 52867 and 50-4, which had the similar values of inulin content. According to Puangbut et al. (2012), planting date and temperature during growing season were the main factors affecting the variation in inulin content in Jerusalem artichoke. They reported the range of inulin contents between 58.1 and 68.9%, and CN 52867 had highest inulin content. In a large collection of 79 genotypes, Puttha et al. (2012) reported the range of inulin contents from 55.3 to 74.0%. JA 37 had the highest inulin content and the lowest was found in JA 4. Genotype and environment play a major role in the variation of inulin contents in Jerusalem artichoke, and the ranges found in these reported were much larger than that observed in our study.

Materials and methods

Plant materials and experimental design

The effect of storage time at 5 °C in a refrigerator on inulin content of four Jerusalem artichoke varieties was studied. The varieties of Jerusalem artichoke included JA 89, HEL 65, CN 52867 and hybrid variety, 50-4 (JA 102 × JA 89) (8), which were developed from the Jerusalem artichoke project of Khon Kaen University, Khon Kaen, Thailand. The Jerusalem artichoke varieties were grown under field condition in 2017 at Rajamangala University of Technology Tawan-ok, Chonburi, Thailand. Four Jerusalem artichoke varieties were assigned as factor A, and six durations of storage (0, 1, 2, 3, 4 and 5 months) were assigned as factor B. The 24 treatment combinations were arranged in a completely randomized design with four replications. After harvest, Jerusalem artichoke tubers were cleaned in tap water and surface-dried to prevent the spread of disease during storage, and then divided into two groups. The first group was instantly prepared in absolute ethanol for analyzing of inulin content (as control). The second group of the tubers was packed separately in 80 sealable polyethylene (PE) bags (4 varieties×4 replications×5 storage times), and later stored at 5 °C in a refrigerator. Every month after storage, the Jerusalem artichoke tubers were taken for analysis of inulin content, and the method for chemical analysis was shortly described below.

Sample preparation and analysis of inulin content

The tubers were sliced lengthwise into thin pieces of about 1 to 2 mm. The sliced samples of 50 grams were soaked in 100 ml absolute ethanol at 10 °C for 24 h to prevent enzymatic degradation of inulin in the tubers. Ethanol solution was drained out and the samples were immediately stored at -20 °C in sealable PE bags until analysis. The samples were oven-dried at 60 °C for 12 hours or until the weights were constant. The dried samples were ground into fine powder and maintained in desiccators until inulin extraction. The sample of each bag was divided into three replications each of which has two gram, and the samples were further used for inulin analysis. Inulin was extracted by reverse osmosis method using water as an extracting agent in a 25 ml flask at 80 °C for 20 min on a water bath. The samples were shaken well, set aside to reduce heat, and filtered with filter paper (Whatman no. 1). The extracted sample of 500 µl was transferred to a 25 ml volumetric flask, hydrolyzed with 750 µl of hydrochloric acid (3% v/v HCl), adjusted with reverse osmotic water, and heated in hot water at 97 ± 2 °C for 45 min on a hot plate. After the samples were cooled at room temperature, they were used for chemical analysis. Fructose was determined by spectrophotometry using periodate reaction (Saengkanuk et al. 2011). Inulin content was calculated based on fructose, ignoring trace amount of glucose and reducing free fructose, glucose and sucrose. The inulin analysis results were shown as the percentage of inulin content on dry weight basis.

Data analysis

Data were analyzed according to a 4×6 factorial design by using Statistix 8 software program (Statistix 8 2003). Least significant difference (LSD) was used to compare means at 0.05 probability level.

Conclusion

Storage of Jerusalem artichoke tubers for long durations did reduce inulin content in the tubers. However, the reduction rate was slow, and small reduction in inulin content was observed during five months of storage at 5 °C in a refrigerator. Therefore, consuming freshly harvested tubers is highly recommended. Inulin content in the storage tubers was also affected by Jerusalem artichoke variety, and JA 89 had highest inulin content across storage durations.

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